THALASSAEMIA INTERNATIONAL FEDERATION

5th Edition

GUIDELINES FOR THE MANAGEMENT OF TRANSFUSION-DEPENDENT β-THALASSAEMIA (TDT)

PUBLISHERS: THALASSAEMIA INTERNATIONAL FEDERATION

Editors: Taher, A.T.
Farmakis, D.
Porter, J.B.
Cappellini, M.D.
Musallam, K.M.

“Cure sometimes, treat often, comfort always.”

Hippocrates (460-357 B.C.)

“The good physician treats the disease; the great physician treats the patient who has the disease.”

Sir William Osler (1849-1919)

Disclaimer: Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not reflect those of the European Union or HaDEA. Neither the European Union nor the granting authority can be held responsible for them.

Printed in Nicosia, Cyprus.

5th Edition

GUIDELINES FOR THE MANAGEMENT OF TRANSFUSION-DEPENDENT β-THALASSAEMIA (TDT)

PUBLISHERS: THALASSAEMIA INTERNATIONAL FEDERATION

Editors: Taher, A.T.
Farmakis, D.
Porter, J.B.
Cappellini, M.D.
Musallam, K.M.

TABLE OF CONTENTS

CONTRIBUTORS AND AFFILIATIONS 14

GRADING OF RECOMMENDATIONS 20

FORWARD 22

CHAPTER 1 | GENETIC BASIS, PATHOPHYSIOLOGY, AND DIAGNOSIS 24

1. INTRODUCTION 24
2. HAEMOGLOBIN TYPES, GLOBIN GENES, AND GLOBIN SYNTHESIS 24
3. CLASSIFICATION AND EPIDEMIOLOGY 26
4. MOLECULAR UNDERSTANDING 29
5. GENOTYPE-PHENOTYPE CORRELATION 30

5.1. β-Thalassaemia
5.2. Haemoglobin E/β-thalassaemia
5.3. Haemoglobin Lepore

6. PATHOPHYSIOLOGY 32

6.1. Ineffective erythropoiesis
6.2. Dysregulated iron metabolism
6.3. Clinical manifestations of ineffective erythropoiesis, anaemia, and iron overload

7. DIAGNOSIS 35

7.1. Clinical diagnosis
7.2. Haematological diagnosis
7.3. Qualitative and quantitative haemoglobin analysis
7.4. Molecular analysis

KEY POINTS AND RECOMMENDATIONS 38
REFERENCES 39

CHAPTER 2 | BLOOD TRANSFUSION 44

1. INTRODUCTION 44
2. AIMS OF BLOOD TRANSFUSION 44
3. PRACTICAL CONSIDERATIONS FOR TRANSFUSION THERAPY 45

3.1. Criteria for initiating transfusion therapy
3.2. Patient blood management, clinical decision-making, and informed consent

3.2.1. What is patient blood management?
3.2.2. How does patient blood management apply in the setting of transfusion-dependent β-thalassaemia?

3.3. Product selection for transfusion
3.4. Maintenance and adjustment of transfusions
3.5. Volume to be transfused
3.6. Rate of transfusion
3.7. Specific patient groups

3.7.1. Very low haemoglobin at presentation
3.7.2. Pregnant patients
3.7.3. Cardiac failure
3.7.4. Haemoglobin E/β-thalassaemia
3.7.5. Non-transfusion-dependent β-thalassaemia
3.8. Transfusional iron loading
4. BLOOD: FROM COLLECTION TO ADMINISTRATION 52
4.1. Haemovigilance
4.2. Blood donation and testing for transfusion-transmitted infections
4.3. Blood component specification and preparation
4.3.1. Leucodepletion
4.4. New technologies
4.4.1. Pathogen inactivation systems
4.4.2. Manufactured red cells
4.4.3. Universal red cells
4.5. Blood products for special patient populations
4.5.1. Washed red cells
4.5.2. Cryopreserved (frozen) red cells
4.5.3. Red cells obtained by donor apheresis
4.6. Storage of donor red cell units
4.7. Age and type of product to be transfused
4.8. Compatibility testing
4.9. Transfusion and the spleen
5. ADVERSE REACTIONS 59
5.1. Febrile non-haemolytic transfusion reactions
5.2. Allergic reactions
5.3. Acute haemolytic reactions
5.4. Alloimmunisation
5.5. Delayed transfusion reactions
5.6. Autoimmune haemolytic anaemia
5.7. Transfusion-related acute lung injury
5.8. Transfusion-associated graft-versus-host disease
5.9. Transfusion-associated circulatory overload
5.10. Transfusion-transmitted infections
6. QUALITY OF LIFE AND ECONOMIC BURDEN OF TRANSFUSION 63
6.1. Economic burden of transfusion
6.2. Quality of life
KEY POINTS AND RECOMMENDATIONS 65
REFERENCES 66

CHAPTER 3 | IRON OVERLOAD AND CHELATION 70

1. INTRODUCTION 70
2. THE RATE OF IRON LOADING 71
3. TOXICITY FROM IRON OVERLOAD 71

3.1. Iron toxicity mechanisms
3.2. Distribution and consequences of transfusional iron overload
4. MONITORING OF IRON OVERLOAD 74
4.1. Serum ferritin
4.2. Liver iron concentration
4.2.1. Methods of measuring liver iron concentration
4.3. Myocardial iron estimation: T2* and other tools
4.4. Cardiac function
4.5. Monitoring of other organ function and iron-mediated damage
4.6. 24-hour urinary iron estimation
4.7. Plasma non-transferrin-bound iron and labile plasma iron
5. AIMS OF IRON CHELATION THERAPY 80
6. SOURCES OF CHELATABLE IRON 81
7. CHEMICAL AND PHARMACOLOGICAL PROPERTIES OF LICENSED IRON CHELATOR 81
8. PRACTICAL PRESCRIBING OF INDIVIDUAL CHELATORS 83
8.1. Deferoxamine monotherapy
8.1.1. Evidence of beneficial effects
8.1.2. Effects on serum ferritin
8.1.3. Effects on liver iron
8.1.4. Effects on cardiac iron
8.1.5. Effects on heart function
8.1.6. Recommended treatment regimen: standard therapy
8.1.7. Recommended treatment regimen: rescue therapy
8.1.8. Recommended treatment regimen: emergency therapy
8.2. Deferiprone monotherapy
8.2.1. Effects on serum ferritin
8.2.2. Effects on liver iron
8.2.3. Effects on cardiac iron
8.2.4. Effects on heart function
8.2.5. Compliance with deferiprone
8.2.6. Long-term benefits of deferiprone monotherapy
8.2.7. Recommended treatment regimen
8.3. Deferasirox monotherapy
8.3.1. Chemistry and pharmacology
8.3.2. Effects on serum ferritin
8.3.3. Dose effects on iron balance
8.3.4. Effect on cardiac iron
8.3.5. Effects on heart function
8.3.6. Convenience and impact on quality of life
8.3.7. Film-coated tablet and sprinkle granule formulations
8.3.8. Recommended treatment regimen: standard therapy
8.3.9. Recommended treatment regimen: rescue therapy

9. COMBINATION THERAPIES 100
9.1. Pharmacology of combination therapies
9.2. Combined deferoxamine and deferiprone
9.2.1. Effects of on serum ferritin
9.2.2. Effects on liver iron
9.2.3. Effects on cardiac iron and heart function
9.2.4. Long-term effects on survival
9.2.5. Safety
9.2.6. Possible treatment regimens
9.3. Combined deferasirox and deferoxamine
9.4. Combined deferasirox and deferiprone
10. WHICH CHELATION REGIMEN, WHEN, AND HOW MUCH? 102
10.1. Standard therapy for obtaining iron balance
10.2. Iron load too high or increasing: rescue therapy to achieve negative iron balance
10.3. Mild increase in cardiac iron (myocardial T2* 10-20 ms): rescue therapy to remove cardiac iron
10.4. Myocardial T2* <10 ms: rescue therapy for cardiac iron overload
10.5. Patients with heart failure: reverse heart failure
10.6. Downward adjustment of chelator dose if body iron falls rapidly or reaches low levels
KEY POINTS AND RECOMMENDATIONS 105
REFERENCES 107

CHAPTER 4 | CARDIOVASCULAR DISEASE 122

1. INTRODUCTION 122
2. PATHOPHYSIOLOGY 122
3. CARDIOVASCULAR CONSEQUENCES OF CHRONIC ANAEMIA 123
4. CARDIOVASCULAR COMPLICATIONS 125
4.1. Cardiac dysfunction
4.1.1. Acute heart failure in iron overload
4.1.2. Chronic ventricular dysfunction and heart failure with reduced ejection fraction (HFrEF)
4.1.3. Restrictive physiology and heart failure with preserved ejection fraction
4.2. Pulmonary hypertension
4.3. Arrhythmia
4.4. Other forms of cardiovascular disease
4.4.1. Thromboembolism
4.4.2. Pericardial disease
4.4.3. Valvular disease
4.4.4. Non-iron-related cardiomyopathy
4.4.5. Arteriosclerosis
4.4.6. Cancer
5. CARDIAC DIAGNOSTICS 137
5.1. Electrocardiogram

5.2. Echocardiography
5.3. Cardiac magnetic resonance imaging
5.4. Blood biomarkers
5.5. Cardiac catheterisation
6. PREVENTION AND SURVEILLANCE 142
7. GENERAL APPROACH TO CARE 143
KEY POINTS AND RECOMMENDATIONS 144
REFERENCES 145

CHAPTER 5 | LIVER DISEASE 152

1. INTRODUCTION 152
2. DIAGNOSIS AND QUANTIFICATION OF LIVER DAMAGE IN PATIENTS WITH THALASSAEMIA 153
3. IRON-MEDIATED LIVER DISEASE 153
4. THALASSAEMIA AND HEPATITIS B VIRUS 153
5. THALASSAEMIA AND HEPATITIS C VIRUS 155
6. CIRRHOSIS AND RELATED COMPLICATIONS 157
7. HEPATOCELLULAR CARCINOMA 157
7.1. Incidence, aetiology, and pathophysiology
7.2. Screening for hepatocellular carcinoma
7.3. Hepatocellular carcinoma treatment
7.4. Liver transplant for hepatocellular carcinoma in β-thalassaemia patients
KEY POINTS AND RECOMMENDATIONS 162
REFERENCES 164

CHAPTER 6 | GROWTH ABNORMALITIES, ENDOCRINE, AND BONE DISEASE 172

1. INTRODUCTION 172
2. HEIGHT AND GROWTH DISORDERS 173
2.1. Epidemiology
2.2. Clinical presentation and diagnosis
2.3. Management and monitoring
2.4. Other considerations and unmet needs
3. DISORDERS OF PUBERTAL DEVELOPMENT 174
3.1. Epidemiology
3.2. Clinical presentation and diagnosis
3.3. Management and monitoring
4. FEMALE HYPOGONADISM 177
4.1. Epidemiology
4.2. Clinical presentation and diagnosis
4.3. Management and monitoring
4.4. Other considerations and unmet needs
5. MALE HYPOGONADISM 181
5.1. Epidemiology
5.2. Clinical presentation and diagnosis

5.3. Management and monitoring
5.4. Other considerations and unmet needs
6. HYPOTHYROIDISM 186
6.1. Epidemiology
6.2. Clinical presentation and diagnosis
6.3. Management and monitoring
6.4. Other considerations and unmet needs
7. GLUCOSE METABOLISM DISORDERS 189
7.1. Epidemiology
7.2. Clinical presentation/progression and diagnosis
7.3. Management and monitoring
7.4. Other considerations and unmet needs
8. HYPOCORTICOSURRENALISM 191
8.1. Epidemiology
8.2. Clinical presentation and diagnosis
8.3. Management and monitoring
8.4. Other considerations and unmet needs
9. HYPOPARATHYROIDISM 193
9.1. Epidemiology
9.2. Clinical presentation and diagnosis
9.3. Management and monitoring
9.4. Other considerations and unmet needs
10. GROWTH HORMONE DEFICIENCY IN ADULTS 195
10.1. Epidemiology
10.2. Clinical presentation and diagnosis
10.3. Management and monitoring
10.4. Other considerations and unmet needs
11. BONE DISEASE 197
11.1. Epidemiology
11.2. Clinical presentation and diagnosis
11.3. Management and monitoring
11.4. Other considerations and unmet needs
KEY POINTS AND RECOMMENDATIONS 202
REFERENCES 217

CHAPTER 7 | FERTILITY AND PREGNANCY 226

1. INTRODUCTION 226
2. FEMALE FERTILITY 226
2.1. Diagnosis and evaluation of subfertility
2.2. Induction of ovulation
2.3. Fertility preservation
3. PREGNANCY 229
3.1. Pre-pregnancy planning

3.2. Evaluating and optimising thalassaemia care
3.2.1. Cardiac iron and function
3.2.2. Liver iron and function
3.2.3. Endocrine evaluation
3.2.4. Infections
3.2.5. Medication review
4. RISKS ASSOCIATED WITH PREGNANCY AND OVERALL MANAGEMENT 232
4.1. Management of pregnancy
4.1.1. Monitoring and medications
4.1.2. Iron chelation and cardiac monitoring
4.1.3. Endocrine monitoring
4.1.4. Monitoring for foetal growth restriction
4.1.5. Transfusion requirements
4.1.6. Antenatal anticoagulation management
4.2. Managing delivery and postpartum care
4.2.1. *Timing and mode of delivery*
4.2.2. Resumption of iron chelation therapy and breastfeeding
4.2.3. Venous thromboembolism prophylaxis
4.2.4. Postpartum care and support
5. MALE FERTILITY 238
5.1. Evaluation
5.2. Treatment
5.2.1. Hormone replacement treatment
5.2.2. Induction of spermatogenesis
5.2.3. Assisted reproductive treatment
KEY POINTS AND RECOMMENDATIONS 242
REFERENCES 246

CHAPTER 8 | OTHER COMPLICATIONS 250

1. INTRODUCTION 250
2. CHOLELITHIASIS 250
3. MALIGNANCY 251
4. SKIN MANIFESTATIONS 251
5. LEG ULCERS 252
6. RENAL DISEASE 252
7. SPLENOMEGALY 254
8. EXTRAMEDULLARY HAEMATOPOIESIS 254
8.1. Clinical presentation
8.1.1. Hepatosplenic
8.1.2. Paraspinal
8.1.3. Thoracic
8.1.4. Abdominal
8.1.5. Craniospinal
8.2. Diagnosis

8.3. Differential diagnosis
8.4. Management
8.4.1. Transfusion
8.4.2. Hydroxyurea
8.4.3. Radiotherapy
8.4.4. Surgery
9. INFECTIOUS DISEASE 258
9.1. Transfusion-transmitted infections
9.2. Transfusion-related immune modulation
9.3. Storage defects of transfused red blood cells
9.4. Transfusional iron overload-related risks of infection
9.5. Infections and iron chelation therapy
9.6. Splenectomy-related risks of infection
9.6.1. Pre/post-splenectomy vaccinations
9.6.2. Post-splenectomy antibiotic prophylaxis
9.6.3. Patient education
9.6.4. Management of infections in splenectomised patients
9.7. Disease-related risks of infection
9.8. Further details on specific bacterial infections
9.8.1. Yersinia enterocolitica
9.8.2. Klebsiella spp.
9.9. Further details on specific viral infections
9.9.1. Human parvovirus B19
9.9.2. Human immunodeficiency virus
9.9.3. Cytomegalovirus infection
9.9.4. West Nile virus
9.9.5. Viral hepatitis
9.9.6. COVID-19
9.10. Further details on specific fungal infections
9.10.1. Mucor spp.
9.10.2. Phytiosum insidiosi
9.11. Further details on specific parasitic infections
9.11.1. Malaria
KEY POINTS AND RECOMMENDATIONS 274
REFERENCES 278

CHAPTER 9 | ORAL AND DENTAL CARE 290

1. INTRODUCTION 290
2. ORO-FACIAL FEATURES 291
2.1. Changes to the facial/jaw bones
2.2. Oral soft tissue/gingival changes
2.3. Dental changes
3. MEDICAL RISK ASSESSMENT/CONSIDERATIONS FOR THE DELIVERY OF DENTAL CARE 295

3.1. Chronic anaemia
3.2. Transfusion-transmitted infections
3.3. Iron overload and tissue deposition
3.4. Infections
3.5. Cardiomyopathy
3.6. Depression
3.7. Medication-related osteonecrosis of the jaw
4. ORAL/DENTAL TREATMENT/SURGERY MODIFICATIONS 297
4.1. Close liaison with the haematology team
4.2. Preventative programme
4.3. Early management of oral/dental infections
4.4. Management of the teeth/dentures
4.5. Dental treatment modality
4.6. Dental assessment prior to commencement of antiresorptive therapy
5. DENTAL CARE PATHWAYS 299
KEY POINTS AND RECOMMENDATIONS 300
REFERENCES 301

CHAPTER 10 | NUTRITION 304

1. INTRODUCTION 304
2. ENERGY/CALORIC INTAKE 305
3. VITAMINS AND MINERALS 305
3.1. Zinc
3.2. Iron
3.3. Calcium and vitamin D
3.4. Folate
3.5. Vitamin E
3.6. Vitamin C
4. ANTIOXIDANT THERAPY 309
5. BOTANICALS 309
5.1. Tea
5.2. L-Carnitine
5.3. Wheat grass
5.4. Silymarin
5.5. Curcumin
KEY POINTS AND RECOMMENDATIONS 312
REFERENCES 315

CHAPTER 11 | LIFESTYLE AND QUALITY OF LIFE 322

1. INTRODUCTION 322
2. WHAT IS THE AIM OF OPTIMAL CARE? 322
3. THE HOPE: OFFERING HOLISTIC CARE TO PATIENTS 323
4. SELF-EFFICACY 324
5. EXERCISE AND PARTICIPATION IN SPORTS 324
5.1. Assessing β-thalassaemia patients for exercise capacity

5.2. Exercise interventions
5.3. Limitations of physical activity recommendations
6. EDUCATION 326
7. EMPLOYMENT 327
8. MARRIAGE AND REPRODUCTIVE LIFE 328
9. ALCOHOL CONSUMPTION 328
10. SMOKING 329
11. DRUG ABUSE 329
12. QUALITY OF LIFE 329
12.1. Assessing quality of life
12.1.1. Generic Instruments
12.1.2. Disease-specific tools
12.2. Qualitative investigation
KEY POINTS AND RECOMMENDATIONS 332
REFERENCES 333

CHAPTER 12 | PSYCHOLOGICAL SUPPORT 338

1. INTRODUCTION 338
2. THE CHALLENGE OF PSYCHOLOGICAL SUPPORT: WHAT DOES THE LITERATURE TELL US? 338
3. PRACTICAL CONSIDERATIONS 342
3.1. Point of diagnosis
3.2. Start of blood transfusion
3.3. Initiation of iron chelation
3.4. Adolescence and transition to increased self-care
3.5. Transition to adult care
4. PAIN IN THALASSAEMIA PATIENTS 349
5. IMPORTANCE OF SOCIAL SUPPORT THROUGHOUT DEVELOPMENT 350
6. PSYCHOSOCIAL SUPPORT THROUGHOUT THE LIFESPAN AS PART OF STANDARD CARE 351
KEY POINTS AND RECOMMENDATIONS 353
REFERENCES 355

CHAPTER 13 | MULTIDISCIPLINARY CARE AND REFERENCE CENTRES IN ADDRESSING HAEMOGLOBIN DISORDERS 364

1. INTRODUCTION 364
2. MULTIDISCIPLINARY CARE 367
2.1. The place and role of the patient in the multidisciplinary care of their condition
3. PAEDIATRIC-TO-ADULT TRANSITION 369
4. REFERENCE CENTRES FOR HAEMOGLOBIN DISORDERS: THE EU RARE DISEASES EXAMPLE 371
5. THALASSAEMIA INTERNATIONAL FEDERATION COLLABORATING CENTERS 372
6. THALASSAEMIA INTERNATIONAL FEDERATION NETWORKS 379
7. HEALTHCARE COVERAGE AND COST OF CARE 380
8. FINAL THOUGHTS AND VISION FOR THE FUTURE 381
REFERENCES 382

CHAPTER 14 | HAEMATOPOIETIC CELL TRANSPLANTATION 384

1. INTRODUCTION 384
2. TRANSPLANTATION FOR TRANSFUSION-DEPENDENT β-THALASSAEMIA 384
2.1. Haematopoietic cell transplantation from HLA-matched family donors
2.2. Haematopoietic cell transplantation from HLA-matched unrelated donors
2.3. Haploidentical haematopoietic cell transplantation
2.4. Considerations for adults
2.5. Mixed chimerism following haematopoietic cell transplantation for thalassaemia
3. PRE-TRANSPLANT EVALUATION 387
4. CONDITIONING REGIMENS 387
5. SOURCE OF HAEMOPOIETIC CELLS 388
6. FOLLOW UP AFTER HAEMATOPOIETIC CELL TRANSPLANTATION 388
7. COST AND COST-EFFECTIVENESS 388
8. COUNSELLING FOR TRANSPLANTATION 389
9. THE EBMT HAEMOGLOBINOPATHY WORKING PARTY 389
10. HAEMATOPOIETIC CELL TRANSPLANTATION AND GENE THERAPY 389
KEY POINTS AND RECOMMENDATIONS 390
REFERENCES 391

CHAPTER 15 | GENE MANIPULATION 394

1. INTRODUCTION 394
2. GENE ADDITION TECHNIQUES 395
2.1. Gene addition: clinical trial results
3. GENE EDITING TECHNIQUES 397
3.1. Gene editing: clinical trial results
4. THE JOURNEY OF PATIENTS RECEIVING GENE ADDITION AND GENE EDITING PRODUCTS 403
5. GENOTOXICITY RISKS OF GENE THERAPY 404
6. FINANCIAL HURDLES IN ADOPTION 406
KEY POINTS AND RECOMMENDATIONS 407
REFERENCES 411

CHAPTER 16 | NOVEL DISEASE-MODIFYING AGENTS 416

1. INTRODUCTION 417
2. PROGRAMMES HALTED DURING EARLY-PHASE DEVELOPMENT 418
2.1. Tovinontrine (IMR-687)
2.2. Ruxolitinib (INCB018424, INC424)
2.3. Hepcidin mimetics
3. APPROVED AGENTS 419
3.1. Luspatercept (ACE-536)
3.1.1. Mechanism of action
3.1.2. Development programme and trials
3.1.3. Real-world evidence

3.1.4. Practical insights
4. ONGOING CLINICAL DEVELOPMENT PROGRAMMES 423
4.1. Mitapivat (AG-348)
4.2. Etavopivat (FT-4202)
5. FUTURE OUTLOOK 424
KEY POINTS AND RECOMMENDATIONS 425
REFERENCES 427

CHAPTER 17 | THE VALUE OF PATIENTS' ENGAGEMENT: THE EXAMPLE OF THE THALASSAEMIA INTERNATIONAL FEDERATION 434

1. INTRODUCTION 434
2. THE HISTORY OF PATIENT ENGAGEMENT IN THALASSAEMIA: HOW IT ALL BEGAN 435
3. HOW DID THE MEDICAL COMMUNITY PERCEIVE THIS MOVEMENT 436
4. NATIONAL PATIENT/PARENT SUPPORT GROUPS LED TO THE BIRTH OF THE THALASSAEMIA INTERNATIONAL FEDERATION 437
5. KEY OBSERVATIONS CAPTURED THROUGH THE WORK AND ACTIVITIES OF THE THALASSAEMIA INTERNATIONAL FEDERATION 439
6. A STRONG EXAMPLE OF A VERY POWERFUL MOVEMENT: THE HUMAN IMMUNODEFICIENCY VIRUS STORY 439
7. PATIENT ENGAGEMENT WITH REGULATORY AGENCIES 440
8. PATIENT ENGAGEMENT IN RESEARCH 443
9. THE GLOBAL PERSPECTIVE OF PATIENT ENGAGEMENT: THE EXAMPLE OF THE WORLD HEALTH ORGANIZATION 444
10. PATIENT ENGAGEMENT IN THALASSAEMIA INTERNATIONAL FEDERATION ACTIVITIES 445
11. REACHING OUT 447
12. FINAL THOUGHTS 449
REFERENCES 450

SUMMARY OF MONITORING RECOMMENDATIONS 452

TIF'S COMPREHENSIVE EDUCATIONAL PROGRAMME 456

CONTRIBUTORS AND AFFILIATIONS

EDITORS:

Ali T. Taher, MD, PhD, FRCP
Professor of Internal Medicine, Hematology & Oncology; Associate VP for Academic Centers, Development and External Affairs; Director, Naef K. Basile Cancer Institute, American University of Beirut Medical Center, Beirut, Lebanon

Dimitrios Farmakis, MD, PhD, FESC, FHFA
Associate Professor, Department of Cardiology, Athens University Hospital Attikon, National and Kapodistrian University of Athens Medical School, Athens, Greece

John B. Porter, MA, MD, FRCP, FRCPath
Professor of Haematology (Emeritus) and Consultant Haematologist, University College London Hospitals, London, United Kingdom

Maria Domenica Cappellini, MD, FRCP, FACP
Professor of Medicine, Unit of Medicine and Metabolic Disease, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

Khaled M. Musallam, MD, PhD
Group Chief Research Officer (Burjeel Holdings) and Founding Director, Center for Research on Rare Blood Disorders (CR-RBD), Burjeel Medical City, Abu Dhabi, United Arab Emirates; Adjunct Professor, Department of Public Health & Epidemiology, Khalifa University, Abu Dhabi, United Arab Emirates; Adjunct Professor, Division of Hematology/Oncology, Department of Pediatrics, Weill Cornell Medicine, New York, NY, USA

AUTHORS (alphabetical by last name):

Alessio Aghemo, MD, PhD
Professor of Gastroenterology and Chairman, Department of Biomedical Sciences, Humanitas University, Pieve Emanuele, Milan, Italy; Chief Division of Internal Medicine and Hepatology, Internal Medicine Center, Department of Gastroenterology, IRCCS Humanitas Research Hospital, Rozzano, Milan, Italy

Mattia Algeri, MD
Staff Physician, Department of Pediatric Hematology/Oncology and Cell and Gene Therapy, IRCCS Bambino Gesu Children's Hospital, Rome, Italy; Department of Health Sciences, Magna Graecia University, Catanzaro, Italy

Michael Angastiniotis, MD, DCH
Medical Advisor, Thalassaemia International Federation (TIF), Nicosia, Cyprus

Emanuele Angelucci, MD
Head, Hematology and Cellular Therapy Unit, IRCCS Ospedale Policlinico San Martino, Genoa, Italy

Yesim Aydinok, MD
Professor, Department of Pediatric Hematology, Ege University Medical School, Izmir, Turkey

Marina Baldini, MD
Unit of Medicine and Metabolic Disease, Department of Internal Medicine, IRCCS Fondazione Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

Rayan Bou-Fakhredin, MSc, PhD
Researcher, SC Medicina ad Indirizzo Metabolico, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico; Department of Clinical Sciences and Community Health, University of Milan, Milan, Italy

Angelo Loris Brunetta, BSS
Treasurer of the Board of Directors, Thalassaemia International Federation (TIF), Nicosia, Cyprus; Fondazione Italiana "Leonardo Giambrone" per la Guarigione dalla Talassemia e Drepanocitosi (FITHAD), Caserta, Italy; President, Associazione Ligure Thalassemici, Genoa, Italy

Maria Domenica Cappellini, MD, FRCP, FACP
Professor of Medicine, Unit of Medicine and Metabolic Disease, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

Maddalena Casale, MD, PhD
Centre of Paediatric Haematology and Oncology, Department of Woman, Child and General and Specialized Surgery, University of Campania Luigi Vanvitelli, Naples, Italy

Prantar Chakrabarti, MD, DNB, DM
Medical Consultant, Zoho Corporation, Tenkasi, Tamil Nadu, India; Former Professor and Head, Department of Haematology, NRS Medical College Kolkata, Kolkata, India

Thomas D. Coates, MD
Section Head, Hematology, Cancer and Blood Disease Institute, Children's Hospital Los Angeles; Professor of Pediatrics and Pathology, University of Southern California Keck School of Medicine, Los Angeles, CA, USA

George Constantinou, BA
Vice President of the Board of Directors, Thalassaemia International Federation (TIF), Nicosia, Cyprus; Founding Member, United Kingdom Thalassaemia Society (UKTS), London, United Kingdom

Polyxeni Delaporta, MD, PhD
Pediatrician, Thalassemia Unit, First Department of Pediatrics, National and Kapodistrian University of Athens, 'Aghia Sophia' Children's Hospital, ERN-EuroBloodNet Center, Athens, Greece

Androulla Eleftheriou, BSc, MSc, PhD
Executive Director, Thalassaemia International Federation (TIF); President, National Thalassaemia Committee; Ex Head of the Virus Reference Centre of the Cyprus Ministry of Health; Ex Director of the Cyprus WHO Collaborating Centre of the Cyprus Ministry of Health; Ex President, Cyprus Alliance for Rare Disorders, Cyprus

Dimitrios Farmakis, MD, PhD, FESC, FHFA
Associate Professor, Department of Cardiology, Athens University Hospital Attikon, National and Kapodistrian University of Athens Medical School, Athens, Greece

Gian Luca Forni, MD
Senior Consultant "UOC Ematologia" IRCCS, Istituto G. Gaslini; Past President "Società Italiana Talassemie ed Emoglobinopatie" SITE; President "For Anemia Foundation" ETS; Former Head of Regional Center for Hemoglobinopathies, Congenital Anemias and Iron Dysmetabolism, Genoa, Italy

Ellen B. Fung, PhD, RD, CCD
Adjunct Professor, Division of Hematology, Department of Pediatrics, University of California San Francisco (UCSF), San Francisco, CA, USA

Massimiliano Gambella, MD
Hematologist, Hematology and Cellular Therapy Unit, IRCCS Ospedale Policlinico San Martino, Genoa, Italy

Andrea Giusti, MD
Division of Internal Medicine and Metabolic Bone Diseases, Department of Internal Medicine and Cardiology, Villa Scassi Hospital, Genoa, Italy

Anna Grandone, MD, PhD
Centre of Paediatric Endocrinology, Department of Woman, Child and General and Specialized Surgery, Naples, Italy

Dru Haines, MA, MS
Pediatric Nurse Practitioner, Comprehensive Thalassemia Center, Benioff Children's Hospital Oakland, University of San Francisco (UCSF), Oakland, CA, USA

Faiez N. Hattab, BDS, PhD
Professor Emeritus of Cardiology and Paediatric Dentistry, Oskar-Maria-Graf-Ring 1a, Munich, Germany

Antonis Kattamis, MD
Professor of Pediatric Hematology/Oncology; Head, Division of Pediatric Hematology/Oncology; Thalassemia Unit, First Department of Pediatrics, National and Kapodistrian University of Athens, 'Aghia Sophia' Children's Hospital, ERN-EuroBloodNet Center, Athens, Greece

Navdeep Kumar, BDS, FDS, RCS(Eng), PhD, MBA, CMgr, FCMI
Consultant in Special Care Dentistry and Divisional Clinical Director, Royal National ENT & Eastman Dental Hospitals, University College London Hospitals (UCLH) NHS Foundation Trust; Honorary Associate Professor, UCL; Training Programme Director SCD London & KSS; Specialty Advisor Royal College of Surgeons of England; Chair of the Association of Dental Hospitals, London, United Kingdom

Kevin H. M. Kuo, MD, MSc, FRCPC
Associate Professor, Division of Hematology, Department of Medicine, Faculty of Medicine, University of Toronto; Associate Professor (status), Institute of Health Policy, Management and Evaluation, Dalla Lana School of Public Health, University of Toronto, Toronto; Scarborough Health Network, Scarborough, ON, Canada

Carlotta La Floresta, MA
Liver Unit, Department of Medical Sciences, Fondazione "Casa Sollievo della Sofferenza" IRCCS, San Giovanni Rotondo, Italy

Yi Li, MD
Assistant Professor of Urology, Department of Urology and Center for Reproductive Sciences, University of California San Francisco (UCSF), San Francisco, CA, USA

Franco Locatelli, MD, PhD
President, National Council of Health; Full Professor of Pediatrics, Catholic University of the Sacred Heart, Rome, Italy; Director, Department of Pediatric Hematology/ Oncology and Cell and Gene Therapy, IRCCS Bambino Gesu Children's Hospital, Rome, Italy

Aurelio Maggio, MD
Professor and Director, Campus of Haematology Franco and Piera Cutino, UOC Ematologia e Malattie Rare, Centro ERN, Centro HUB for Thalassemia and Haemoglobinipathy, AOOR Villa Sofia-V. Cervello, Palermo, Italy

Alessandra Mangia, MD
Professor and Head, Liver Unit, Department of Medical Sciences, Fondazione "Casa Sollievo della Sofferenza" IRCCS, San Giovanni Rotondo, Italy

Khaled M. Musallam, MD, PhD
Group Chief Research Officer (Burjeel Holdings) and Founding Director, Center for Research on Rare Blood Disorders (CR-RBD), Burjeel Medical City, Abu Dhabi, United Arab Emirates; Adjunct Professor, Department of Public Health & Epidemiology, Khalifa University, Abu Dhabi, United Arab Emirates; Adjunct Professor, Division of Hematology/Oncology, Department of Pediatrics, Weill Cornell Medicine, New York, NY, USA

Lena Oevermann, MD
Pediatric Hemato-Oncologist, Department of Pediatric Oncology and Hematology, Charité-Universitätsmedizin Berlin, Berlin, Germany

Raffaella Origa, MD, PhD
Professor, Department of Medical Sciences and Public Health, University of Cagliari; Ospedale Pediatrico Microcitemico, ASL Cagliari, Cagliari, Italy

Mary Peoples, BS
Clinical Research Coordinator, Comprehensive Thalassemia Center, Benioff Children's Hospital Oakland, University of San Francisco (UCSF), Oakland, CA, USA

Valeria Maria Pinto, MD
Hematologist, Hematology and Cellular Therapy Unit, IRCCS Ospedale Policlinico San Martino, Genoa, Italy

Maurizio Poggi, MD
Endocrinologist, Endocrine Metabolic Unit, Department of Internal Medicine, Sant'Andrea Hospital, Rome, Italy

John B. Porter, MA, MD, FRCP, FRCPath
Professor of Haematology (Emeritus) and Consultant Haematologist, University College London Hospitals (UCLH), London, United Kingdom

Nicola Pugliese, MD
Gastroenterologist and Hepatologist, Department of Biomedical Sciences, Humanitas University, Pieve Emanuele, Milan, Italy; Division of Internal Medicine and Hepatology, Department of Gastroenterology, IRCCS Humanitas Research Hospital, Rozzano, Milan, Italy

Manel Ratnayake, MD
Consultant Haematologist, National Thalassaemia Centre, Kurunegala, Sri Lanka

Stefano Rivella, PhD
Kwame Ohene-Frempong Chair on Sickle Cell Anemia and Professor of Pediatrics, Division of Hematology, Department of Pediatrics; Raymond G. Perelman Center for Cellular and Molecular Therapeutics; Penn Center for Musculoskeletal Disorders, The Children's Hospital of Philadelphia; Leader of the RNA Gene Therapeutics Group, Cell and Molecular Biology Affinity Group, Penn Institute for RNA Innovation, Penn Institute for Regenerative Medicine, University of Pennsylvania, Philadelphia, PA, USA

Farzana Sayani, MD, MSs
Medical Director, Red Blood Cell Disorders Program, Division of Medical Oncology and Haematology, University Health Network; Associate Professor, Temerty Faculty of Medicine, University of Toronto, Toronto, Canada; Founding Director, Penn Comprehensive Adult Thalassemia Program, Hospital of University of Pennsylvania; Adjunct Associate Professor, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

Marisa Schweber-Koren, LCSW
Medical Social Worker, Comprehensive Thalassemia Center, Benioff Children's Hospital Oakland, University of San Francisco (UCSF), Oakland, CA, USA

Farrukh Shah, MD, FRCP, FRCPath
Consultant Hematologist, Sheikh Khalifa Medical City, Abu Dhabi, United Arab Emirates

Sujit Sheth, MD
Professor of Clinical Pediatrics, Division of Hematology/Oncology, Department of Pediatrics, Weill Cornell Medicine, New York, NY, USA

Sylvia Titi Singer, MD
Professor of Pediatrics, Department of Hematology/Oncology, University of California San Francisco (UCSF) Benioff Children's Hospital Oakland, Oakland, CA, USA

James Smith, MD, MS
Clinical Professor of Urology, Department of Urology and Center for Reproductive Sciences, University of California San Francisco (UCSF), San Francisco, CA, USA

Pietro Sodani, MD
Hematologist, Unità Operativa Complessa Internal Medicine and Hematology, Azienda Ospedaliera Ospedali Riuniti Marche Nord, Ospedale Santo Croce, Fano, Italy

Maria Carla Sollaino, MD, PhD
Laboratorio di Genetica e Genomica, Ospedale Pediatrico Microcitemico, ASL Cagliari, Cagliari, Italy

Ali T. Taher, MD, PhD, FRCP
Professor of Internal Medicine, Hematology & Oncology; Associate VP for Academic Centers, Development and External Affairs; Director, Naef K. Basile Cancer Institute, American University of Beirut Medical Center, Beirut, Lebanon

Vip Viprakasit, MD, DPhil(Oxon), FRCPT
Professor of Pediatrics; Director, Thalassemia Research Program; Division of Hematology & Oncology, Department of Pediatrics & Thalassemia Center, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand

Evangelia Vlachodimitropoulou, MBBS, BSc, MRCOG, PhD
Consultant in Obstetrics, Maternal & Fetal Medicine, Department of Obstetrics and Gynaecology, King's College Hospital, London, United Kingdom

J. Malcolm Walker, MD, FRCP
Professor of Cardiology, Institute of Cardiovascular Science, University College London (UCL); Consultant Cardiologist, UCLH; Clinical Director, Hatter Cardiovascular Institute ICS & UCL, London, United Kingdom

Erica Wood, AO
Professor and Head, Transfusion Research Unit, Public Health and Preventive Medicine, Monash University, Melbourne; Consultant Haematologist, Department of Haematology, Monash Health, Clayton, Australia; co-chair of the WHO Advisory Group on Blood Regulation, Availability and Safety; member of WHO's Expert Advisory Panel on Transfusion Medicine, WHO Working Group on Haemovigilance Tools, and WHO Guideline Development Group on Anaemia

John C. Wood, MD, PhD
Director, Cardiovascular MRI, Division of Cardiology, Children's Hospital Los Angeles; Professor of Pediatrics and Radiology, University of Southern California Keck School of Medicine, Los Angeles, CA, USA

Robert Yamashita, MA, PhD
Clinical Study Coordinator, Comprehensive Thalassemia Center, Benioff Children's Hospital Oakland, University of San Francisco (UCSF), Oakland, CA, USA

ACKNOWLEDGMENTS:

The Publishers and Editors would like to thank all authors and reviewers of the previous editions for their past contributions which were invaluable in the development of the current update. Special thanks also go to Katia Pelides, MEd, MSc (Senior Educational Programme Officer, Thalassaemia International Federation) for her tireless support and coordination.

GRADING OF RECOMMENDATIONS

Where relevant, recommendations in these guidelines are accompanied by ratings of the level of evidence and/or class of recommendations based on the below criteria:

LEVEL OF EVIDENCE:

- **Grade A:** Data derived from multiple randomised clinical trials or meta-analyses.
- **Grade B:** Data derived from a single randomised clinical trial or large non-randomised studies.
- **Grade C:** Consensus of the experts and/or small studies, retrospective studies, or registries.

CLASS OF RECOMMENDATION:

- **Class I:** There is evidence and/or general agreement that a given treatment or procedure is beneficial, useful, effective.
- **Class II:** There is conflicting evidence and/or a divergence of opinion about the usefulness/efficacy of the given treatment or procedure.
  - **Class IIa:** The weight of evidence is in favor of usefulness/efficacy and therefore should be considered.
  - **Class IIb:** The usefulness/efficacy is less well established by evidence/opinion and therefore may be considered.
- **Class III:** There is evidence or general agreement that the given treatment or procedure is not useful/effective, and in some cases may be harmful.

FORWARD

Dear Colleagues,

On behalf of the Board of Directors of the Thalassaemia International Federation (TIF) and the Editors of this 5th Edition of the 'Guidelines for The Management of Transfusion-Dependent β-Thalassaemia (TDT)', it is both an honour and a privilege to present this introductory foreword.

We are fortunate to live in an era where haemoglobin disorders, once considered fatal childhood afflictions, are now preventable, manageable, and even curable. This remarkable transformation has shifted the narrative of thalassaemia from despair to hope, ushering in an age of unprecedented scientific and medical advancements.

Compelling evidence, including increased survival rates, decreased mortality and morbidity, and improved quality of life and social integration, unequivocally illustrates that the battle against thalassaemia is nearing victory. The advent of groundbreaking therapies in the past decade – including two gene therapies and one disease-modifying treatment approved for clinical use – alongside numerous others in various stages of development, heralds a brighter future for patients worldwide.

Regrettably, these advancements remain largely confined to nations with robust economies, where comprehensive health and social care systems provide universal access to treatment. In stark contrast, the vast majority of patients – estimated by TIF to be less than 10% of the global patient population – are unable to benefit from these developments. This disparity underscores the urgent need to empower countries, particularly those with high disease prevalence, to prioritise disease-specific policies for prevention and care.

The World Health Organization (WHO), through and not confined to its resolutions on disease management, blood safety, patient safety, congenital anomalies and birth defects, human genomics in global health, and access to essential medicines and health products, offers a robust framework for national efforts. Similarly, the European Union (EU)'s exemplary initiatives on rare diseases since the 1990s provide invaluable guidance that transcends regional boundaries. These initiatives provide a foundation and model for other countries beyond the EU to build upon, highlighting their significant benefits – first and foremost for patients as well as for the sustainability and resilience of health and social care systems. It is our hope that, as nations strive to achieve the United Nations' 2030 Sustainable Development Goals, meaningful progress will be realised, albeit unevenly and with great inequalities, across the globe.

TIF remains steadfast in its commitment to supporting national patient and parent organisations, the healthcare professionals' community, and policymakers. The preparation, publication, translation, and free distribution of Guidelines such as this one is a cornerstone of our educational mission. Widely recognised for their significant impact on the care of patients with these disorders, these Guidelines serve as critical resources for healthcare providers and a foundation upon which national policies and practices can be built.

We are profoundly grateful for the tireless efforts of national thalassaemia associations across the world – members of TIF – whose voluntary contributions have complemented and, in some cases, exceeded governmental initiatives. We humbly urge governments worldwide to lend their support

in addressing the many and multiple unmet needs of patients with these disorders, especially those in underdeveloped regions. This is particularly crucial given the immense geopolitical, environmental, and public health challenges that the world is facing today, which severely threaten health and social care systems and disproportionately affect patients with such disorders, who rely daily on highly specialised health and social services to manage their lifelong dependencies.

The role of patient advocates in shaping national, regional, and international strategies cannot be overstated. Since its inception in 1986, TIF has been fighting for the inclusion of patients in decision-making processes. This 5th Edition of the TDT Guidelines proudly introduces a new chapter dedicated to the value of patient engagement, emphasising the transformative power of empowering patients as informed and effective advocates for their needs. Additionally, patients have contributed to the review of various other chapters in this book, ensuring that issues such as lifestyle and mental health are comprehensively addressed through the understanding and recognition of the patients' perspective. TIF remains committed to continuing and expanding its support for the patients it represents, through strengthening its active and meaningful collaboration with national associations, decision-making bodies, healthcare professionals, industry leaders, and other key stakeholders.

We strongly encourage healthcare professionals who study these Guidelines to advocate for their adoption and implementation within their centres and countries by supporting and guiding national competent health authorities in their country about their immense value. Evidence-based practices outlined in these Guidelines are vital for enabling early diagnosis, effective management, and timely intervention. They also serve as a roadmap for integrating novel therapies that are under development or already approved into care frameworks, ensuring that all TDT patients, regardless of location, culture, religion, and language have access to optimal standards of care – a basic and fundamental human right.

This updated 5th Edition features seventeen meticulously crafted chapters, authored by leading experts with decades of experience in TDT management. It offers insights into cutting-edge therapies and highlights considerations for care in resource-limited settings, providing a lifeline for underserved regions.

In closing, allow us to extend our heartfelt gratitude to the key Editors and each and every Chapter Author, whose unwavering dedication and expertise in their respective fields have been instrumental in advancing the field of thalassaemia care and in making possible the development and completion of this upgraded edition of Guidelines. This publication, and indeed most of TIF's educational endeavours, stand as a testament to their exceptional commitment.

On behalf of the Thalassaemia International Federation

Dr. Panos Englezos, hon. PhD
President, TIF
Dr. Androulla Eleftheriou BSC, MSc, PhD
Executive Director, TIF

On behalf of the Editors

Prof. Maria Domenica Cappellini, MD, FRCP, FACP
Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

01 GENETIC BASIS, PATHOPHYSIOLOGY, AND DIAGNOSIS

Authors: **Raffaella Origa[1], Sujit Sheth[2], Maria Carla Sollaino[3], Stefano Rivella[4]**

[1.] Department of Medical Sciences and Public Health, University of Cagliari; Ospedale Pediatrico Microcitemico, ASL Cagliari, Cagliari, Italy

[2.] Division of Hematology/Oncology, Department of Pediatrics, Weill Cornell Medicine, New York, NY, USA

[3.] Laboratorio di Genetica e Genomica, Ospedale Pediatrico Microcitemico, ASL Cagliari, Cagliari, Italy

[4.] Division of Hematology, Department of Pediatrics; Raymond G. Perelman Center for Cellular and Molecular Therapeutics; Penn Center for Musculoskeletal Disorders, The Children's Hospital of Philadelphia; RNA Gene Therapeutics Group, Cell and Molecular Biology Affinity Group, Penn Institute for RNA Innovation, Penn Institute for Regenerative Medicine, University of Pennsylvania, Philadelphia, PA, USA

Competing interest: RO reports being a member of scientific advisory boards for Bristol Myers Squibb and Vertex Pharmaceuticals; and speaker honoraria from Chiesi, Bristol Myers Squibb and Vertex Pharmaceuticals. SS reports consultancy fees from Agios Pharmaceuticals, Bristol Myers Squibb and Novo Nordisk; being a member of a clinical trial steering committee for Vertex Pharmaceuticals; and research funding (for clinical trials) from Agios, Bristol Myers Squibb, Novo Nordisk and Regeneron. SR reports being a member of the scientific advisory board for Ionis Pharmaceuticals; has acted as a consultant for Disc Medicine, Protagonist Therapeutics, La Jolla Pharmaceutical Company, Meira GTx, GSK, Bristol Myers Squibb, Incyte, Cambridge Healthcare Res, Celgene Corporation, Catenion, First Manhattan Co., FORMA Therapeutics, Ghost Tree Capital, Keros Therapeutics, Noble insight, Sanofi Aventis U.S., Slingshot Insight, Spexis AG, Techspert.io, BVF Partners L.P., Rallybio LLC, venBio Select LLC, ExpertConnect LLC and LifeSci Capital; and is a co-inventor for patents US8058061 B2 C12N 20111115 and US7541179 B2 C12N 20090602. The remaining authors have no competing interests to disclose. All competing interests are outside the present work.

1. INTRODUCTION

The landscape of β-thalassaemia management continues to evolve over the years. This has only been possible through improved understanding of the molecular basis of the disease, underlying pathophysiology and associated clinical manifestations, as well as accurate diagnosis and patient classification to inform optimal management approaches [1-3]. In this Chapter, we revisit the genetic basis, pathophysiology, and diagnosis of β-thalassaemia and introduce the specific phenotype, transfusion-dependent β-thalassaemia (TDT) - the focus of these Guidelines. Additional information related to the various disease processes, manifestations, and management aspects of TDT are outlined in respective Chapters. Guidelines for management of non-transfusion-dependent β-thalassaemia (NTDT), and α-thalassaemia are covered separately in dedicated publications from the Thalassaemia International Federation (TIF) [4, 5].

2. HAEMOGLOBIN TYPES, GLOBIN GENES, AND GLOBIN SYNTHESIS

Oxygen is transported from the lungs to the tissues by a highly specialised protein molecule, haemoglobin, located in blood's red cells. Each red blood cell contains approximately 300 million molecules of this protein, corresponding to about 30 picograms in weight per cell. Each molecule

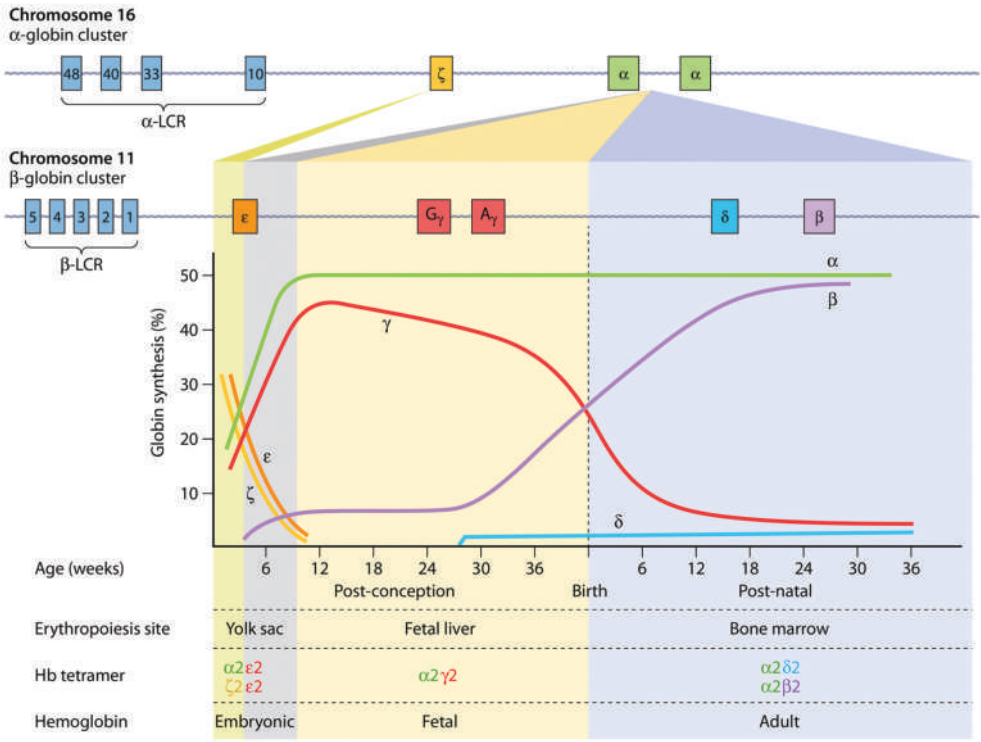
of haemoglobin is formed by two pairs of identical sub-units, the globin chains. These globin chains are named with letters of the Greek alphabet and belong to two groups: the α-globin cluster, comprising the ζ- and α-globin chains, and the β-globin cluster, comprising the globin chains ε, γ, β, and δ. The globin chains have a highly specialised structure, enabling efficient oxygen binding to the heme molecule in the lung alveoli and the regulated, gradual release of oxygen to the tissues. The amino acid sequence of the globin chains is coded by genes located on chromosomes 16 (the α-globin gene cluster) and 11 (the β-globin gene cluster). Surrounding the structural genes, either upstream (5') or downstream (3') of the DNA sequence, are several regulatory nucleotide sequences. These sequences control gene expression by determining which genes are activated or deactivated and influencing the efficiency of their expression. In adult life, most globin synthesis occurs in erythroblasts within the bone marrow. Globin chains must have the correct structure and be regulated so that the number of α-globin chains precisely matches that of the β-globin chains. The above conditions are not met when there is a complete or partial defect in one or more 'allelic' globin genes [6].

The globin chains appear sequentially during ontogeny and, after pairing, form the following four major types of haemoglobin:

- 'Embryonic' haemoglobins, which are detectable from the 3rd to the 10th week of gestation and represent ζ2ε2 (Hb Gower 1), α2ε2 (Hb Gower 2), ζ2γ2 (Hb Portland 1) and ζ2β2 tetramers (Hb Portland 2).
- 'Foetal' haemoglobin (HbF), which constitutes the predominant oxygen carrier during pregnancy and is an α2γ2 tetramer.
- 'Adult' haemoglobin (HbA, α2β2), which replaces HbF shortly after birth.
- A minor adult component, HbA2 (α2δ2).

The transition of different haemoglobin species at various stages of human development is known as 'haemoglobin switching', and it is illustrated in Figure 1 [7]. Under normal conditions, the red blood cells of the adult human contain approximately 97-98% of HbA, 2-3% of HbA2, and traces of HbF.

Figure 1. *Human β-like globin gene regulation during development. The two monomers of α-like globin expressed from chromosome 16 pair with two monomers of β-like globin to form a functional haemoglobin tetramer. The components of the haemoglobin change during development. The embryonic (ε) part of β-like globin is expressed during the first 6 weeks of gestation and forms embryonic haemoglobin by pairing with α-globin. After 6 weeks, ε-globin expression declines and is replaced by γ-globin to form foetal haemoglobin. The final haemoglobin switching takes place a few weeks before birth when the γ-globin expression is replaced by β-globin to form adult haemoglobin. Abbreviations: Hb, haemoglobin; LCR, locus control region. Reproduced with permission from [7].*



3. CLASSIFICATION AND EPIDEMIOLOGY

The term 'thalassaemia' refers to a group of blood diseases characterised by decreased or absent synthesis of one or more of the normal globin chains. According to the chain whose synthesis is impaired, the thalassaemias are called α-, β-, γ-, δ-, δβ-, or εγδβ-thalassaemia. Most thalassaemias are inherited as recessive traits, but some rare forms are transmitted in an autosomal dominant manner.

From a clinical point of view, the most relevant types are α- and β-thalassaemia, resulting from the decrease of one of the two types of polypeptide chains (α or β) that form the normal adult human haemoglobin molecule (HbA, α2β2). β-Thalassaemia, the focus of these Guidelines, constitutes a significant public health problem in countries around the Mediterranean Sea, the Middle East and Trans-Caucasus, India, and the Far East [8].

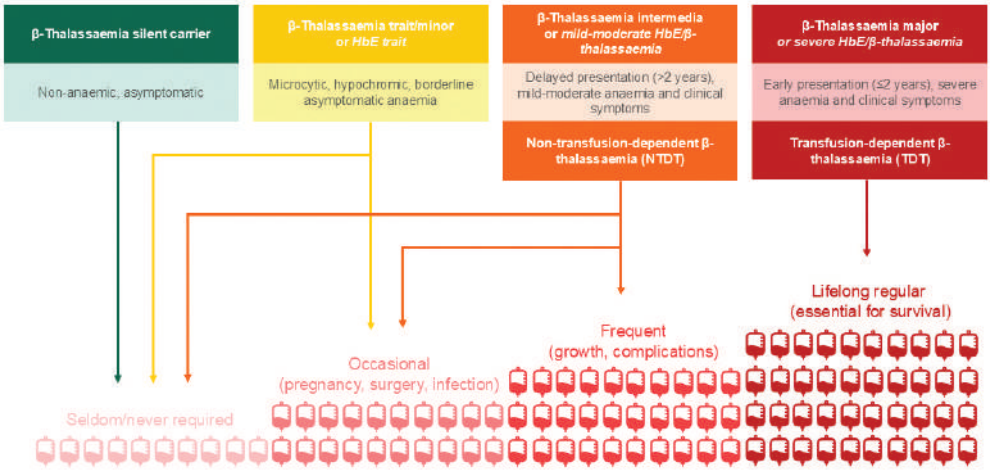
The highest carrier frequency of β-thalassaemia is reported in the Maldives (18%), Cyprus (14%), Sardinia (10.3%), and Southeast Asia (3-5%). The high gene frequency in these regions is most likely related to the selective pressure from *Plasmodium falciparum* malaria. However, population migration and intermarriage between different ethnic groups have introduced β-thalassaemia in almost every country of the world, including Northern Europe, North and South America, the Caribbean, and Australia [9].

Dominantly inherited β-thalassaemia, unlike the common recessive forms of β-thalassaemia, has been described in dispersed geographical regions, with many mutations occurring spontaneously [10, 11].

As autosomal recessive conditions, heterozygotes of β-thalassaemia are usually asymptomatic and require no treatment while homozygotes and compound heterozygotes for β-thalassaemia alleles result in β-thalassaemia syndromes or diseases [1, 12]. In addition, the combination of β-thalassaemia alleles with other structural variants associated with different haemoglobinopathies (e.g., haemoglobin E, C, or S) also gives rise to various pathologic conditions. Haemoglobin E is the most common abnormal haemoglobin in Southeast Asia, with a carrier frequency of up to 50% in some regions. It is also prevalent in parts of the Indian subcontinent, including India, Pakistan, Bangladesh and Sri Lanka [13, 14]. Since pathologic manifestations of haemoglobin E mutations mimic β-thalassaemia, patients with haemoglobin E/β-thalassaemia are usually covered under management recommendations of 'β-thalassaemia' which is the case in these Guidelines, with specific variations mentioned as relevant. The interaction of β-thalassaemia with haemoglobin S results in a syndrome closely resembling other sickling disorders (sickle cell disease), which are outside the scope of these Guidelines.

Three main forms of β-thalassaemia have been classically recognised, often referred to as 'conventional phenotypes': β-thalassaemia major variably referred to as 'Cooley's anaemia' and 'Mediterranean anaemia', β-thalassaemia intermedia, and β-thalassaemia minor also called 'β-thalassaemia trait', 'β-thalassaemia carrier', or 'heterozygous β-thalassaemia'. These terms were primarily based on the broad genotype (β-thalassaemia major/intermedia being homozygotes or compound heterozygotes for β-thalassaemia alleles, or heterozygotes with α-globin gene duplications, or having dominantly inherited β-thalassaemia; and β-thalassaemia minor being heterozygote), age of presentation, and severity of anaemia and symptoms on presentation (Figure 2) [1, 12]. Patients with haemoglobin E/β-thalassaemia are similarly conventionally classified as having major/intermedia, or more commonly as being mild, moderate, or severe [1-3].

Figure 2. *Transfusion requirement in various β-thalassaemia phenotypes. Abbreviations: Hb, haemoglobin. Modified with permission from [1, 12].*



More recently, based on their clinical severity and transfusion requirement, β-thalassaemia syndromes are more often phenotypically classified into two main groups TDT and NTDT (Figure 2) [1, 12]. Patients requiring regular, lifelong blood transfusion for survival are indicated as TDT. Most of these TDT patients, without adequate transfusion support, would suffer several complications and a markedly shortened life span. This category usually includes the conventional phenotypes β-thalassaemia major and severe haemoglobin E/β thalassaemia, and is the main focus of these Guidelines. The NTDT patient group includes β-thalassaemia intermedia and mild/moderate haemoglobin E/β thalassaemia [12]. This classification was adopted in recognition of the role of transfusion (or lack of) in shaping the underlying pathophysiology. It also helped revisit management approaches for these different forms, and inform eligibility and endpoints for clinical trials. It should be noted that the NTDT/TDT classification is 'fluid' and patients can transition from one form to another. In a recent large study from Italy, around 13% of NTDT patients had 'phenoconversion' to TDT over an observation period of 10 years [15]. The key triggers have been low haemoglobin levels and multimorbidity [15], noting that prescription of regular transfusion can also be a patient or physician choice, especially considering the growing evidence on the long-term harms of untreated anaemia in NTDT patients and the benefit of regular transfusion in this patient population with regards to lowering morbidity and mortality rates [16-19]. Although there is no set criteria in terms of amount and duration of regular transfusion to distinguish patients as TDT, it is commonly adopted that patients receiving 6 or more red blood cell units over a period of 6 months (with ≤6-week transfusion-free) or regular transfusions over 1-3 years should be classified and managed as TDT, with some form of 'acknowledgement' of their 'new' regular transfusion status for any consideration of lifelong exposure [20].

Data on the epidemiology of clinically significant forms are limited. In a recent systematic literature review, overall, the estimated prevalence of TDT and NTDT followed the predicted pattern of being higher in the Middle East, Asia, and Mediterranean than in Europe or North America. However, population-based prevalence estimates were not found for many countries, and there was heterogeneity in case definitions, diagnostic methodology, type of thalassaemia reported, and details on transfusion requirements [21]. TIF provides a Global Thalassaemia Review [22], a deliverable of the joint work with the World Health Organisation (WHO), which can be used as a resource for understanding thalassaemia burden across geographies. A call for development of more robust national registries has been frequently made by TIF [23]. Survival estimates for TDT mostly stem from single-centre or individual country studies. What can be concluded from available data is that: 1) survival in TDT patients has improved over the years, with survival estimates approaching the general population in recent birth cohorts in Western countries, 2) the case is not echoes in resource-limited countries where high disease burden and early mortality continue to be observed [24, 25].

The approach to screening and prevention for β-thalassaemia is dependent on the frequency of the specific mutations in the region, the available resources, cultural and religious issues, and the age of the targeted population. Public awareness and education, public surveillance and population screening, extended family screening of first-born child, premarital screening and genetic counselling, prenatal diagnosis, and family planning are among the strategies commonly applied in screening programmes. It should be part of a generalised programme to educate and screen the at-risk population for thalassaemia disorders and improve the quality of life and management of affected patients [8]. Further details and resources on prevention programmes can be found at the TIF website [26].

4. MOLECULAR UNDERSTANDING

The degree of globin chain imbalance is determined by the nature of the mutation of the β-globin gene (*HBB)*. β^0 refers to the complete absence of production of β-globin directed by the affected allele. β^+ refers to alleles with some residual production of β-globin (often around 10%). In β^{++} the reduction in β-globin production is very mild [27]. Almost 330 beta thalassaemia alleles have now been characterised. A complete list with designation of mutation severity is available at the HbVar Globin Gene Server (https://globin.bx.psu.edu/hbvar/menu.html) [28, 29].

Non-deletional forms of β-thalassaemia account for the vast majority of the β-thalassaemia alleles. They include single base substitutions, small insertions, or deletions within the gene or its immediate flanking sequences. Rarely β-thalassaemia is the result of gross gene deletion. These alterations differ according to the mechanism by which they affect gene function: transcription, RNA processing, or translation. Transcriptional mutations involve promoter regulatory elements (either CACCC or TATA box) with mild or very mild reductions in β-globin production (β^+, β^{++}, or silent mutations). Other mutations can lead to aberrant RNA processing, including those in the splice junction (β^0 mutations), consensus splice sites, cryptic splice sites, and RNA cleavage-Poly A signal (β^+ mutations). Mutants affecting the translation of the β-globin mRNA include mutations of the initiation codon, nonsense codon mutations, and frameshift mutations, all resulting in the complete absence of production of β-globin (β^0 mutations) [27, 30].

It is important to remember that dominantly inherited β-thalassaemia comprises a distinct set of mutations (including missense mutations, deletion or insertion of intact codons, premature termination, and frameshift mutations) affecting the *HBB* gene. These mutations are associated with typical haematological features of β-thalassaemia, such as increased HbA2 levels and imbalanced α/β-globin chain biosynthesis in heterozygotes, and cause a disease phenotype even when present on heterozygosity [10, 11].

Despite marked molecular heterogeneity, the prevalent molecular defects are limited in each at-risk population, in which 4 to 10 variants usually account for most of *HBB* disease-causing alleles.

Haemoglobin E is the most common structural variant with thalassaemic properties, and behaves largely like a β^+ mutation. It is characterised by the substitution of lysine for glutamic acid at position 26 of the β-globin chain. The mutation G>A at codon 26 of the β-globin gene not only produces the amino acid substitution but also activates a cryptic splice site at codon 24-25, leading to an alternative splicing pathway. The overall result is the production of reduced amounts of the variant haemoglobin E; haemoglobin E constitutes 25-30% of total haemoglobin in haemoglobin E carriers, instead of the expected 50%. In other words, the codon 26 G>A mutation results both in a qualitative and a quantitative β-globin gene defect [13, 14].

5. GENOTYPE-PHENOTYPE CORRELATION

5.1. β-Thalassaemia

The extent of α/β-globin chain imbalance is the primary determinant of clinical severity in β-thalassaemia. Therefore, any factor that can reduce this imbalance leads to a lower degree of α-globin chain precipitation and may improve the clinical outcome.

One of the most common and consistent mechanisms for milder clinical severity is homozygosity or compound heterozygosity for two β^+ thalassaemia mild or silent mutations. Examples of these alleles are the silent -101 C>T and the mild IVS-1–6 T>C mutation in the Mediterranean population, the -28 A>G in Southeast Asian population, and the –29 A>G in Africans. Survival variation based on β-thalassaemia genotype severity has been recently illustrated [31, 32]. Other secondary genetic factors that can ameliorate the phenotype are the co-inheritance of α-thalassaemia or of genetic determinants that increase γ-globin chain production and HbF. Deletional and non-deletional hereditary persistence of foetal haemoglobin (HPFH) mutations, associated with high HbF levels in carriers, result in mild β-thalassaemia intermedia when combined with severe β-thalassaemia alleles [27, 30]. Changes in the ubiquitin-proteasome system or expression of α-haemoglobin stabilising protein which can help detoxify and tolerate a modest pool of free α-globin have also been shown to ameliorate disease severity [33-38].

A mild phenotype may also be determined by co-inheritance of genetic determinants associated with γ-globin chain production, mapping outside the β-globin gene cluster. Several genome-wide association studies (GWAS) have identified two quantitative trait loci (*BCL11A* on chromosome 2p16 and *HBS1L-MYB* intergenic region on chromosome 6q23) that account for 20%-30% of the common variation in HbF levels in healthy adults which are associated with a mild β-thalassaemia intermedia phenotype and with a delayed need for transfusion in patients with homozygous β^0-thalassaemia [39-46].

In some instances, heterozygous β-thalassaemia can lead to the β-thalassaemia intermedia phenotype instead of the asymptomatic carrier state. Most of these patients have an excess of functional α-globin genes (α-globin gene triplication or quadruplication), which increases the imbalance in the ratio of α/non-α globin chain synthesis [47]. In addition, as previously mentioned, rare mutations that result in the synthesis of extremely unstable β-globin variants, which precipitate in erythroid precursors causing ineffective erythropoiesis, may be associated with β-thalassaemia intermedia in heterozygotes (dominant thalassaemia) [10, 11].

Several tertiary genetic modifiers that can influence the clinical expression of specific complications in β-thalassaemia have been identified. The most studied polymorphism is the presence of (TA)7 in the promoter region of the uridine diphosphate-glucuronosyltransferase gene, which, in the homozygous state, is associated with Gilbert syndrome and the development of cholelithiasis in individuals with TDT and NTDT [48]. Another modifier is the apolipoprotein E ε4 allele [49]. The presence of this allele seem to increase the risk of left ventricular failure in homozygous β-thalassaemia. Less defined modifying factors are genes coding for HFE-associated hereditary haemochromatosis and genes involved in bone metabolism [27, 30, 50].

5.2. Haemoglobin E/β-thalassaemia

Heterozygotes for haemoglobin E are clinically normal and manifest only minimal changes in red blood cell indices, with the presence of haemoglobin E on haemoglobin analyses. Homozygotes for haemoglobin E are also clinically silent and may be only mildly anaemic [13, 14].

Patients with haemoglobin E/β-thalassaemia, which is common in Southeast Asia, have clinical manifestations that are variable in severity – from NTDT to TDT [27]. These are usually classified into three categories:

- Mild haemoglobin E/β-thalassaemia: this is observed in about 15% of all cases in Southeast Asia. This group of patients maintains haemoglobin levels between 9 and 12 g/dL and usually does not develop clinically significant problems at an early age. However, some patients suffer from growth failure, iron overload, and other complications similar to those of NTDT patients.
- Moderate haemoglobin E/β-thalassaemia: most haemoglobin E/β-thalassaemia cases fall into this category. Haemoglobin levels remain at 6-7 g/dL and the clinical symptoms are similar to β-thalassaemia intermedia (NTDT). Transfusions are not required unless an infection precipitates further anaemia.
- Severe haemoglobin E/β-thalassaemia: the haemoglobin level can be as low as 4-5 g/dL. Patients in this group manifest symptoms similar to those of TDT and are treated as TDT patients.

A disease scoring system that helps classify patients into mild, moderate, and severe has been proposed [51].

The reasons for this variability have only been partially defined, including the type of β-thalassaemia mutation (β^+ or β^0), co-inheritance of α-thalassaemia, and an innate propensity for postnatal γ-globin expression. Still, even subjects with seemingly identical genotypes may exhibit clinical manifestations of vastly different severity [13, 52-61]. It should be noted that patients with haemoglobin E/β-thalassaemia also show different phenotypic severity at particular stages of development. Advancing age has an independent and direct effect on the

background level of erythropoietin (EPO) production in response to anaemia [62-64]. A notable environmental factor influencing phenotype in patients with haemoglobin E/β-thalassaemia is infection with malaria, particularly *Plasmodium vivax* [65]

5.3. Haemoglobin Lepore

Haemoglobin Lepore is another structural β variant resulting from a fusion of the δ and β globin genes. The homozygous state of haemoglobin Lepore or haemoglobin Lepore co-inherited with β- thalassaemia can result in moderate to severe transfusion-dependent β-thalassaemia syndromes [27, 30].

6. PATHOPHYSIOLOGY

Understanding the pathophysiology and clinical manifestations of β-thalassaemia is largely based on untreated patients with NTDT. Transfusion therapy in TDT, when adequate, ameliorates the underlying disease process but gives way to various clinical morbidities primarily attributed to secondary iron overload, as will be featured in subsequent Chapters in these Guidelines. TDT patients who do not receive adequate transfusions and achieve target haemoglobin levels, however, could still experience the various manifestations of uncontrolled disease [66].

Normally, under conditions of poor oxygenation, erythropoiesis may be expanded significantly to increase oxygen delivery to the tissues. This physiological adaptation is defined as stress erythropoiesis and is characterised by an imbalance of erythroid proliferation and differentiation, resulting in an expansion of the erythroid progenitor pool that eventually leads to increased production of red cells [67].

6.1. Ineffective erythropoiesis

In β-thalassaemia, the reduced ability of erythrocytes to differentiate, survive, circulate and deliver oxygen stimulates a state of chronic stress erythropoiesis in the attempt to compensate for the poor production of enucleated red blood cells. The basic defect in β-thalassaemia is a reduced or absent production of β-globin chains with a relative excess of α-globin chains, which accumulate in the erythroid precursors, leading to the formation of alpha globin aggregates and haemichromes and a consequent state of substantial oxidative stress which contributes to reduced differentiation and apoptosis. The inability of this larger number of erythroid progenitor cells to generate sufficient red blood cells is defined as ineffective erythropoiesis which is the primary driver for chronic anaemia in β-thalassaemia [67].

The formation of α-globin aggregates has cytotoxic effects that lead to sequestration of heat shock protein 70 (Hsp70), leaving GATA1 accessible for caspase-3 cleavage. It results in apoptosis at the polychromatophilic stage of erythroid maturation and the generation of reactive oxygen species (ROS) [68, 69].

Moreover, elevated levels of certain members of the transforming growth factor β (TGF-β) family, such as growth differentiation factor 11 (GDF11) and others, have been observed. It has been proposed that they play a role in ineffective erythropoiesis through the activation of class II activin receptors A (ActRIIA) and B (ActRIIB) and the consequent activation of the inhibitory SMAD2/3 pathway leading to reduced erythroid differentiation and a lack of mature erythrocytes. However, the full mechanism is still far from being fully elucidated [70-72].

Anaemia varies in extent depending on the degree of α/β-globin chain imbalance and it is exacerbated by the reduced half-life of the red cells and their haemolysis in the circulatory stream since they are prematurely destroyed by the spleen.

Ineffective erythropoiesis and chronic anaemia lead to increased iron absorption from the intestine, which results in primary iron overload (see below).

6.2. Dysregulated iron metabolism

Erythropoiesis and iron levels are tightly linked to preserve a healthy balance of iron utilisation for the generation of new erythroblasts and iron recycling from senescent erythrocytes. Under conditions of ineffective erythropoiesis, the erythroid factor erythroferrone (ERFE) produced by erythroid progenitors promotes increased intestinal iron absorption [73, 74]. ERFE acts on the liver to suppress *HAMP* expression and reduce hepcidin synthesis [75, 76]. The iron supply to the erythroid cells is modulated by the expression of transferrin receptor 1 (TfR1). Interestingly, in sera of thalassaemia patients, soluble TfR1 is proportional to the amount of ineffective erythropoiesis. A second transferrin receptor (TfR2) is also expressed in erythroid cells. However, its primary role appears to be in coordinating iron levels with EPO sensitivity and may also play a role in modulating ineffective erythropoiesis [77, 78].

Red blood cell progenitors that fail to mature increase the total iron pool of the body through direct and indirect mechanisms, while pathologically high levels of free iron support expansion in the number of early erythroid progenitors. Although the virtually unlimited availability of iron represents a boost factor for erythropoiesis, toxic, free iron increases ROS production, diminishing red blood cell functionality and lifespan and perpetuating a vicious circle. Haemolysis makes a significant contribution to the total amount of circulating iron [79, 80].

Advances in understanding this dynamic relationship between ineffective erythropoiesis and dysregulated iron metabolism with increased intestinal iron absorption (primary iron overload) [81], helped explain why so many NTDT patients go on to develop cumulative and considerable iron overload, characterised by increased hepatocyte iron loading and elevated liver iron concentration with serum ferritin levels relatively lower than what would be seen in transfusional (secondary) iron overload [82, 83].

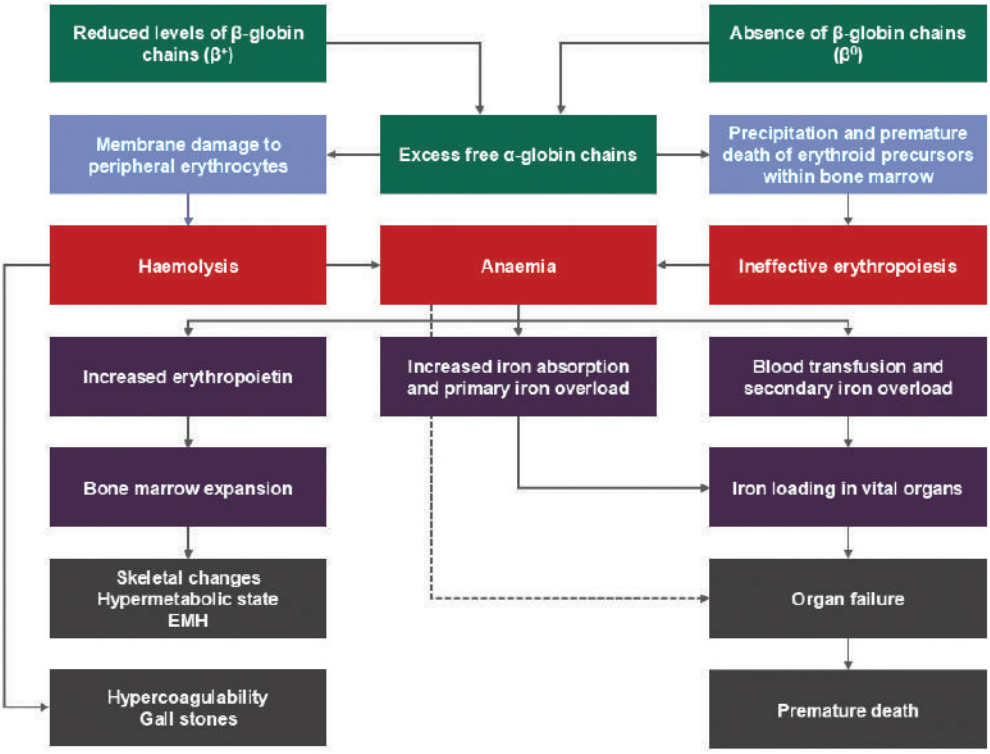
In TDT, when native and transfused red blood cells are broken down, their haemoglobin is degraded, releasing iron into the bloodstream. This iron is initially bound and detoxified by the plasma iron transporter protein transferrin. Over time, the iron-binding capacity of transferrin is exceeded, and excess iron is deposited in organs and tissues, leading to iron overload and organ damage with hepatic fibrosis, cirrhosis, and eventually liver failure, cardiomyopathy and heart failure, diabetes mellitus, hypogonadism, hypothyroidism, and other hormonal deficiencies. Iron deposition in the bone marrow can further impair erythropoiesis.

6.3. Clinical manifestations of ineffective erythropoiesis, anaemia, and iron overload

The volume of blood to be infused will be determined by the number/volume of red cells per The clinical manifestations associated with ineffective erythropoiesis are fully expressed in NTDT patients [84]. The increased production of erythropoietin may produce hyperplasia of erythroid marrow in medullary and extramedullary sites with characteristic deformities of the skull and face, cortical thinning and pathological fractures of long bones, extramedullary erythropoietic

tissue masses, and splenomegaly [1, 2]. The lipid membrane composition of abnormal red blood cells may result in a hypercoagulable state leading to thrombotic complications and pulmonary hypertension, especially in splenectomised adults [85, 86]. Retarded growth may occur as a result of chronic anaemia and the excessive metabolic burden imposed by erythroid expansion. Anaemia may produce cardiac enlargement and severe cardiac failure, and has been associated with overall increased risks of morbidity and mortality [16]. Haemolysis contributes to the increased concentration and amount of unconjugated bilirubin and the development of calcium bilirubin gallstones. Finally, expanded iron absorption leads to iron overload, especially in the liver, which, when severe and prolonged, can cause fibrosis, hepatocellular carcinoma, and endocrine complications [16]. Regular transfusions limit but do not eliminate ineffective erythropoiesis and its complications, which are more likely in the case of sub-optimally and inadequately transfused patients, who have a significantly increased risk of morbidity and mortality [87, 88]. Adequately transfused patients on the other hand, will be mainly at risk of end-organ damage due to secondary iron overload, especially if sub-optimally chelated (see Chapter 3). The pathophysiology of β-thalassaemia is summarised in Figure 3.

Figure 3. *Pathophysiology of β-thalassaemia. Abbreviations: EMH, extramedullary haematopoiesis.*

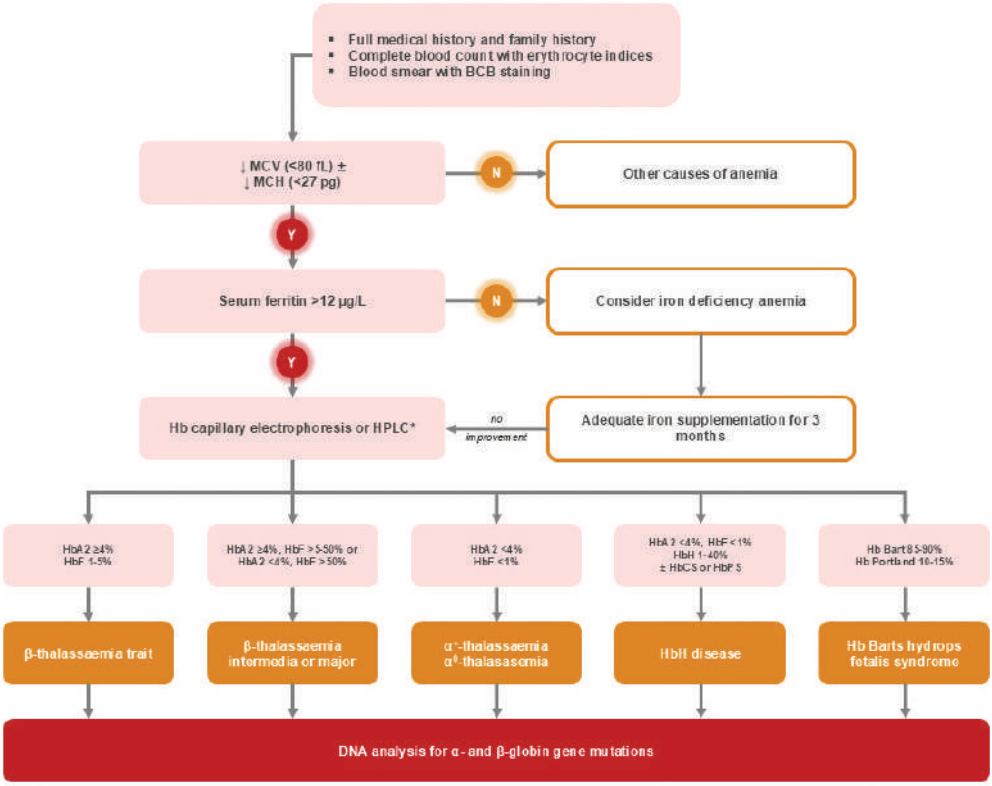


The increased knowledge on pathophysiology and clinical manifestations underlies the innovative therapies recently/being developed in thalassaemia including additive gene therapy and genome editing (see Chapter 15) and several disease-modifying therapies (see Chapter 16).

7. DIAGNOSIS

Since almost all thalassaemic conditions present with hypochromic microcytic anaemia, a diagnosis of thalassaemia should be considered in all individuals displaying such abnormal red blood cell features. However, it is crucial first to exclude iron deficiency anaemia, which remains common in many parts of the world [89]. A diagnostic algorithm is provided in Figure 4 [90].

Figure 4. *Algorithm for the diagnosis of thalassaemia. *Could also detect co-inherited structural haemoglobin variants indicating HbE, HbS, HbC and other disorders. Abbreviations: BCB, brilliant cresyl blue; Hb, haemoglobin; HbA2, adult haemoglobin 2; HbCS, haemoglobin Constant Spring; HbF, foetal haemoglobin; HbH, haemoglobin H; HbPS, haemoglobin Paksé; HPLC, high-performance liquid chromatography; MCH, mean corpuscular haemoglobin; MCV, mean corpuscular volume; N, no; Y, yes. Reproduced with permission from [90].*



7.1. Clinical diagnosis

Carriers of thalassaemia are usually clinically asymptomatic but sometimes have mild anaemia. Clinical presentation of TDT usually occurs between 6 and 24 months with severe microcytic anaemia, mild jaundice, and hepatosplenomegaly. Affected infants fail to thrive and become progressively pale. Feeding problems, irritability, recurrent bouts of fever due to a hypermetabolic state or intercurrent infection, and progressive enlargement of the abdomen caused by spleen and liver enlargement may occur. In developed countries, if prenatal diagnosis has not been performed, the diagnosis of TDT is established at this stage, and a regular transfusion programme is initiated (see Chapter 2 for indications and considerations for initiating regular transfusions in β-thalassaemia). The classic clinical picture of TDT is currently seen only in some developing countries where the resources for performing long-term transfusion programmes are unavailable. The clinical picture in patients who are untreated or poorly transfused is characterised by growth retardation, pallor, jaundice, poor musculature, genu valgum, hepatosplenomegaly, leg ulcers, development of masses from extramedullary haematopoiesis, and skeletal changes resulting from expansion of the bone marrow. Skeletal changes include deformities in the long bones of the legs and typical craniofacial changes including thalassaemic facies (bossing of the skull, prominent malar eminence, depression of the bridge of the nose, and hypertrophy of the maxillae, which tends to expose the upper teeth). If a chronic transfusion regimen is not started, patients with TDT usually die within the first few years of life from high-output heart failure [1, 2, 27]. Individuals with NTDT present symptoms later than those with TDT, have milder anaemia and, by definition, do not require or only occasionally require transfusions. Further details on clinical presentation of NTDT are provided in a dedicated TIF Guidelines [4].

7.2. Haematological diagnosis

Heterozygous carriers of β-thalassaemia usually display low mean cell haemoglobin (MCH), low mean cell volume (MCV), and an increased level of HbA2 which may be associated with low normal or slightly subnormal haemoglobin levels. A peripheral blood film shows less severe erythrocyte morphological changes than affected individuals and erythroblasts are usually not seen. AT presentation, TDT is characterised by reduced haemoglobin level (<7 g/dL), MCV >50 fL and <70 fL and MCH >12 and <20 pg. NTDT is characterised by haemoglobin level between 7 and 10 g/dL, MCV between 50 and 80 fL, and MCH between 16 and 27 pg. The peripheral blood film in affected individuals demonstrates red blood cell morphological changes with microcytosis, hypochromia, anisocytosis, poikilocytosis and nucleated red blood cells (erythroblasts, NRBC). The number of erythroblasts is related to the degree of anaemia and is markedly increased after splenectomy. In general, these abnormal red blood cell morphological features are shared among different types of thalassaemia syndromes, including interactions with structural haemoglobin variants such as haemoglobin E/β thalassaemia. Haemoglobin E can be easily detected using a special dye, dichlorophenolindophenol (DCIP) [1, 2, 13, 14, 27].

7.3. Qualitative and quantitative haemoglobin analysis

Capillary electrophoresis, high-performance liquid chromatography (HPLC), and cellulose acetate electrophoresis identify the amount and type of haemoglobin present. The haemoglobin pattern varies by β-thalassaemia type. In β^0-thalassaemia homozygotes, HbA is absent, and HbF constitutes 92-95% of the total haemoglobin. In β^+-thalassaemia homozygotes and β^+/β^0 compound

heterozygotes HbA levels are between 10 and 30% according to the variable degree of reduction of β-globin chain synthesis, and HbF is 70-90%. HbA2 is variable in β-thalassaemia homozygotes/compound heterozygotes, and it is enhanced in β-thalassaemia carriers. In haemoglobin E/β-thalassaemia patients, haemoglobin E constitutes between 30% and 70% of the haemoglobin with the remainder being HbF [1, 2, 13, 14, 27].

7.4. Molecular analysis

Molecular analysis may be required to confirm the diagnosis of haeomglobin E and identify the specific β-thalassaemia genotype. Since the prevalent pathogenic variants are limited in each at-risk population, targeted analysis for pathogenic variants based on ancestry may be considered first. Commonly occurring mutations of the β-globin gene are detected by polymerase chain reaction (PCR)-based procedures. The most commonly used methods are reverse dot blot analysis or primer-specific amplification, with a set of probes or primers complementary to the most common mutations in the population from which the affected individual originated. β-globin gene sequence analysis using Sanger method may be considered first if the affected individual is not of an ancestry at high risk or if targeted analysis reveals only one or no pathogenic variant. If the results are inconclusive, gene-targeted deletion/duplication analysis (multiplex ligation-dependent probe amplification [MLPA]) can follow.

Currently, available thalassaemia diagnostic kits often utilise next-generation sequencing (NGS) technology. Unlike Sanger sequencing, NGS methods do not require prior knowledge of the sequence of target regions, thereby eliminating the need for target-specific DNA primers. The method involves multiplex PCR amplification to generate a DNA fragment target amplicon library, followed by PCR and ligation of each amplicon to sequencing adapters that include unique index sequences. The sample pool is then sequenced using NGS chemistry. This approach can detect single nucleotide variants (SNVs), insertions and deletions (indels), and copy number variations (CNVs) in the globin genes. Additionally, regions positioned upstream and downstream of these genes are targeted to detect large deletions or duplications, such as those found in forms of εγδβ-thalassaemia.

KEY POINTS AND RECOMMENDATIONS

1. A diagnosis of thalassaemia should be considered in all those who have hypochromic microcytic anaemia (**Grade C, Class I**).
2. In the diagnostic work-up for hypochromic microcytosis, iron deficiency anaemia should always be excluded (**Grade C, Class I**).
3. Molecular analysis is not required to confirm the diagnosis of a β-thalassaemia carrier, but it is necessary to confirm the α-thalassaemia carrier status (**Grade C, Class I**).
4. An α-globin gene triplication or quadruplication should be taken into consideration in heterozygous β-thalassaemia subjects with a β-thalassaemia intermedia phenotype (**Grade C, Class I**).
5. Haematological parameters, including red cell indices and morphology, followed by separation and measurement of haemoglobin fractions, are the basis for the identification of β-thalassaemia carriers (**Grade C, Class I**).
6. Since the prevalent pathogenic variants of the β-globin gene are limited in each at-risk population, a polymerase chain reaction (PCR) method designed to detect the common specific mutation simultaneously should be used initially (**Grade C, Class IIa**).
7. β-globin gene sequence analysis may be considered first if the affected individual is not of an ancestry at high risk or if targeted analysis reveals only one or no pathogenic variant (**Grade C, Class IIa**).
8. Methods that may be used to detect rare or unknown deletions include Southern blotting (now fallen into abeyance), quantitative PCR, long-range PCR and, above all, multiplex ligation-dependent probe amplification (MLPA) (**Grade C, Class IIa**).
9. Considerations of phenotype should not only be based on genotype but should take clinical presentation and disease severity as observed over a duration of time (**Grade C, Class IIb**).
10. Patients who receive 6 or more red blood cell units over 6 months with ≤6-week transfusion-free period or receiving frequent transfusions for >1 year can be classified as transfusion-dependent β-thalassaemia for purposes of management approach or clinical trial eligibility (**Grade C, Class IIb**).

REFERENCES

1. Taher AT, Musallam KM, Cappellini MD. beta-Thalassemias. N Engl J Med 2021;384(8):727-743.
2. Kattamis A, Kwiatkowski JL, Aydinok Y. Thalassaemia. Lancet 2022;399(10343):2310-2324.
3. Origa R. beta-Thalassemia. Genet Med 2017;19(6):609-619.
4. Taher A, Musallam K, Cappellini MD. Guidelines for the Management of Non-Transfusion-Dependent β-Thalassaemia. 3rd ed. Nicosia, Cyprus: Thalassaemia International Federation, 2023.
5. Amid A, Lal A, Coates TD, Fucharoen S. Guidelines for the management of α-thalassemia. Nicosia, Cyprus: Thalassaemia International Federation, 2023.
6. Steinberg MH, Forget BG, Higgs DR, Weatherall DJ. Disorders of hemoglobin : genetics, pathophysiology, and clinical management. 2nd ed. New York: Cambridge University Press, 2009.
7. Venkatesan V, Srinivasan S, Babu P, Thangavel S. Manipulation of Developmental Gamma-Globin Gene Expression: an Approach for Healing Hemoglobinopathies. Mol Cell Biol 2020;41(1).
8. Weatherall DJ. The inherited diseases of hemoglobin are an emerging global health burden. Blood 2010;115(22):4331-4336.
9. Weatherall DJ. The Evolving Spectrum of the Epidemiology of Thalassemia. Hematol Oncol Clin North Am 2018;32(2):165-175.
10. Efremov GD, Simjanovska L, Plaseska-Karanfilska D, Stanojevic E, Petkov GH. Hb Jambol: a new hyperunstable hemoglobin causing severe hemolytic anemia. Acta Haematol 2007;117(1):1-7.
11. Kanathezhath B, Hazard FK, Guo H, Kidd J, Azimi M, Kuypers FA, Vichinsky EP, Lal A. Hemoglobin Hakkari: an autosomal dominant form of beta thalassemia with inclusion bodies arising from de novo mutation in exon 2 of beta globin gene. Pediatr Blood Cancer 2010;54(2):332-335.
12. Musallam KM, Rivella S, Vichinsky E, Rachmilewitz EA. Non-transfusion-dependent thalassemias. Haematologica 2013;98(6):833-844.
13. Galanello R. Recent advances in the molecular understanding of non-transfusion-dependent thalassemia. Blood Rev 2012;26 Suppl 1:S7-S11.
14. Fucharoen S, Weatherall DJ. The hemoglobin e thalassemias. Cold Spring Harb Perspect Med 2012;2(8).
15. Musallam KM, Barella S, Origa R, Ferrero GB, Lisi R, Pasanisi A, Longo F, Gianesin B, Forni GL, Webthal p. 'Phenoconversion' in adult patients with beta-thalassemia. Am J Hematol 2024;99(3):490-493.
16. Musallam KM, Sheth S, Cappellini MD, Forni GL, Maggio A, Taher AT. Anemia and iron overload as prognostic markers of outcomes in beta-thalassemia. Expert Rev Hematol 2024;17(9):631-642.
17. Musallam KM, Taher AT, Cappellini MD, Hermine O, Kuo KHM, Sheth S, Viprakasit V, Porter JB. Untreated Anemia in Nontransfusion-dependent beta-thalassemia: Time to Sound the Alarm. Hemasphere 2022;6(12):e806.
18. Musallam KM, Vitrano A, Meloni A, Pollina SA, Karimi M, El-Beshlawy A, Hajipour M, Di Marco V, Ansari SH, Filosa A, Ricchi P, Ceci A, Daar S, Vlachaki E, Singer ST, Naserullah ZA, Pepe A, Scondotto S, Dardanoni G, Bonifazi F, Sankaran VG, Vichinsky E, Taher AT, Maggio A. Survival and causes of death in 2,033 patients with non-transfusion-dependent beta-thalassemia. Haematologica 2021;106(9):2489-2492.
19. Taher AT, Musallam KM, Karimi M, El-Beshlawy A, Belhoul K, Daar S, Saned MS, El-Chafic AH, Fasulo MR, Cappellini MD. Overview on practices in thalassemia intermedia management aiming for lowering complication rates across a region of endemicity: the OPTIMAL CARE study. Blood 2010;115(10):1886-1892.
20. Musallam KM, Cappellini MD, Viprakasit V, Kattamis A, Rivella S, Taher AT. Revisiting the non-transfusion-dependent (NTDT) vs. transfusion-dependent (TDT) thalassemia classification 10 years later. Am J Hematol 2021;96(2):E54-E56.
21. Musallam KM, Viprakasit V, Lombard L, Gilroy K, Rane A, Vinals L, Tam C, Rizzo M, Coates TD. Systematic review and evidence gap assessment of the clinical, quality of life, and economic burden of alpha-thalassemia. EJHaem 2024;5(3):541-547.

22. Eleftheriou A, Angastiniotis M. Global Thalassaemia Review 2023. Nicosia, Cyprus: Thalassaemia International Federation, 2023.
23. Farmakis D, Angastiniotis M, El Ghoul MM, Cannon L, Eleftheriou A. Thalassaemia Registries: A Call for Action. A Position Statement from the Thalassaemia International Federation. Hemoglobin 2022;46(4):225-232.
24. Forni GL, Gianesin B, Musallam KM, Longo F, Rosso R, Lisi R, Gamberini MR, Pinto VM, Graziadei G, Vitucci A, Bonetti F, Musto P, Piga A, Cappellini MD, Borgna-Pignatti C, Webthal p. Overall and complication-free survival in a large cohort of patients with beta-thalassemia major followed over 50 years. Am J Hematol 2023;98(3):381-387.
25. Kattamis A, Forni GL, Aydinok Y, Viprakasit V. Changing patterns in the epidemiology of beta-thalassemia. Eur J Haematol 2020;105(6):692-703.
26. Angastiniotis M, Petrou M, Loukopoulos D, Modell B, Farmakis D, Englezos P, Eleftheriou A. The Prevention of Thalassemia Revisited: A Historical and Ethical Perspective by the Thalassemia International Federation. Hemoglobin 2021;45(1):5-12.
27. Galanello R, Origa R. Beta-thalassemia. Orphanet J Rare Dis 2010;5:11.
28. Giardine B, Borg J, Viennas E, Pavlidis C, Moradkhani K, Joly P, Bartsakoulia M, Riemer C, Miller W, Tzimas G, Wajcman H, Hardison RC, Patrinos GP. Updates of the HbVar database of human hemoglobin variants and thalassemia mutations. Nucleic Acids Res 2014;42(Database issue):D1063-1069.
29. Kountouris P, Lederer CW, Fanis P, Feleki X, Old J, Kleanthous M. IthaGenes: an interactive database for haemoglobin variations and epidemiology. PLoS One 2014;9(7):e103020.
30. Danjou F, Anni F, Galanello R. Beta-thalassemia: from genotype to phenotype. Haematologica 2011;96(11):1573-1575.
31. Musallam KM, Vitrano A, Meloni A, Addario Pollina S, Di Marco V, Hussain Ansari S, Filosa A, Ricchi P, Ceci A, Daar S, Vlachaki E, Singer ST, Naserullah ZA, Pepe A, Scondotto S, Dardanoni G, Karimi M, El-Beshlawy A, Hajipour M, Bonifazi F, Vichinsky E, Taher AT, Sankaran VG, Maggio A, International Working Group on T. Primary HBB gene mutation severity and long-term outcomes in a global cohort of beta-thalassaemia. Br J Haematol 2022;196(2):414-423.
32. Kountouris P, Michailidou K, Christou S, Hadjigavriel M, Sitarou M, Kolnagou A, Kleanthous M, Telfer P. Effect of HBB genotype on survival in a cohort of transfusion-dependent thalassemia patients in Cyprus. Haematologica 2021;106(9):2458-2468.
33. Gell D, Kong Y, Eaton SA, Weiss MJ, Mackay JP. Biophysical characterization of the alpha-globin binding protein alpha-hemoglobin stabilizing protein. J Biol Chem 2002;277(43):40602-40609.
34. Kihm AJ, Kong Y, Hong W, Russell JE, Rouda S, Adachi K, Simon MC, Blobel GA, Weiss MJ. An abundant erythroid protein that stabilizes free alpha-haemoglobin. Nature 2002;417(6890):758-763.
35. Kong Y, Zhou S, Kihm AJ, Katein AM, Yu X, Gell DA, Mackay JP, Adachi K, Foster-Brown L, Louden CS, Gow AJ, Weiss MJ. Loss of alpha-hemoglobin-stabilizing protein impairs erythropoiesis and exacerbates beta-thalassemia. J Clin Invest 2004;114(10):1457-1466.
36. Khandros E, Thom CS, D'Souza J, Weiss MJ. Integrated protein quality-control pathways regulate free alpha-globin in murine beta-thalassemia. Blood 2012;119(22):5265-5275.
37. Lechauve C, Keith J, Khandros E, Fowler S, Mayberry K, Freiwan A, Thom CS, Delbini P, Romero EB, Zhang J, Motta I, Tillman H, Cappellini MD, Kundu M, Weiss MJ. The autophagy-activating kinase ULK1 mediates clearance of free alpha-globin in beta-thalassemia. Sci Transl Med 2019;11(506).
38. Viprakasit V, Tanphaichitr VS, Chinchang W, Sangkla P, Weiss MJ, Higgs DR. Evaluation of alpha hemoglobin stabilizing protein (AHSP) as a genetic modifier in patients with beta thalassemia. Blood 2004;103(9):3296-3299.
39. Menzel S, Garner C, Gut I, Matsuda F, Yamaguchi M, Heath S, Foglio M, Zelenika D, Boland A, Rooks H, Best S, Spector TD, Farrall M, Lathrop M, Thein SL. A QTL influencing F cell production maps to a gene encoding a zinc-finger protein on chromosome 2p15. Nat Genet 2007;39(10):1197-1199.

40. Sankaran VG, Menne TF, Xu J, Akie TE, Lettre G, Van Handel B, Mikkola HK, Hirschhorn JN, Cantor AB, Orkin SH. Human fetal hemoglobin expression is regulated by the developmental stage-specific repressor BCL11A. Science 2008;322(5909):1839-1842.
41. Liu N, Hargreaves VV, Zhu Q, Kurland JV, Hong J, Kim W, Sher F, Macias-Trevino C, Rogers JM, Kurita R, Nakamura Y, Yuan GC, Bauer DE, Xu J, Bulyk ML, Orkin SH. Direct Promoter Repression by BCL11A Controls the Fetal to Adult Hemoglobin Switch. Cell 2018;173(2):430-442 e417.
42. Basak A, Munschauer M, Lareau CA, Montbleau KE, Ulirsch JC, Hartigan CR, Schenone M, Lian J, Wang Y, Huang Y, Wu X, Gehrke L, Rice CM, An X, Christou HA, Mohandas N, Carr SA, Chen JJ, Orkin SH, Lander ES, Sankaran VG. Control of human hemoglobin switching by LIN28B-mediated regulation of BCL11A translation. Nat Genet 2020;52(2):138-145.
43. Uda M, Galanello R, Sanna S, Lettre G, Sankaran VG, Chen W, Usala G, Busonero F, Maschio A, Albai G, Piras MG, Sestu N, Lai S, Dei M, Mulas A, Crisponi L, Naitza S, Asunis I, Deiana M, Nagaraja R, Perseu L, Satta S, Cipollina MD, Sollaino C, Moi P, Hirschhorn JN, Orkin SH, Abecasis GR, Schlessinger D, Cao A. Genome-wide association study shows BCL11A associated with persistent fetal hemoglobin and amelioration of the phenotype of beta-thalassemia. Proc Natl Acad Sci U S A 2008;105(5):1620-1625.
44. Galanello R, Sanna S, Perseu L, Sollaino MC, Satta S, Lai ME, Barella S, Uda M, Usala G, Abecasis GR, Cao A. Amelioration of Sardinian beta0 thalassemia by genetic modifiers. Blood 2009;114(18):3935-3937.
45. Danjou F, Francavilla M, Anni F, Satta S, Demartis FR, Perseu L, Manca M, Sollaino MC, Manunza L, Mereu E, Marceddu G, Pissard S, Joly P, Thuret I, Origa R, Borg J, Forni GL, Piga A, Lai ME, Badens C, Moi P, Galanello R. A genetic score for the prediction of beta-thalassemia severity. Haematologica 2015;100(4):452-457.
46. Martyn GE, Wienert B, Yang L, Shah M, Norton LJ, Burdach J, Kurita R, Nakamura Y, Pearson RCM, Funnell APW, Quinlan KGR, Crossley M. Natural regulatory mutations elevate the fetal globin gene via disruption of BCL11A or ZBTB7A binding. Nat Genet 2018;50(4):498-503.
47. Origa R, Sollaino MC, Borgna-Pignatti C, Piga A, Feliu Torres A, Masile V, Galanello R. alpha-globin gene quadruplication and heterozygous beta-thalassemia: a not so rare cause of thalassemia intermedia. Acta Haematol 2014;131(3):162-164.
48. Galanello R, Cipollina MD, Dessi C, Giagu N, Lai E, Cao A. Co-inherited Gilbert's syndrome: a factor determining hyperbilirubinemia in homozygous beta-thalassemia. Haematologica 1999;84(2):103-105.
49. Economou-Petersen E, Aessopos A, Kladi A, Flevari P, Karabatsos F, Fragodimitri C, Nicolaidis P, Vrettou H, Vassilopoulos D, Karagiorga-Lagana M, Kremastinos DT, Petersen MB. Apolipoprotein E epsilon4 allele as a genetic risk factor for left ventricular failure in homozygous beta-thalassemia. Blood 1998;92(9):3455-3459.
50. Origa R, Galanello R. Pathophysiology of beta thalassaemia. Pediatr Endocrinol Rev 2011;8 Suppl 2:263-270.
51. Sripichai O, Makarasara W, Munkongdee T, Kumkhaek C, Nuchprayoon I, Chuansumrit A, Chuncharunee S, Chantrakoon N, Boonmongkol P, Winichagoon P, Fucharoen S. A scoring system for the classification of beta-thalassemia/Hb E disease severity. Am J Hematol 2008;83(6):482-484.
52. Olivieri NF, Pakbaz Z, Vichinsky E. HbE/beta-thalassemia: basis of marked clinical diversity. Hematol Oncol Clin North Am 2010;24(6):1055-1070.
53. Olivieri NF, Muraca GM, O'Donnell A, Premawardhena A, Fisher C, Weatherall DJ. Studies in haemoglobin E beta-thalassaemia. Br J Haematol 2008;141(3):388-397.
54. Winichagoon P, Thonglairoam V, Fucharoen S, Wilairat P, Fukumaki Y, Wasi P. Severity differences in beta-thalassaemia/haemoglobin E syndromes: implication of genetic factors. Br J Haematol 1993;83(4):633-639.
55. Premawardhena A, Fisher CA, Fathiu F, de Silva S, Perera W, Peto TE, Olivieri NF, Weatherall DJ. Genetic determinants of jaundice and gallstones in haemoglobin E beta thalassaemia. Lancet 2001;357(9272):1945-1946.
56. Olivieri NF, Pakbaz Z, Vichinsky E. Hb E/beta-thalassaemia: a common & clinically diverse disorder. Indian J Med Res 2011;134:522-531.

57. Sharma V, Saxena R. Effect of alpha-gene numbers on phenotype of HbE/beta thalassemia patients. Ann Hematol 2009;88(10):1035-1036.
58. Nuinoon M, Makarasara W, Mushiroda T, Setianingsih I, Wahidiyat PA, Sripichai O, Kumasaka N, Takahashi A, Svasti S, Munkongdee T, Mahasirimongkol S, Peerapittayamongkol C, Viprakasit V, Kamatani N, Winichagoon P, Kubo M, Nakamura Y, Fucharoen S. A genome-wide association identified the common genetic variants influence disease severity in beta0-thalassemia/hemoglobin E. Hum Genet 2010;127(3):303-314.
59. Ma Q, Abel K, Sripichai O, Whitacre J, Angkachatchai V, Makarasara W, Winichagoon P, Fucharoen S, Braun A, Farrer LA. Beta-globin gene cluster polymorphisms are strongly associated with severity of HbE/beta(0)-thalassemia. Clin Genet 2007;72(6):497-505.
60. Perera S, Allen A, Silva I, Hapugoda M, Wickramarathne MN, Wijesiriwardena I, Allen S, Rees D, Efremov DG, Fisher CA, Weatherall DJ, Premawardhena A. Genotype-phenotype association analysis identifies the role of alpha globin genes in modulating disease severity of beta thalassaemia intermedia in Sri Lanka. Sci Rep 2019;9(1):10116.
61. Traivaree C, Monsereenusorn C, Rujkijyanont P, Prasertsin W, Boonyawat B. Genotype-phenotype correlation among beta-thalassemia and beta-thalassemia/HbE disease in Thai children: predictable clinical spectrum using genotypic analysis. J Blood Med 2018;9:35-41.
62. O'Donnell A, Premawardhena A, Arambepola M, Allen SJ, Peto TE, Fisher CA, Rees DC, Olivieri NF, Weatherall DJ. Age-related changes in adaptation to severe anemia in childhood in developing countries. Proc Natl Acad Sci U S A 2007;104(22):9440-9444.
63. Allen A, Fisher C, Premawardhena A, Peto T, Allen S, Arambepola M, Thayalsutha V, Olivieri N, Weatherall D. Adaptation to anemia in hemoglobin E-ss thalassemia. Blood 2010;116(24):5368-5370.
64. Premawardhena A, Fisher CA, Olivieri NF, de Silva S, Arambepola M, Perera W, O'Donnell A, Peto TE, Viprakasit V, Merson L, Muraca G, Weatherall DJ. Haemoglobin E beta thalassaemia in Sri Lanka. Lancet 2005;366(9495):1467-1470.
65. O'Donnell A, Premawardhena A, Arambepola M, Samaranayake R, Allen SJ, Peto TE, Fisher CA, Cook J, Corran PH, Olivieri NF, Weatherall DJ. Interaction of malaria with a common form of severe thalassemia in an Asian population. Proc Natl Acad Sci U S A 2009;106(44):18716-18721.
66. Taher AT, Weatherall DJ, Cappellini MD. Thalassaemia. Lancet 2018;391(10116):155-167.
67. Cazzola M. Ineffective erythropoiesis and its treatment. Blood 2022;139(16):2460-2470.
68. Arlet JB, Ribeil JA, Guillem F, Negre O, Hazoume A, Marcion G, Beuzard Y, Dussiot M, Moura IC, Demarest S, de Beauchene IC, Belaid-Choucair Z, Sevin M, Maciel TT, Auclair C, Leboulch P, Chretien S, Tchertanov L, Baudin-Creuza V, Seigneuric R, Fontenay M, Garrido C, Hermine O, Courtois G. HSP70 sequestration by free alpha-globin promotes ineffective erythropoiesis in beta-thalassaemia. Nature 2014;514(7521):242-246.
69. Guillem F, Dussiot M, Colin E, Suriyun T, Arlet JB, Goudin N, Marcion G, Seigneuric R, Causse S, Gonin P, Gastou M, Deloger M, Rossignol J, Lamarque M, Choucair ZB, Gautier EF, Ducamp S, Vandekerckhove J, Moura IC, Maciel TT, Garrido C, An X, Mayeux P, Mohandas N, Courtois G, Hermine O. XPO1 regulates erythroid differentiation and is a new target for the treatment of beta-thalassemia. Haematologica 2020;105(9):2240-2249.
70. Goldstein JM, Sengul H, Messemer KA, Fernandez-Alfara M, Garbern JC, Kristl AC, Lee RT, Wagers AJ. Steady-state and regenerative hematopoiesis occurs normally in mice in the absence of GDF11. Blood 2019;134(20):1712-1716.
71. Guerra A, Oikonomidou PR, Sinha S, Zhang J, Lo Presti V, Hamilton CR, Breda L, Casu C, La P, Martins AC, Sendamarai AK, Fleming M, Rivella S. Lack of Gdf11 does not improve anemia or prevent the activity of RAP-536 in a mouse model of beta-thalassemia. Blood 2019;134(6):568-572.
72. Camaschella C. GDF11 is not the target of luspatercept. Blood 2019;134(6):500-501.

73. Kautz L, Jung G, Du X, Gabayan V, Chapman J, Nasoff M, Nemeth E, Ganz T. Erythroferrone contributes to hepcidin suppression and iron overload in a mouse model of beta-thalassemia. Blood 2015;126(17):2031-2037.
74. Kautz L, Jung G, Valore EV, Rivella S, Nemeth E, Ganz T. Identification of erythroferrone as an erythroid regulator of iron metabolism. Nat Genet 2014;46(7):678-684.
75. Ganz T. Hepcidin--a regulator of intestinal iron absorption and iron recycling by macrophages. Best Pract Res Clin Haematol 2005;18(2):171-182.
76. Origa R, Cazzola M, Mereu E, Danjou F, Barella S, Giagu N, Galanello R, Swinkels DW. Differences in the erythropoiesis-hepcidin-iron store axis between hemoglobin H disease and beta-thalassemia intermedia. Haematologica 2015;100(5):e169-171.
77. Di Modica SM, Tanzi E, Olivari V, Lidonnici MR, Pettinato M, Pagani A, Tiboni F, Furiosi V, Silvestri L, Ferrari G, Rivella S, Nai A. Transferrin receptor 2 (Tfr2) genetic deletion makes transfusion-independent a murine model of transfusion-dependent beta-thalassemia. Am J Hematol 2022;97(10):1324-1336.
78. Nai A, Lidonnici MR, Rausa M, Mandelli G, Pagani A, Silvestri L, Ferrari G, Camaschella C. The second transferrin receptor regulates red blood cell production in mice. Blood 2015;125(7):1170-1179.
79. Guerra A, Parhiz H, Rivella S. Novel potential therapeutics to modify iron metabolism and red cell synthesis in diseases associated with defective erythropoiesis. Haematologica 2023;108(10):2582-2593.
80. Lin S, Zheng Y, Chen M, Xu L, Huang H. The interactions between ineffective erythropoiesis and ferroptosis in beta-thalassemia. Front Physiol 2024;15:1346173.
81. Camaschella C, Pagani A. Advances in understanding iron metabolism and its crosstalk with erythropoiesis. Br J Haematol 2018;182(4):481-494.
82. Musallam KM, Cappellini MD, Wood JC, Taher AT. Iron overload in non-transfusion-dependent thalassemia: a clinical perspective. Blood Rev 2012;26 Suppl 1:S16-19.
83. Origa R, Galanello R, Ganz T, Giagu N, Maccioni L, Faa G, Nemeth E. Liver iron concentrations and urinary hepcidin in beta-thalassemia. Haematologica 2007;92(5):583-588.
84. Saliba AN, Musallam KM, Taher AT. How I treat non-transfusion-dependent beta-thalassemia. Blood 2023;142(11):949-960.
85. Taher AT, Cappellini MD, Bou-Fakhredin R, Coriu D, Musallam KM. Hypercoagulability and Vascular Disease. Hematol Oncol Clin North Am 2018;32(2):237-245.
86. Derchi G, Galanello R, Bina P, Cappellini MD, Piga A, Lai ME, Quarta A, Casu G, Perrotta S, Pinto V, Musallam KM, Forni GL, Webthal Pulmonary Arterial Hypertension G. Prevalence and risk factors for pulmonary arterial hypertension in a large group of beta-thalassemia patients using right heart catheterization: a Webthal study. Circulation 2014;129(3):338-345.
87. Musallam KM, Barella S, Origa R, Ferrero GB, Lisi R, Pasanisi A, Longo F, Gianesin B, Forni GL. Pretransfusion hemoglobin level and mortality in adults with transfusion-dependent beta-thalassemia. Blood 2024;143(10):930-932.
88. El-Beshlawy A, Dewedar H, Hindawi S, Alkindi S, Tantawy AA, Yassin MA, Taher AT. Management of transfusion-dependent beta-thalassemia (TDT): Expert insights and practical overview from the Middle East. Blood Rev 2024;63:101138.
89. Viprakasit V, Limwongse C, Sukpanichnant S, Ruangvutilert P, Kanjanakorn C, Glomglao W, Sirikong M, Utto W, Tanphaichitr VS. Problems in determining thalassemia carrier status in a program for prevention and control of severe thalassemia syndromes: a lesson from Thailand. Clin Chem Lab Med 2013;51(8):1605-1614.
90. Musallam KM, Cappellini MD, Coates TD, Kuo KHM, Al-Samkari H, Sheth S, Viprakasit V, Taher AT. Alphalpha-thalassemia: A practical overview. Blood Rev 2024;64:101165.

02 BLOOD TRANSFUSION

Authors: **Farrukh Shah[1], Erica Wood[2], Aurelio Maggio[3]**

[1.] Sheikh Khalifa Medical City, Abu Dhabi, United Arab Emirates

[2.] Transfusion Research Unit, Public Health and Preventive Medicine, Monash University, Melbourne; Department of Haematology, Monash Health, Clayton, Australia

[3.] Campus of Haematology Franco and Piera Cutino, UOC Ematologia e Malattie Rare, Centro ERN, Centro HUB for Thalassemia and Haemoglobinipathy, AOOR Villa Sofia-V. Cervello, Palermo, Italy

Competing interest: FS reports advisory boards and lecture fees from Novartis (STAND clinical trial adjudication committee chair), Bristol Meyers Squibb (Celgene Cop), Vertex Pharmaceuticals, Bluebird Bio, Chiesi, Roche, Silence Therapeutics, Agios Pharmaceuticals (IDMC committee RISE UP, ENERGIZE, studies, and ACTIVATE studies), Pharmacosmos (IDMC committee) and Pfizer. EW reports research funding to institution from AbbVie, Amgen, AstraZeneca, BeiGene, Celgene, CSL Behring, Gilead, Janssen, Novartis, Roche, Sanofi, Sobi and Takeda. AM reports being a member of advisory boards for Novartis, Bristol Meyers Squibb, Bluebird Bio and Vertex Pharmaceuticals; and research funding and travel support from Agios Pharmaceuticals. All competing interests are outside the present work.

1. INTRODUCTION

There is no doubt that advances in transfusion practices over the years have transformed outcomes and improved survival in patients with transfusion-dependent β-thalassemia (TDT) [1]. Still, regional variations in transfusion protocols continue to exist, and a considerable number of patients do not have access to adequate transfusions to sustain target haemoglobin levels in many parts of the world [2, 3]. This Chapter provides a holistic review of key topics including aims of blood transfusion and practical considerations when the decision to administer transfusions is made. It also features best practices in blood collection, processing, and storage. Adverse events, beyond iron overload (see Chapter 3), will also be discussed.

2. AIMS OF BLOOD TRANSFUSION

Regular transfusions are currently the mainstay of therapy and required life-long for TDT patients. Providing maximum benefit whilst minimising potential harms is the primary aim. This can be delivered by prioritising the provision of an effective transfusion regimen that results in good growth and development, good energy levels, and sufficient suppression of intra- and extramedullary haematopoiesis. A safe transfusion process will:

- Use a product that is collected, tested, selected, issued, and administered according to established quality and safety regulations and guidance.
- Be administered to patients who have provided fully informed consent and are part of decision-making in their transfusion care.
- Be administered by staff trained in blood transfusion.
- Be performed in an accredited system where adverse reactions and procedural incidents are identified, managed appropriately, and prevented wherever possible (i.e., with a good haemovigilance structure).

3. PRACTICAL CONSIDERATIONS FOR TRANSFUSION THERAPY

3.1. Criteria for initiating transfusion therapy

The decision to initiate a long-term regular transfusion regimen should be driven by the current clinical phenotype of the patient, anticipated short- and longer-term outcomes, and in discussion with the patient and/or parents, as appropriate. The decision should primarily be based on evidence of failure to thrive as manifested by poor feeding, a drop in growth velocity and/or a failure to gain weight, the severity of the anaemia in repeated measurements and in the absence of intercurrent infections, the level of ineffective erythropoiesis and the development (ideally, prevention) of bony changes.

If available, knowledge of the patient's specific diagnosis, including molecular genetics, can help inform the decision to start regular transfusions but it should not be the primary indicator. Once a regular programme of transfusion is started, to ensure good outcomes, it is important that the pretransfusion haemoglobin target is maintained at an optimal threshold.

A transient drop in haemoglobin due to an intercurrent infection should not drive the initiation of a regular transfusion programme and repeat assessment of clinical parameters is important after a single top-up transfusion to ensure decision making is appropriate.

For deciding when to transfuse on a regular basis, the following should be included in the investigations:

- Confirmed diagnosis of thalassaemia
- Laboratory criteria:
    - Haemoglobin level <7 g/dL on two occasions >2 weeks apart in asymptomatic patients with a β^0 β^0 or β^0 β^+ any other genotype known to cause TDT (excluding all other contributory causes such as infections), AND/OR
- Clinical criteria irrespective of haemoglobin level:
    - Significant symptoms of anaemia
    - Poor growth or failure to thrive
    - Complications from excessive intramedullary haematopoiesis such as pathological fractures and facial changes
    - Clinically significant extramedullary haematopoiesis

Most patients with severe genotypes will start on regular transfusion therapy, where this is available, within the first two years of life. Some patients with non-transfusion-dependent β-thalassemia (NTDT) may only need sporadic transfusions for intercurrent illnesses, whilst others may need a short time on regular transfusions to help initiate or complete pubertal growth and development, or a more regular transfusion programme long-term to manage symptomatic anaemia or complications such as extramedullary haematopoiesis or pulmonary hypertension [4]. A recent cohort study from Italy including 305 adults with NTDT (β-thalassemia intermedia), the rate of 'phenoconversion' to TDT was 13.8% over 10 years. Conversion was primarily driven by haemoglobin level (<8.5 g/dL) and comorbidity [5]. The authors also attributed this to higher 'tendency' of treating clinicians to introduce regular transfusions in NTDT patients, owing to the growing evidence on the morbidity and mortality risks associated with untreated anaemia

(especially, haemoglobin <10 g/dL) [6, 7], and improved morbidity and mortality outcomes in patients receiving regular transfusions [8, 9].

In patients with haemoglobin E/β-thalassaemia, some of whom may be transfusion independent despite low haemoglobin levels, a more stringent observation for failure to thrive and monitoring for hepatosplenomegaly should be undertaken, particularly because many patients with haemoglobin E/β-thalassaemia remain clinically well with no relevant symptoms even with haemoglobin values in the 6-7 g/dL range [10, 11]. Once a decision is made to start regular transfusions for patients with haemoglobin E/β-thalassaemia they should be managed in the same way as all transfusion-dependent patients, particularly if transfusion was initiated for complications such as extramedullary haematopoiesis. A lower pretransfusion haemoglobin threshold may be tolerated in patients with haemoglobin E/β-thalassaemia, but careful monitoring and assessment is important to ensure that complications relating to the anaemia are identified early and corrective action taken [10, 12, 13]. Progressive increase of splenomegaly suggests maintaining a higher pretransfusion haemoglobin level with an increase of blood transfusions.

3.2. Patient blood management, clinical decision-making, and informed consent

3.2.1. What is patient blood management?

Various definitions exist for patient blood management (PBM), but all emphasise the centrality of the patient in terms of decision-making around whether transfusion support is required, consideration of alternatives to minimise transfusion requirements (where these alternatives exist and are accessible), optimisation of the patient's own blood (such as red cell mass), and personalised care delivery to meet the individual patient's needs. While much of the published literature in PBM focuses on transfusion support in elective surgery, the principles apply equally to all patients who may need transfusion support, including patients with TDT. The International Society of Blood Transfusion (ISBT) defines PBM as "an evidence-based, multidisciplinary approach aimed at optimising the care of patients who might need transfusion. It puts the patient at the heart of decisions made around blood transfusion, promoting appropriate use of blood and blood components and the timely use of alternatives where available. PBM represents an international initiative in best practice for transfusion medicine." Implementation of PBM is endorsed as a policy priority by the World Health Organization (WHO) [14]. A range of resources for setting up a PBM programme is available from the ISBT and WHO websites.

3.2.2. How does patient blood management apply in the setting of transfusion-dependent β-thalassaemia?

PBM is patient-focused, with the aim of meeting the needs of the patient and family and aiming to develop and deliver a personalised care plan in collaboration with the patient and family. PBM is ideally suited to support patients with TDT, many of whom are currently anticipating a lifelong requirement for transfusion support, although new therapies now available in some countries or in clinical trials may substantially change the need for transfusion in the coming years for many patients (see Chapter 15 and Chapter 16).

A programme of regular transfusions requires major commitment from patients and families, including frequent appointments for collection of pretransfusion samples as well as administration of blood, and monitoring for (and prevention of, wherever possible) short- and

longer-term complications. Transfusion carries substantial costs (either to the patient, or the community, or both, depending on the health system) and risks – not just limited to the possibility of transfusion-transmitted infections. However, in the past, patients or parents have often been 'told' what will happen in terms of transfusion, based on why transfusion support might be needed (for example, to correct anaemia and suppress extramedullary haematopoiesis), without asking for their input or considering their views on how the transfusions might be best delivered, or how to measure the impact of transfusion on clinical outcomes, including quality of life and functional outcomes (such as improving symptoms of anaemia).

Using the principles of PBM, patients and families should receive not just a clear explanation of why transfusion is being recommended, but what [if any] alternatives are available; what benefits, risks and costs might be involved with the various alternatives; how the process will work; and how to respond if an adverse event is suspected. There should be documentation of the discussion and informed consent/agreement to proceed from the medical decision-maker (usually a parent for young children). A personalised transfusion plan should be developed and documented, which should be reviewed periodically and updated as needed, incorporating feedback from patients and families.

3.3. Product selection for transfusion

As transfusion is lifesaving and currently will typically be life-long, it is important to reduce the risk of alloimmunisation to red cell antigens; hence, prior to the first transfusion it is recommended that detailed red cell phenotype and/or genotype is obtained, as available. The following should be undertaken wherever possible:

- Before embarking on transfusion therapy, patients should have extended red cell antigen typing that includes at least ABO typing, and Rh C, c, D, E, e, and Kell (K, k) although preferably a full red cell phenotype/genotype panel).
- If the patient is already transfused, antigen typing can be performed using molecular rather than serological testing.
- All patients with thalassaemia should be transfused with ABO and Rh (C, c, D, E, e) and K compatible blood to avoid alloimmunisation against these antigens.
- There should be a valid group and antibody screen available on each occasion prior to transfusion being administered.

In regions where there is significant heterogeneity between the donor and recipient populations, it may be appropriate to extend antigen matching in line with specific local population requirements [15-18].

3.4. Maintenance and adjustment of transfusions

The recommended target pretransfusion haemoglobin level for most patients is 9.5-10.5 g/dL. This is typically achieved by administering red cells every 2 to 5 weeks. In most patients, this transfusion regimen promotes normal growth, allows normal physical activities, adequately suppresses bone marrow activity, and minimises transfusional iron accumulation [19, 20]. Recently, a large retrospective cohort study from Italy looked at the association of pretransfusion haemoglobin level (period average) and mortality in 779 TDT patients followed for up to 10

years [21]. There was a significant and steady decrease in thalassaemia-related mortality with increasing pretransfusion haemoglobin levels, and a threshold of <9.3 g/dL was the best predictor of long-term mortality. Multivariate regression analyses indicated an incremental protective effect of increasing pretransfusion haemoglobin level categories (by 0.5 g/dL) compared to a pretransfusion haemoglobin level <9 g/dL, with significant associations noted with levels ≥9.5 g/dL. The association was only observed in patients with adequate iron control [21].

A higher target pretransfusion haemoglobin level of 10-11 g/dL (or as high as practicable) may be appropriate for patients with heart disease including pulmonary hypertension, clinically significant extramedullary haematopoiesis, or other medical conditions, and for those patients who do not achieve adequate suppression of bone marrow activity at the lower haemoglobin level [22-24]. Although shorter intervals between transfusions may reduce overall blood requirements, the choice of interval must consider other factors such as the patient's school or work schedule and other lifestyle factors. The strict control of iron loading in these cases is mandatory since such regimen is associated with an increase in iron intake and burden.

3.5. Volume to be transfused

The volume of blood to be infused will be determined by the number/volume of red cells per unit and the unit haematocrit. These relate to the acceptable haematocrit of the donors, the volume collected at donation, whether it is whole blood or red cells, and the type of anticoagulant used (Table 1). In adults, typically a certain number of units (e.g., two or three) rather than a particular volume of blood is ordered. In children, the recommendation is to transfuse on a volume basis to achieve a target haemoglobin/haematocrit and avoid under- or over-transfusion. For children or for others who may need a specific volume, the following calculation is generally used: **[(Desired – Actual Haemoglobin in g/dL) x Weight in kg x 3.0] / Haematocrit of Red Cell Unit = mL to be transfused [25].**

As an example, using the guidance in Table 1, in order to raise haemoglobin level by 4 g/dL in a patient weighing 40 kg and receiving blood with a haematocrit of 60%, 800 mL would be required. This calculation assumes a total blood volume of 70 mL/kg body weight.

Post-transfusion haemoglobin can be measured when evaluating the effects of changes in the transfusion regimen, the degree of hypersplenism, or unexplained changes in response to transfusion.

To achieve a pretransfusion haemoglobin of 9.5-10.5 g/dL, it is often usual to aim for a post-transfusion haemoglobin of 13-15 g/dL. This overall approach to transfusion has been shown to promote normal growth, to allow normal physical activities, to adequately suppress bone marrow activity and to minimise transfusional iron accumulation in most patients [19].

Table 1. *Guideline for choosing how much blood to transfuse.*

		HAEMATOCRIT OF DONOR RED CELLS			
		50%	**60%**	**75%**	**80%**
TARGET INCREASE IN HAEMOGLOBIN LEVEL	2 g/dL	12 mL/kg	10 mL/kg	8 mL/kg	7.5 mL/kg
	3 g/dL	18 mL/kg	15 mL/kg	12 mL/kg	11.2 mL/kg
	4 g/dL	24 mL/kg	20 mL/kg	16 mL/kg	15 mL/kg

3.6. Rate of transfusion

The optimal rate of transfusion administration has not been subjected to a prospective study and in practice depends on both clinical factors and on the type of component issued. For example, leucodepletion is widely used worldwide with the intention to decrease febrile non-haemolytic reactions and other consequences, but a unit of leucodepleted red cells is typically a smaller volume than non-leucodepleted red cells. Other factors include the use of red cell additive solutions (which will influence the unit haematocrit), the patient status, and the starting and desired haemoglobin.

Children are generally transfused at a rate of 5 mL/kg/hour, hence a stable, non-febrile, non-bleeding child weighing 50 kg can receive 250 mL of red cells in one hour. In adults, standard transfusion practice has generally recommended transfusion of a unit of red cells (average volume 260 mL) over 90 minutes, but data from two thalassaemia centres in London suggests that in carefully selected adults >45 kg, free of cardiac disease, transfusion of one unit of red cells over one hour is safe and three units can be administered safely at this rate. Patients should be clinically reviewed between each unit of red cells. Particular caution should be taken with smaller patients, patients with cardiac iron overload and a low cardiac ejection fraction, or those with a very low initial haemoglobin level.

3.7. Specific patient groups

3.7.1. Very low haemoglobin at presentation

Patients who present with severe anaemia need to be transfused with great care to avoid precipitating heart failure. If this is an initial presentation, then cardiovascular compensation is already present and a slow increase in haemoglobin will not cause any harm. Standard practice is to give around 10-15 mL/kg until a 2-3 g/dL increase in haemoglobin is attained and then to give a top-up transfusion a few days later to bring the haemoglobin to an acceptable baseline.

For example, a 10 kg child presenting with a haemoglobin of 2.5 g/dL may be given red cells at 15 mL/kg (150 mL) on day of presentation and then another 5-10 mL/kg 24 to 48 hours later. The first transfusion will improve the haemoglobin to close to 5.5 g/dL and then a further transfusion given 24-48 hours later should improve the haemoglobin to around 7-8 g/dL. The following week, a top-up transfusion of 10 mL/kg may be safely given to bring the haemoglobin up to 8-9 g/dL. Once the new baseline haemoglobin is reached, assessment should be performed to see if the child will need to be on a long-term transfusion programme or if this was due to an intercurrent illness, and will guide the next steps.

Transfusion in acutely unwell children with severe anaemia should always be done with specialist support and careful assessment with volumes of >20 mL/kg avoided in the acutely febrile child [26, 27].

In the adult setting, severe anaemia is more likely to be seen in patients with β-thalassaemia intermedia during an intercurrent illness. They are more likely to present earlier with less severe anaemia; however, they are also more likely to have some degree of cardiovascular compromise as the anaemia will usually have developed over a shorter period. Full assessment and appropriate support of cardiovascular aspects need to be provided, and transfusion should proceed in a stepwise manner with small volumes (e.g., 1 or 2 units of red cells) and assessment and decision for next transfusion based on how the patient is doing clinically.

3.7.2. Pregnant patients

In pregnancy (see Chapter 7), in the first trimester there is expansion of the physiological plasma volume and cardiac output increases continuously during pregnancy, peaking at around 24 weeks gestation at around 40% or higher above pre-pregnancy cardiac output. Twin pregnancies are associated with an additional 15% higher cardiac output [28]. Maternal anaemia is associated with an increased risk of intrauterine growth retardation and premature delivery [29-31]. Maintaining pretransfusion haemoglobin at 10 g/dL is important in reducing both maternal cardiovascular stress and improving foetal outcomes [29, 32].

In NTDT patients, anaemia can become problematic during pregnancy. The decision to transfuse during a pregnancy needs to be highly individualised based on maternal symptoms, foetal developmental milestones, previous transfusions and complications/antibodies. If transfusion is started during the pregnancy for symptomatic maternal anaemia or IUGR then transfusion should continue to ensure optimal benefit is provided with pretransfusion thresholds similar to the transfusion-dependent patients. In patients who maintain a haemoglobin above 8 g/dL during pregnancy without foetal complications, then delivery can safely proceed without transfusion. In patients who have haemoglobin below 8 g/dL, consideration can be given to the patient having a top-up transfusion in preparation for delivery at around 37-38 weeks of gestation. In all NTDT patients, crossmatched blood should be available for delivery.

3.7.3. Cardiac failure

These patients will be unwell with symptoms from heart failure; however, anaemia must be managed as this will make the cardiac compromise worse if allowed to progress. Patients should be transfused and pretransfusion haemoglobin maintained at 10-11 g/dL or higher in these patients to ensure good oxygenation of the myocardium. Iron overload must be strictly monitored in this cohort of patients.

3.7.4. Haemoglobin E/β-thalassaemia

The transfusion schedule outlined above, and target haemoglobin levels have been shown to minimise iron loading, suppress bone marrow expansion, and improve survival in Italian patients with β-thalassemia major [19-21]. The optimal regimen for patients with (severe) haemoglobin E/β-thalassaemia has not been studied in such detail; hence, the same approach as for other TDT patients has traditionally been followed. It is important to note that the phenotypic variation in haemoglobin E/β-thalassaemia is wide, and recent publications support a more individualised

approach for decision-making around target haemoglobin levels. Haemoglobin targets should be primarily guided by a patient's symptoms and quality of life issues rather than the haemoglobin level itself.

3.7.5. Non-transfusion-dependent β-thalassaemia

As previously mentioned, NTDT patients (including those with β-thalassaemia intermedia or mild-moderate haemoglobin E/β-thalassaemia) may require either a short-term period of transfusion (e.g., to support transition through puberty) or longer-term transfusion to manage symptomatic anaemia particularly or specific morbidities in older patients [4]. In patients who are being transfused for symptomatic anaemia, transfusion thresholds may be adjusted to support improvement in symptoms and quality of life rather than a specific haemoglobin target. In patients being transfused to manage complications related to anaemia such as extramedullary haematopoiesis, then transfusion thresholds should be as per TDT recommendations. Patients needing transfusion to support growth and transition through puberty should be managed as per TDT target haemoglobin levels until puberty is complete and then a planned reduction in transfusion volume and frequency undertaken; however, if the patient does not tolerate the anaemia after being on a transfusion programme for several years, then they can continue regular transfusions and be managed as per TDT guidelines for all aspects of their care.

3.8. Transfusional iron loading

There are national and international guidelines regarding the keeping of transfusion records (e.g., European Union Commission Directives 2004/33/EC, 2005/61/EC, 2005/62/EC, 2009/135/EC, 2014/110/EU, (EU) 2016/1214, 2011/38/EU, 2002/98/EC; available at: https://eur-lex.europa.eu/eli/dir/). Historically, for patients with β-thalassaemia, additional records would be kept. These include the volume or weight of the administered units, the haematocrit of the units or the average haematocrit of units with similar anticoagulant-preservative solutions, and the patient's current weight at the time of the transfusion (especially for children). With this information, it is possible to calculate the annual blood requirements as volume of transfused blood or pure red cells (haematocrit 100%) per kg of body weight. The latter (pure red cells per kg of body weight) when multiplied by 1.08, the estimated amount of iron per mL of red cells yields an approximate value for transfusional iron that the patient receives per kg of body weight in a year. Table 2 shows a detailed example of how the daily rate of iron loading (mg/kg/day) is calculated. The rate of transfusional iron loading may be very important in choosing the appropriate dose of an iron chelator among other indicators of iron overload (see Chapter 3). For example, the recommended dose of the chelator deferasirox is based in part on the daily or annual rate of transfusional iron loading. Nowadays, this level of calculation is not often done, although it may be useful in situations where there has been a change in blood requirement, development of hypersplenism, or where access to accurate magnetic resonance measurements of iron loading is poor.

Table 2. *Calculation of annual blood requirements and transfusional iron loading (example).*

Patient and transfusion details	• Weight: 40 kg • Transfusion amount and schedule: 600 mL every 4 weeks • Average haematocrit of transfused red cells: 60%
Annual blood requirement	• 13 transfusions x 600 mL/40 kg = 195 mL/kg per year
Annual pure red cell requirement	• 195 mL/kg per year x 60% (average haematocrit) = 117 mL/kg per year
Annual transfusional iron loading	• 117 mL/kg per year x 1.08 mg iron per mL pure red cells = 126 mg iron per year
Daily transfusional iron loading	• 126 mg iron per year/365 days = 0.34 mg/kg

In adults where the volume of blood administered is often not collected and transfusion recorded as a unit of blood transfused, an alternative formula can be used to calculate the rate of iron loading (mg/kg/day): **[Units of Blood Transfused (annum) x 200] / [Patient Weight in kg x 365]**. Recent guidelines from the British Society for Haematology (BSH) recommend this is used for ensuring that if patients are failing to respond to chelation, the dose of chelation / regime is adjusted to ensure both iron input and iron chelator doses are synchronous.

4. BLOOD: FROM COLLECTION TO ADMINISTRATION

4.1. Haemovigilance

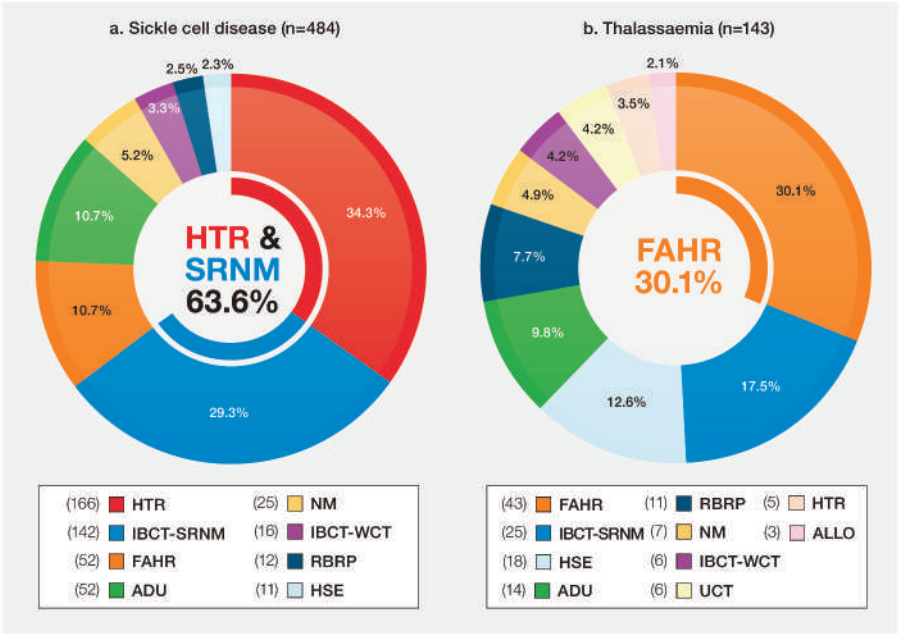
Haemovigilance is the set of surveillance procedures covering the entire blood transfusion chain, from the donation and processing of blood and its components, through to their provision and transfusion to patients, and including their follow-up.

It includes the monitoring, reporting, investigation and analysis of adverse events related to the donation, processing, and transfusion of blood, and taking action to prevent their occurrence or recurrence. The reporting systems play a fundamental role in enhancing patient safety by learning from failures and then putting in place system changes to prevent them in the future [33] (https://ihn-org.com/about/haemovigilance/). The haemovigilance system should involve all relevant stakeholders and should be coordinated between the blood transfusion service, hospital clinical staff and transfusion laboratories, hospital transfusion committees, the national regulatory agency, and national health authorities. The resulting modifications to transfusion policies, standards and guidelines, as well as improvements to processes in blood services and transfusion practices in hospitals, lead to improved patient safety [34].

Good haemovigilance is key to the delivery of safe and effective transfusion in any setting and must be in place in the delivery of blood transfusion to those with TDT. The Serious Hazards of Transfusion (SHOT) haemovigilance scheme in the United Kingdom has been collecting data

specifically on risks for patients with haemoglobinopathies and published its first 5-year cumulative dataset for this patient group in 2014. This highlighted the risk of serious outcomes in multi-transfused patients with ABO incompatible transfusions, major morbidity, alloimmunisation, and death all occurring. Subsequently, each annual SHOT report has a section on haemoglobinopathy patients and hazards related to transfusion. All existing reports have highlighted the critical importance of communication with the transfusion laboratory that the patient has a haemoglobinopathy to ensure that appropriate phenotype matching is undertaken, and historical antibodies are reviewed. Issues with informed consent and positive patient identification remain a cause for preventable morbidity with patients still having incorrect blood transfused either due to specific requirements not being met (due to poor communication with the laboratory) or wrong unit transfused due to failure of the positive patient identification process. Cumulative data on transfusion hazards in haemoglobinopathy patients were summarised in the 2023 report and although febrile, allergic, or hypotensive reactions (FAHR) are the primary reports to SHOT, incorrect blood component transfused (IBCT) remains a major cause of morbidity either due to specific requirements not being met or wrong component being transfused, comprising 21.7% of reports in thalassaemia patients (Figure 1) [35].

Figure 1.*Cumulative data for adverse transfusion events in patients with haemoglobin disorders 2010 to 2023. (a) Sickle cell disease (n=484). (b) Thalassaemia (n=143). Reproduced with permission from [35].*



ADU=avoidable, delayed or under or overtransfusion; ALLO=alloimmunisation; FAHR=febrile, allergic or hypotensive reactions; HTR=haemolytic transfusion reactions; IBCT-SRNM=incorrect blood component transfused-specific requirements not met; IBCT-WCT=IBCT-wrong component transfused; NM=near miss; TACO=transfusion-associated circulatory overload; TAD=transfusion-associated dyspnoea; TTI=transfusion-transmitted infection; UCT=uncommon complications of transfusion

Categories with 2 or fewer reports are not included in the figures

IBCT incidences are avoidable harm and can be reduced by positive patient identification both at time of pretransfusion blood sampling and at initiation of transfusion. Positive patient identification should always be done with the patient or accompanying adult for children. Patients do not always understand why the identification process is so critical for safety, assuming - as do many staff - that the main risks of transfusion are related to the safety of the product; however, when patients and family members are properly informed of the real risks of transfusion, they can be more active participants in improving the safety of the process.

4.2. Blood donation and testing for transfusion-transmitted infections

To safeguard the health of patients with TDT, blood should be obtained from carefully selected, low-risk, voluntary, non-remunerated blood donors and should be collected, processed, stored, and distributed by dedicated, blood transfusion centres with established quality assurance systems in place. National blood policies and good manufacturing processes help to ensure high-quality products are available when and where patients need them, minimise wastage of blood products and other resources, and protect the health of community blood donors.

Avoidance of transfusion-transmitted infections (TTI) is a high priority, as patients with repeated donor exposure are at greatest risk for exposure to, and consequences of, such infections. Adherence to the directives and guidelines of national or international regulatory authorities or professional societies, help minimise the risk of TTI. WHO recommends, at a minimum, donation screening for human immunodeficiency viruses (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), and syphilis. In many countries, screening for other infectious diseases such as human T-cell lymphotropic virus (HTLV) type I/II, malaria, toxoplasma, hepatitis A, hepatitis E, West Nile virus, and Chagas disease is also conducted. Further updated information and standards are available through websites of the European Union, WHO, and Association for the Advancement of Bood and Biotherapies (AABB).

4.3. Blood component specification and preparation

The WHO has identified the need for standardised manufacturing processes, good clinical and manufacturing practices (GCP and GMP), cold chain management, proper labelling, and traceability from donor to patient ("vein–to-vein") and haemovigilance systems to ensure consistent, high-quality, and reliable blood components suitable for transfusion to patients.

4.3.1. Leucodepletion

Reduction to 1 x106/L or less leucocytes per unit is considered the critical threshold for eliminating adverse reactions attributed to contaminating white cells (Table 3) [36]. In countries where there is universal prestorage leucodepletion, the incidence of febrile non-haemolytic transfusion reactions, HLA sensitisation, and transfusion transmitted infections are lower [37]. Methods for leucoreduction include:

- Pre-storage filtration of whole blood is the preferred method for leucoreduction. This method of leucocyte removal offers high efficiency filtration and provides consistently low residual leucocytes in the processed red cells and high red cell recovery. Red cell components are obtained by centrifugation of the leucodepleted whole blood and removal of donor plasma.
- Pretransfusion, laboratory filtration refers to the filtration at the blood bank laboratory of red cells, prepared from donor whole blood.

- Bedside filtration refers to the red cell unit that is filtered at the bedside at the time of transfusion. This method does not allow optimal quality control because the techniques used for bedside filtration may be highly variable. It is now not used where pre-storage leucoreduction is available.

Table 3. *Known adverse effects of leucocytes in blood products.*

Reaction	Causative agent
Febrile non-haemolytic transfusion reaction	HLA-antibodies in patients, cytokine reactions produced by donor leucocytes
HLA-alloimmunisation	HLA-alloimmunisation in response to exposure to donor HLA antigens
Transfusion-transmitted infections	Cell-associated infectious agents such as cytomegalovirus

4.4. New technologies

4.4.1. Pathogen inactivation systems

These are being developed but are currently only in clinical trials and none are routinely being deployed for red cells by blood services currently. It is possible that these may become part of standard manufacturing of products such as plasma and platelets. There is less clinical trial evidence for benefit/red cell survival data for red blood cell transfusions and the cost per unit of red cells would be significantly higher than standard units of red cells that are leucodepleted and tested according to current standards and guidelines.

4.4.2. Manufactured red cells

There has been a great deal of interest in generating red cells from immortalised cell lines and therefore allowing patients with rare blood groups to have closely matched cells, as well as the potential for development of a universal 'red cell' that can be used for all transfusions. Currently, a proof-of-principle, phase 1 clinical trial (REcovery and survival of STem cell Originated REd cells, RESTORE; ISRCTN42886452) run by the United Kingdom's National Health Service Blood and Transplant (NHSBT) using manufactured red cells is only able to transfuse 5-10 mL of red cells produced from a manufacturing process and has been shown to be safe. However, the technology needs a great deal of enhancement to produce the volume of red cells needed for a blood transfusion and will also need the development of a number of immortalised cell lines from suitable donors with rare blood groups.

4.4.3. Universal red cells

Many blood services globally are interested in developing this, but it is likely that universal plasma and platelets will be available many years before technology is sufficiently advanced and available to develop a universal red cell product.

4.5. Blood products for special patient populations

4.5.1. Washed red cells

These may be beneficial for patients with TDT who have repeated severe allergic transfusion reactions or for patients with immunoglobulin A (IgA) deficiency, in which the recipient's pre-formed antibody to IgA may result in an anaphylactic reaction. Washing of the donor product removes plasma proteins that constitute the target of antibodies in the recipient. Washing may be accomplished using manual or automated techniques. Washed red cells that are not suspended in storage solution must be transfused within 24 hours, and this shorter shelf life creates the possibility of wastage if patients are not available for transfusion at the time the blood is prepared. Suspension in SAGM after washing allows for shelf life as long as 14 days if a closed circuit is used.

Washing alone usually does not result in adequate leucocyte reduction and should not be used as a substitute for leucoreduction. Instead, washing should be used in conjunction with filtration. In addition, washing of red cell units removes some erythrocytes from the transfusion product, and it is therefore valuable to monitor post-transfusion haemoglobin levels to ensure attainment of the target haemoglobin level.

4.5.2. Cryopreserved (frozen) red cells

This is the component derived from whole blood in which red cells are frozen, preferably within 7 days of collection and using a cryopreservant. It can be stored at -60°C to -80°C in an electrical freezer, when a high-glycerol method is used, or alternatively at -140°C to -150°C in vapor phase liquid nitrogen, when a low-glycerol method is used.

This product is used to maintain a supply of rare donor units for patients who have unusual red cell antibodies or who are missing common red cell antigens. Their shelf life of 1-7 days depends on whether they were washed in an open or closed system and whether they were re-suspended in SAGM. The shorter shelf life again creates the possibility of wastage. Approximately, 20% of the donor cells are lost in the washing after the freezing process. There is no good evidence about how long these can be stored although in NHS Blood and Transplant they are now kept for 30 years.

4.5.3. Red cells obtained by donor apheresis

This refers to the collection of two units of red cells from the same donor, usually used for transfusion of one patient. The reduction of donor exposures may decrease the risk of transmission of infections and developing alloantibodies and other transfusion-related complications. This approach creates significant logistical problems as the donors need higher haematocrits, can attend less frequently for donation, and the collections are performed using apheresis techniques. In addition, the collection of two separate bags and other factors may create an organisational challenge in ensuring that both units go to the same patient.

4.6. Storage of donor red cell units

The anticoagulant preservative solutions used in blood collection have been developed to prevent coagulation and to permit storage of red cells without significant loss of metabolic integrity. All the currently used solutions contain sodium citrate, citric acid and glucose, and

some of them also contain adenine, guanosine, and phosphate (e.g., citrate phosphate dextrose adenine [CPDA-1]]). As shown in Table 4, the introduction of additives solutions (AS) such as AS-1, AS-3, and AS-5 presently permits storage of red cells for up to 42 days.

The maximum duration of storage, as noted on each unit, varies with the type of preparation. However, all the storage solutions should achieve a mean 24-hour post-transfusion survival of no less than 75% of the transfused red cells. The actual half-life of donor red cells after transfusion is not routinely tested for different additives and for different lengths of storage.

The haemoglobin oxygen release function which is extremely important in TDT is impaired during normal storage due to progressive loss of 2,3-biphosphoglycerate (2,3-BPG, previously known as 2,3-diphosphoglycerate, 2,3-DPG). However, the rapid repletion of 2,3-BPG after transfusion generally compensates for the loss of function during storage.

Table 4. *Storage time for anticoagulant preservative solutions with and without additive solution.*

Solution type	Shelf life (days)
CPD	21
CP2D	21
CPDA-1	35
CPD, CP2D, or CPDA-1 with AS-1 (adsol), AS-3 (nutricel), or AS-5	35-42

Abbreviations: CPD, citrate phosphate dextrose; CP2D, double dextrose; CPDA-1 citrate phosphate dextrose adenine; AS, additive solution.

4.7. Age and type of product to be transfused

Many centres have historically used blood that is less than 2 weeks old for TDT patients. In Italy for example, the National Transfusion Centre recommends using fresh blood for TDT patients, defined as blood that is less than 10 days old. During the COVID-19 pandemic there was a reduction in blood donor availability and many patients were transfused red cells that were greater than 14 days old with no reports of major complications. The development of red cell antibodies has always been a major concern for patients, blood services, and health authorities, but the prioritisation of fresh blood over better phenotype matching may increase the risk of developing new red cell antibodies [15].

In countries such as the United Kingdom, there has been a change in prioritising a product for transfusion to patients with haemoglobinopathies. The recommendations are as follows: 1) Optimally match for red cell blood groups and existing antibodies, 2) choose a unit based on best match and then, 3) based on age of the unit.

This essentially has resulted in patients being given slightly older but better matched red cells. This is an important patient safety aspect especially as blood services shift to genotype testing

of donors and patients and this increases the likelihood of better matched blood with lower rates of alloimmunisation. In resource-limited settings or areas without highly regulated blood banks, units less than 14 days old should be used for patients if phenotype matching is not available. Patients should still be as optimally matched for Rh and Kell blood groups, if possible, with a better matched unit prioritised over the age of the red cell unit.

4.8. Compatibility testing

Development of one or more specific red cell antibodies (alloimmunisation) is an important complication of chronic transfusion therapy [38-40]. However, the prevalence of alloantibodies varies widely among centres and may be related to the genetic homogeneity of the population, strategies for antigen matching, and other factors. It is important to monitor patients carefully for the development of new antibodies. Anti-E, anti-C and anti-Kell alloantibodies are the most common. However, 5-10% of patients develop alloantibodies against other erythrocyte antigens or develop warm or cold allo- and/or autoantibodies of unidentified specificity. It may be appropriate to extend antigen matching in line with specific local population requirements [16].

Most blood banks currently perform a screen for new antibodies and an indirect antiglobulin test (IAT) crossmatch before each transfusion. Where patients are not pregnant and have no history of alloimmunisation, an electronic issue may be used. Here, in eligible patients with a negative antibody screen, blood is issued without an IAT crossmatch being performed. This is only appropriate in blood banks that adhere to strict regulations regarding computer systems, sample labelling, and other critical issues [41]. Using either approach, new clinically significant antibodies must be identified so that blood lacking the corresponding antigen(s) is selected.

A complete and detailed record of antigen typing, current, and historical red cell antibodies and transfusion reactions must be maintained for each patient and should be readily available if the patient is transfused at a different centre. Transfusion of blood from first-degree relatives should be avoided because of the risks of transfusion-associated graft-versus-host disease (unless irradiation of the blood components is performed) and the risk of developing antibodies that might adversely affect the outcome of a later stem cell transplant. The length of time between the sample acquisition and antibody screen and the transfusion of blood for regularly transfused patients is usually 72 hours but may be as long as one week in centres with full Rh and Kell antigen matching in patients who are regularly transfused [42]. In patients who are irregularly transfused or start transfusion later in life, the risk of alloimmunisation may be greater (Table 5) [40, 43], so t the interval between sample for antibody screen and transfusion should preferably not be longer than 72 hours.

Table 5. *Age at first transfusion and alloimmunisation in thalassaemia.*

Age at first transfusion	Alloimmunisation rate	Reference
<1 years	7.7%	[43]
≥1 years	27.9%	
<3 years	20.9%	[40]
≥3years	47.5%	

4.9. Transfusion and the spleen

Average transfusion requirements are about 30% higher in non-splenectomised (0.43 mg/kg/day) than splenectomised TDT patients (0.33 mg/kg/day) [44]. In a study of TDT patients who required more than 250 mL of red cells/kg/year, splenectomy decreased the annual iron loading by an average of 39% [45]. With modern chelation regimes, this is seldom a justification for splenectomy unless blood transfusion rates increase into unmanageable ranges, in the context of an enlarging spleen. Hypertransfusion decreases the rate of splenic enlargement [46] and may diminish the extent to which the spleen contributes to an increased blood transfusion requirement [47] thus preventing the need for a splenectomy.

Specific thresholds of annual transfusion requirements that would lead to consideration of splenectomy are difficult to establish because earlier studies did not specify the haematocrit levels of the transfused blood and because the potential reduction in transfusional iron loading after splenectomy must be weighed against the long-term consequences of asplenia including sepsis, thrombosis, and pulmonary hypertension [48, 49]. With access and tolerance to good chelation regimens, splenectomy should not be needed for iron control.

For patients who are persistently anaemic a trial of hypertransfusion with a pretransfusion haemoglobin target set at >10.5 g/dL may help shrink the spleen/settle the hypersplenism. Splenectomy should only be considered in TDT patients following a multidisciplinary discussion and clear documentation on risks and benefits. Post-splenectomy transfusion plans should be agreed with the patient along with a clear plan on monitoring for pulmonary hypertension, and prevention and management of other post-splenectomy complications.

5. ADVERSE REACTIONS

Beyond transfusional iron overload (see Chapter 3), blood transfusion exposes the patient to a variety of risks and adverse events. Thus, it is vital to continue to improve blood safety and to find ways of reducing transfusion requirements and the number of donor exposures. Adverse events reporting should be embedded within a haemovigilance framework. As mentioned earlier, the SHOT yearly reports from the United Kingdom are an excellent resource for those interested in adverse event rates, and are usually accompanied by a section on haemoglobinopathies [35].

5.1. Febrile non-haemolytic transfusion reactions

Febrile non-haemolytic transfusion reactions were common in past decades, but have been dramatically reduced by leucoreduction, especially pre-storage leucoreduction, which sharply reduces cytokine accumulation and leucocyte alloimmunisation. In the absence of effective leucoreduction, patients experiencing such reactions should be given antipyretics before their transfusions. Since fever may accompany a haemolytic transfusion reaction or the administration of a unit with bacterial contamination, these other causes should always be considered in a patient who develops fever during administration of red cells.

5.2. Allergic reactions

Allergic reactions are usually due to plasma proteins and range from mild to severe. Milder reactions include urticaria, itching, and flushing, and they are generally mediated by IgE. More severe reactions, such as stridor, bronchospasm, hypotension, or other symptoms of anaphylaxis may occur, especially in patients with IgA deficiency and anti-IgA antibodies. Allergic reactions have been reported in patients who receive units of blood from donors who have been exposed to something that the patient is allergic to, for example, a donor eating strawberries prior to donating blood received by someone who is allergic to strawberries.

Occasional mild allergic reactions can often be prevented using antihistamines or corticosteroids before transfusion. Recurrent allergic reactions can be markedly reduced by washing the red cells to remove the donor plasma. Patients with IgA deficiency and severe allergic reactions may require blood from IgA-deficient donors.

5.3. Acute haemolytic reactions

Acute haemolytic reactions begin within minutes or sometimes hours of initiating a transfusion and are typically characterised by the abrupt onset of fever, chills, lower back pain, a sense of impending doom, dyspnoea, haemoglobinuria, and shock. Clinical features may be more difficult to recognise in patients who are unconscious, anaesthetised, or otherwise unable to report their symptoms. These unusual reactions most commonly arise from errors in patient identification or blood typing and compatibility testing. The risk of receiving the wrong blood is greater for a patient with TDT who travels to another centre or is admitted to a hospital not familiar with his/her case and medical history. Haemolytic reactions in these patients can still be avoided by:

- The use of optimal methods for identifying the patients and labelling of the sample when blood is obtained for crossmatch.
- Proper linkage of the sample to the donor unit in the blood bank.
- Adherence to standard protocols for screening for antibodies and carrying out the necessary compatibility protocols.
- Use of multiple patient identifiers before transfusing the blood.

In many transfusion units, two staff members check the identification of the unit and the recipient prior to beginning the transfusion although this has been replaced in some resource-rich countries by validated electronic systems to manage patient and unit identification. If signs and symptoms suggest an acute haemolytic reaction, the transfusion should be stopped immediately, and the blood group, patient, and product identity re-checked urgently. Intravenous

fluids should be administered to maintain intravascular volume. Diuretics may help to preserve renal function. Disseminated intravascular coagulation (DIC) may require additional measures and specialist advice. The blood bank should also be alerted to the possibility of an undetected alloantibody.

5.4. Alloimmunisation

As described earlier, alloimmunisation is a common complication of frequent transfusion therapy, occurring in as many as 10-20% of patients with thalassaemia. Alloimmunisation is more common in children who begin transfusion therapy after 1-3 years of age than in those who begin transfusion therapy earlier. This may reflect the fact that such people are often transfused in an emergency (therefore often not at the hospital where they are known to have thalassaemia, and therefore inadequately matched) or when immune activated (i.e., when they are unwell). Some evidence also suggests that new alloantibodies develop more frequently after splenectomy [38]. The use of extended antigen-matched donor blood is effective in reducing the rate of alloimmunisation [15].

5.5. Delayed transfusion reactions

Delayed transfusion reactions usually manifest 5-14 days after transfusion and are characterised by unexpected levels of post-transfusion anaemia, as well as malaise and jaundice. These reactions may be due to an alloantibody that was not detectable at the time of transfusion or to the development of a new antibody. A sample should be sent to the blood bank to investigate the presence of a new antibody and to repeat crossmatching of the last administered unit(s). This life-threatening condition is more frequently seen in patients who are intermittently transfused such as those with NTDT and sickle cell disease. It may be very severe in patients with sickle cell disease because haemoglobin levels may go down to 3-4 g/dL. Awareness and early diagnosis is critical in ensuring timely interventions and improving outcomes. Treatments include intravenous immunoglobulin, erythropoietin and rituximab. Continuation of transfusion in this condition may further decrease haemoglobin level, and should be avoided when possible [50].

5.6. Autoimmune haemolytic anaemia

Autoimmune haemolytic anaemia is a very serious complication of transfusion therapy that usually, but not always, occurs in patients with alloantibodies [51], although may be unrelated to transfusion. Even red cells from seemingly compatible units (i.e., those units that do not contain the antigen to which there is a known alloantibody) may demonstrate markedly shortened survival, and the haemoglobin concentration may fall well below the usual pretransfusion level because of destruction of both the donor's and the recipient's red cells. The serologic evaluation by the blood bank usually shows an antibody that reacts with a wide range of test cells and fails to show specificity for a particular antigen. Steroids, immunosuppressive drugs, and intravenous immunoglobulins are used for the clinical management of this complication [52, 53].

Autoimmune haemolytic anaemia occurs more frequently in patients who begin transfusion therapy later in life [54], and this complication should be carefully considered before instituting transfusion therapy for teenagers and adults with NTDT.

5.7. Transfusion-related acute lung injury

Transfusion-related acute lung injury (TRALI) is a potentially severe complication that is usually caused by specific anti-neutrophil or anti-HLA antibodies that activate the patient's neutrophils but may also be due to non-antibody-related accumulation of pro-inflammatory mediators during storage of donor red cells [55, 56]. This complication is characterised by dyspnoea, tachycardia, fever, and hypotension during or (typically) within six hours of transfusion. Hypoxaemia is present and the chest radiograph shows bilateral infiltrates typical of pulmonary oedema, although there is no reason to suspect volume overload. Management includes oxygen, administration of steroids, and diuretics, and when needed, assisted ventilation. Response is typically prompt if the situation is recognised and patient is adequately managed, which may require intensive care support, but fatalities do occur.

5.8. Transfusion-associated graft-versus-host disease

Transfusion-associated graft-versus-host disease (TA-GVHD) is caused by viable lymphocytes present in donor red cell units. It is a rare but often fatal complication of transfusion. Immunosuppressed patients are at particular risk, but TA-GVHD may also occur in immunocompetent recipients of red cells from a haploidentical donor such as a family member. TA-GVHD usually occurs within 1-4 weeks of transfusion and is characterised by fever, rash, liver dysfunction, diarrhoea, and pancytopenia due to bone marrow failure. To reduce the risk of TA-GVHD, donated blood from a family member should be avoided, or if used, should always be irradiated before transfusion. Leucodepletion alone is inadequate for the prevention of this complication.

5.9. Transfusion-associated circulatory overload

Transfusion-associated circulatory overload (TACO) may occur in the presence of recognised or unrecognised cardiac dysfunction, or when the rate of transfusion is inappropriately fast, but it has been reported in patients without any recognised pretransfusion susceptibility. Signs and symptoms include dyspnoea and tachycardia, and the clinical examination and chest radiograph show the classic findings of pulmonary oedema. Treatment focuses on volume reduction (for example, via diuresis) and cardiac support, as required.

5.10. Transfusion-transmitted infections

TTI including viruses, bacteria, and parasites remain a major risk worldwide in blood transfusion (see Chapter 8). Blood traceability rules are important and enshrined in law in several countries so that 'look back' can occur when blood is contaminated (see earlier reference to European Union Commission Directives). Even in countries where residual risk of transmission through blood transfusion of clinically significant pathogens (HIV, HBV, HCV, and syphilis) has been reduced to minimal levels, problems continue to exist or emerge because:

- Laboratory tests may fail to identify viruses during the window period or because of imperfect sensitivity.
- The clinical significance of newly identified infectious agents is not always completely understood, and donors are not (yet) screened for these agents.
- Newly emerging infectious agents such as coronaviruses, hepatitis E, highly virulent influenza strains, and prions may constitute serious threats.

- There is currently no evidence that SARS-COV-2 is transmitted by blood transfusions, however, donor deferral due to recent illness or the logistics of donation during a pandemic has affected blood stocks in many countries.
- Absence of widely accepted or routinely used tests for bacterial, viral, and other pathogens (e.g., Yersinia enterocolitica, hepatitis A, toxoplasmosis, malaria, and babesiosis).

In many regions of the world in which thalassaemia is most common, continued transmission of HBV, HCV and HIV underscores the importance of promoting the quality systems of national blood transfusion services, including voluntary blood donations, careful donor selection, and donor blood screening, and the consistent use of immunisations such as hepatitis B vaccine.

6. QUALITY OF LIFE AND ECONOMIC BURDEN OF TRANSFUSION

6.1. Economic burden of transfusion

TDT patients require on average between 2 to 4 units of red cells every 2 to 5 weeks. This places a considerable strain on local healthcare resources especially in regions where thalassaemia is highly prevalent. The burden of managing TDT impacts the health service provision at the institutional and governmental levels, and presents social, personal, and financial challenges for families of patients living with TDT.

Challenges at national and governmental levels are primarily related to maintaining an adequate blood supply for routine transfusions for chronic patients as well as managing a regional or national transfusion service for non-thalassaemic patients. In endemic countries, this can present very serious challenges to health services in managing supply and demand [57].

Many countries with less developed healthcare services are heavily reliant on paid donors or family based directed donations to provide blood for patients [58]. Other countries with large populations of thalassaemia patients need non-governmental organisations to conduct blood drives and provide transfusion for TDT patients, but these will often also require family and friends to donate replacement units [58]. Nations where there is a centralised/regional blood services are more able to manage supply and demand aspects as donation rates are frequently higher in those countries and regions due to better resources and oversight in those services [59].

Blood transfusions are expensive; the mean direct cost of one unit of leucoreduced red cells in the USA was estimated at USD 223 in 2008 [60]. A more recent study used time-driven activity-based costing performed over a 1-month period, capturing every step of the transfusion pathway for patients with TDT at a designated provider of specialist thalassaemia services in Australia. The total per-unit cost was USD 695.59, with approximately 40% of cost being for procurement of the red cell unit, with process costs accounting for 55%. The single largest contributor to process costs was attributed to iron chelation medication (approximately 80%) [61, 62]. These costs may potentially rise as blood services are subjected to the impact of removing plasticisers from blood collection and administration packs as well as the impact of global supply chain issues.

The financial burden on the family caring for a patient with TDT may be considerable, and this is dependent on the healthcare system in which the patient resides. If there is no health service provision that provides full coverage for the blood transfusion visits (day care cost as well as

cost of blood units) either through an insurance base system or governmental funding, then patients and their families will need to pay for all the cost of life saving blood transfusions unless they are supported by non-governmental organisations and thalassaemia societies. Conversations should happen to help direct families to where they can get help with costs.

Modell and Darlinson reported in 2008 that globally, 22,500 deaths occurred annually because TDT patients were not able to receive adequate transfusions [63]. The uncounted financial impact on families may be very significant with parents needing time off work to take their child for blood transfusion, pay for transportation to centres for crossmatching and transfusion as well as needing to purchase iron chelation therapy and other ancillary costs such a blood tests and scans for monitoring [64]. This all comes on top of the considerable healthcare resource utilisation and costs attributed to complications attributed to transfusion therapy, especially those secondary to poorly controlled iron overload [65, 66].

6.2. Quality of life

Blood transfusion is a life-saving intervention in TDT. Symptoms of bone pain and fatigue as well as shortness of breath are significantly improved by blood transfusion, although these are often poorly documented or quantified. Aiming for a pretransfusion haemoglobin of 9.5-10.5 g/dL is effective in preventing complications, reducing symptoms, and improving quality of life. Patients with significant symptoms related to anaemia should have their pretransfusion haemoglobin optimised. The whole family is impacted by the initiation and ongoing transfusion regime, and this includes mental and emotional health of parents and siblings of the affected child. Psychological support should be offered to parents and siblings to help them adjust to life with a child affected by TDT (see Chapter 12).

KEY POINTS AND RECOMMENDATIONS

1. Confirm the diagnosis of thalassaemia, perform appropriate clinical and laboratory assessment, and obtain informed consent prior to initiation of transfusion (**Grade C, Class IIa**).
2. The decision to initiate a long-term regular transfusion regimen should not only be driven by patient genotype or haemoglobin level, and should not be driven by a transient drop in haemoglobin due to an intercurrent infection. It should consider the current clinical phenotype of the patient and anticipated short- and longer-term outcomes, and should be taken in discussion with the patient or parents (**Grade C, Class IIa**).
3. Use careful donor selection and screening, favouring voluntary, regular, non-remunerated blood donors (**Grade C, Class IIa**).
4. Before first transfusion, perform extended red cell antigen typing of patients at least for Rh C, c, D, E, e and Kell (K, k), and if available a full red cell pheno/genotype (**Grade C, Class IIa**).
5. At each transfusion, give ABO, Rh(D) compatible blood. The goal for all blood transfusions to thalassaemia patients is units that are also matched for C, c, E, e and Kell antigens (**Grade C, Class IIa**).
6. If units are optimally matched, then fresher units can be chosen over older units in less regulated blood banks but in highly regulated blood banks then a first-in first-out principle is reasonable (**Grade C, Class IIb**).
7. Before each transfusion, perform a screen for new antibodies and an indirect antiglobulin test crossmatch, or in centres that meet regulatory requirements, perform an electronic crossmatch where allowed (**Grade C, Class I**).
8. Use leucodepleted packed red cells. Pre-storage filtration is strongly recommended, but blood bank pretransfusion filtration is acceptable. Bedside filtration is only acceptable if there is no capacity for pre-storage filtration or blood bank pretransfusion filtration (**Grade C, Class I**).
9. Use washed red cells for patients who have severe allergic reactions (**Grade C, Class IIa**).
10. Transfuse every 2-5 weeks, targeting a pretransfusion haemoglobin of 9.5-10.5 g/dL (**Grade C, Class I**).
11. A higher target pretransfusion haemoglobin level of 10-11 g/dL (or as high as practicable) may be appropriate for patients with heart disease including pulmonary hypertension, clinically significant extramedullary haematopoiesis, or other medical conditions, and for those patients who do not achieve adequate suppression of bone marrow activity at the lower haemoglobin level (**Grade C, Class I**).
12. Maintaining pretransfusion haemoglobin at 10 g/dL is important in reducing both maternal cardiovascular stress and improving foetal outcomes in pregnant women (**Grade C, Class I**).
13. Keep the post-transfusion haemoglobin below 13-15 g/dL (**Grade C, Class IIa**).
14. Haemovigilance and adverse events reporting are key to the safety of blood transfusion. Keep a record of red cell antibodies, transfusion reactions, and annual transfusion requirements for each patient (**Grade C, Class**

REFERENCES

1. Forni GL, Gianesin B, Musallam KM, Longo F, Rosso R, Lisi R, Gamberini MR, Pinto VM, Graziadei G, Vitucci A, Bonetti F, Musto P, Piga A, Cappellini MD, Borgna-Pignatti C, Webthal p. Overall and complication-free survival in a large cohort of patients with beta-thalassemia major followed over 50 years. Am J Hematol 2023;98(3):381-387.
2. Viprakasit V, Gattermann N, Lee JW, Porter JB, Taher AT, Habr D, Martin N, Domokos G, Cappellini MD. Geographical variations in current clinical practice on transfusions and iron chelation therapy across various transfusion-dependent anaemias. Blood Transfus 2013;11(1):108-122.
3. El-Beshlawy A, Dewedar H, Hindawi S, Alkindi S, Tantawy AA, Yassin MA, Taher AT. Management of transfusion-dependent beta-thalassemia (TDT): Expert insights and practical overview from the Middle East. Blood Rev 2024;63:101138.
4. Taher A, Musallam K, Cappellini MD. Guidelines for the Management of Non-Transfusion-Dependent β-Thalassaemia. 3rd ed. Nicosia, Cyprus: Thalassaemia International Federation, 2023.
5. Musallam KM, Barella S, Origa R, Ferrero GB, Lisi R, Pasanisi A, Longo F, Gianesin B, Forni GL, Webthal p. 'Phenoconversion' in adult patients with beta-thalassemia. Am J Hematol 2024;99(3):490-493.
6. Musallam KM, Vitrano A, Meloni A, Pollina SA, Karimi M, El-Beshlawy A, Hajipour M, Di Marco V, Ansari SH, Filosa A, Ricchi P, Ceci A, Daar S, Vlachaki E, Singer ST, Naserullah ZA, Pepe A, Scondotto S, Dardanoni G, Bonifazi F, Sankaran VG, Vichinsky E, Taher AT, Maggio A, International Working Group on T. Risk of mortality from anemia and iron overload in nontransfusion-dependent beta-thalassemia. Am J Hematol 2022;97(2):E78-E80.
7. Musallam KM, Cappellini MD, Daar S, Taher AT. Morbidity-free survival and hemoglobin level in non-transfusion-dependent beta-thalassemia: a 10-year cohort study. Ann Hematol 2022;101(1):203-204.
8. Musallam KM, Vitrano A, Meloni A, Pollina SA, Karimi M, El-Beshlawy A, Hajipour M, Di Marco V, Ansari SH, Filosa A, Ricchi P, Ceci A, Daar S, Vlachaki E, Singer ST, Naserullah ZA, Pepe A, Scondotto S, Dardanoni G, Bonifazi F, Sankaran VG, Vichinsky E, Taher AT, Maggio A. Survival and causes of death in 2,033 patients with non-transfusion-dependent beta-thalassemia. Haematologica 2021;106(9):2489-2492.
9. Taher AT, Musallam KM, Karimi M, El-Beshlawy A, Belhoul K, Daar S, Saned MS, El-Chafic AH, Fasulo MR, Cappellini MD. Overview on practices in thalassemia intermedia management aiming for lowering complication rates across a region of endemicity: the OPTIMAL CARE study. Blood 2010;115(10):1886-1892.
10. Olivieri NF, Muraca GM, O'Donnell A, Premawardhena A, Fisher C, Weatherall DJ. Studies in haemoglobin E beta-thalassaemia. Br J Haematol 2008;141(3):388-397.
11. Fucharoen S, Ketvichit P, Pootrakul P, Siritanaratkul N, Piankijagum A, Wasi P. Clinical manifestation of beta-thalassemia/hemoglobin E disease. J Pediatr Hematol Oncol 2000;22(6):552-557.
12. Mettananda S, Pathiraja H, Peiris R, Wickramarathne N, Bandara D, de Silva U, Mettananda C, Premawardhena A. Blood transfusion therapy for β-thalassemia major and hemoglobin E β-thalassemia: Adequacy, trends, and determinants in Sri Lanka. Pediatr Blood Cancer 2019;66(5):e27643.
13. Mettananda S, Pathiraja H, Peiris R, Bandara D, de Silva U, Mettananda C, Premawardhena A. Health related quality of life among children with transfusion dependent β-thalassaemia major and haemoglobin E β-thalassaemia in Sri Lanka: a case control study. Health Qual Life Outcomes 2019;17(1):137.
14. World Health Organization. The urgent need to implement patient blood management: policy brief. Geneva, Switzerland: World Health Organization, 2021.
15. Compernolle V, Chou ST, Tanael S, Savage W, Howard J, Josephson CD, Odame I, Hogan C, Denomme G, Shehata N. Red blood cell specifications for patients with hemoglobinopathies: a systematic review and guideline. Transfusion 2018;58(6):1555-1566.
16. Cheng CK, Lee CK, Lin CK. Clinically significant red blood cell antibodies in chronically transfused patients: a survey of Chinese thalassemia major patients and literature review. Transfusion 2012;52(10):2220-2224.

17. Chou ST, Liem RI, Thompson AA. Challenges of alloimmunization in patients with haemoglobinopathies. Br J Haematol 2012;159(4):394-404.
18. Chou ST, Alsawas M, Fasano RM, Field JJ, Hendrickson JE, Howard J, Kameka M, Kwiatkowski JL, Pirenne F, Shi PA, Stowell SR, Thein SL, Westhoff CM, Wong TE, Akl EA. American Society of Hematology 2020 guidelines for sickle cell disease: transfusion support. Blood Adv 2020;4(2):327-355.
19. Cazzola M, Borgna-Pignatti C, Locatelli F, Ponchio L, Beguin Y, De Stefano P. A moderate transfusion regimen may reduce iron loading in beta-thalassemia major without producing excessive expansion of erythropoiesis. Transfusion 1997;37(2):135-140.
20. Cazzola M, De Stefano P, Ponchio L, Locatelli F, Beguin Y, Dessi C, Barella S, Cao A, Galanello R. Relationship between transfusion regimen and suppression of erythropoiesis in beta-thalassaemia major. Br J Haematol 1995;89(3):473-478.
21. Musallam KM, Barella S, Origa R, Ferrero GB, Lisi R, Pasanisi A, Longo F, Gianesin B, Forni GL. Pretransfusion hemoglobin level and mortality in adults with transfusion-dependent beta-thalassemia. Blood 2024;143(10):930-932.
22. Pennell DJ, Udelson JE, Arai AE, Bozkurt B, Cohen AR, Galanello R, Hoffman TM, Kiernan MS, Lerakis S, Piga A, Porter JB, Walker JM, Wood J. Cardiovascular function and treatment in beta-thalassemia major: a consensus statement from the American Heart Association. Circulation 2013;128(3):281-308.
23. Teawtrakul N, Jetsrisuparb A, Pongudom S, Sirijerachai C, Chansung K, Wanitpongpun C, Fucharoen S. Epidemiologic study of major complications in adolescent and adult patients with thalassemia in Northeastern Thailand: the E-SAAN study phase I. Hematology 2018;23(1):55-60.
24. Subahi EA, Ata F, Choudry H, Iqbal P, AlHiyari MA, Soliman AT, De Sanctis V, Yassin MA. Extramedullary haematopoiesis in patients with transfusion dependent beta-thalassaemia (TDT): a systematic review. Ann Med 2022;54(1):764-774.
25. Davies P, Robertson S, Hegde S, Greenwood R, Massey E, Davis P. Calculating the required transfusion volume in children. Transfusion 2007;47(2):212-216.
26. Maitland K, Kiguli S, Olupot-Olupot P, Opoka RO, Chimalizeni Y, Alaroker F, Uyoga S, Kyeyune-Byabazaire D, M'Baya B, Bates I, Williams TN, Munube D, Mbanya D, Molyneux EM, South A, Walker AS, Gibb DM, George EC. Transfusion management of severe anaemia in African children: a consensus algorithm. Br J Haematol 2021;193(6):1247-1259.
27. Maitland K, Kiguli S, Olupot-Olupot P, Engoru C, Mallewa M, Saramago Goncalves P, Opoka RO, Mpoya A, Alaroker F, Nteziyaremye J, Chagaluka G, Kennedy N, Nabawanuka E, Nakuya M, Namayanja C, Uyoga S, Kyeyune Byabazaire D, M'Baya B, Wabwire B, Frost G, Bates I, Evans JA, Williams TN, George EC, Gibb DM, Walker AS. Immediate Transfusion in African Children with Uncomplicated Severe Anemia. N Engl J Med 2019;381(5):407-419.
28. Hunter S, Robson SC. Adaptation of the maternal heart in pregnancy. Br Heart J 1992;68(6):540-543.
29. Origa R, Piga A, Quarta G, Forni GL, Longo F, Melpignano A, Galanello R. Pregnancy and beta-thalassemia: an Italian multicenter experience. Haematologica 2010;95(3):376-381.
30. Nassar AH, Naja M, Cesaretti C, Eprassi B, Cappellini MD, Taher A. Pregnancy outcome in patients with beta-thalassemia intermedia at two tertiary care centers, in Beirut and Milan. Haematologica 2008;93(10):1586-1587.
31. Luewan S, Srisupundit K, Tongsong T. Outcomes of pregnancies complicated by beta-thalassemia/hemoglobin E disease. Int J Gynaecol Obstet 2009;104(3):203-205.
32. Shah FT, Nicolle S, Garg M, Pancham S, Lieberman G, Anthony K, Mensah AK. Guideline for the management of conception and pregnancy in thalassaemia syndromes: A British Society for Haematology Guideline. Br J Haematol 2024;204(6):2194-2209.
33. de Vries RR, Faber JC, Strengers PF. Haemovigilance: an effective tool for improving transfusion practice. Vox Sang 2011;100(1):60-67.

34. World Health Organization. A guide to establishing a national haemovigilance system. 06 January 2016. Available at: https://www.who.int/publications/i/item/9789241549844. Accessed: 22 Dec 2024.
35. Narayan S, Baker P, Bellamy M, Bentley A, Bolton-Maggs P, Booth C, Brailsford S, Carter-Graham S, Clarke H, Danaee A, Davies J, Davison C, Grey S, Harvala H, Haynes S, Hughes S, Kelly A, Latham T, Malhotra P, McCullagh J, Milser E, Molloy A, New H, Perry T, Peters J, Poles D, Regan F, Tomlinson T, Tuckley V, Watt A, T. Y. The 2023 Annual SHOT Report. Manchester: Serious Hazards of Transfusion (SHOT) Steering Group, 2024.
36. Klein HG, Spahn DR, Carson JL. Red blood cell transfusion in clinical practice. Lancet 2007;370(9585):415-426.
37. Da Ponte A, Bidoli E, Talamini R, Steffan A, Abbruzzese L, Toffola RT, De Marco L. Pre-storage leucocyte depletion and transfusion reaction rates in cancer patients. Transfus Med 2005;15(1):37-43.
38. Thompson AA, Cunningham MJ, Singer ST, Neufeld EJ, Vichinsky E, Yamashita R, Giardina P, Kim HY, Trachtenberg F, Kwiatkowski JL, Thalassemia Clinical Research Network I. Red cell alloimmunization in a diverse population of transfused patients with thalassaemia. Br J Haematol 2011;153(1):121-128.
39. Singer ST, Wu V, Mignacca R, Kuypers FA, Morel P, Vichinsky EP. Alloimmunization and erythrocyte autoimmunization in transfusion-dependent thalassemia patients of predominantly asian descent. Blood 2000;96(10):3369-3373.
40. Spanos T, Karageorga M, Ladis V, Peristeri J, Hatziliami A, Kattamis C. Red cell alloantibodies in patients with thalassemia. Vox Sang 1990;58(1):50-55.
41. Milkins C, Berryman J, Cantwell C, Elliott C, Haggas R, Jones J, Rowley M, Williams M, Win N. Guidelines for pre-transfusion compatibility procedures in blood transfusion laboratories. British Committee for Standards in Haematology. Transfus Med 2013;23(1):3-35.
42. Trompeter S, Baxter L, McBrearty M, Zatkya E, Porter J. Sample acceptance time criteria, electronic issue and alloimmunisation in thalassaemia. Transfus Med 2015;25(6):414-417.
43. Michail-Merianou V, Pamphili-Panousopoulou L, Piperi-Lowes L, Pelegrinis E, Karaklis A. Alloimmunization to red cell antigens in thalassemia: comparative study of usual versus better-match transfusion programmes. Vox Sang 1987;52(1-2):95-98.
44. Cohen AR, Glimm E, Porter JB. Effect of transfusional iron intake on response to chelation therapy in beta-thalassemia major. Blood 2008;111(2):583-587.
45. Graziano JH, Piomelli S, Hilgartner M, Giardina P, Karpatkin M, Andrew M, Lolacono N, Seaman C. Chelation therapy in beta-thalassemia major. III. The role of splenectomy in achieving iron balance. J Pediatr 1981;99(5):695-699.
46. O'Brien RT, Pearson HA, Spencer RP. Transfusion-induced decrease in spleen size in thalassemia major: documentation by radioisotopic scan. J Pediatr 1972;81(1):105-107.
47. Modell B. Management of thalassaemia major. Br Med Bull 1976;32(3):270-276.
48. Taher A, Isma'eel H, Mehio G, Bignamini D, Kattamis A, Rachmilewitz EA, Cappellini MD. Prevalence of thromboembolic events among 8,860 patients with thalassaemia major and intermedia in the Mediterranean area and Iran. Thromb Haemost 2006;96(4):488-491.
49. Taher AT, Cappellini MD, Bou-Fakhredin R, Coriu D, Musallam KM. Hypercoagulability and Vascular Disease. Hematol Oncol Clin North Am 2018;32(2):237-245.
50. de Montalembert M, Dumont MD, Heilbronner C, Brousse V, Charrara O, Pellegrino B, Piguet C, Soussan V, Noizat-Pirenne F. Delayed hemolytic transfusion reaction in children with sickle cell disease. Haematologica 2011;96(6):801-807.
51. Ameen R, Al-Shemmari S, Al-Humood S, Chowdhury RI, Al-Eyaadi O, Al-Bashir A. RBC alloimmunization and autoimmunization among transfusion-dependent Arab thalassemia patients. Transfusion 2003;43(11):1604-1610.
52. Hill QA, Stamps R, Massey E, Grainger JD, Provan D, Hill A. The diagnosis and management of primary autoimmune haemolytic anaemia. Br J Haematol 2017;176(3):395-411.

53. Jäger U, Barcellini W, Broome CM, Gertz MA, Hill A, Hill QA, Jilma B, Kuter DJ, Michel M, Montillo M, Röth A, Zeerleder SS, Berentsen S. Diagnosis and treatment of autoimmune hemolytic anemia in adults: Recommendations from the First International Consensus Meeting. Blood Rev 2020;41:100648.
54. Rebulla P, Modell B. Transfusion requirements and effects in patients with thalassaemia major. Cooleycare Programme. Lancet 1991;337(8736):277-280.
55. Vlaar AP, Juffermans NP. Transfusion-related acute lung injury: a clinical review. Lancet 2013;382(9896):984-994.
56. Swanson K, Dwyre DM, Krochmal J, Raife TJ. Transfusion-related acute lung injury (TRALI): current clinical and pathophysiologic considerations. Lung 2006;184(3):177-185.
57. Shah FT, Sayani F, Trompeter S, Drasar E, Piga A. Challenges of blood transfusions in β-thalassemia. Blood Rev 2019;37:100588.
58. World Health Organisation. Global status report on blood safety and availability 2016. Geneva, Switzerland: World Health Organization, 2017.
59. European Directorate for the Quality of Medicines & HealthCare. Report of the survey on blood supply management 2012. Strasbourg, France: Council of Europe, 2014.
60. Whitaker BI. The 2011 National Blood Collection and Utilization Survey report. USA: Department of Health and Human Services, 2011.
61. McQuilten ZK, Higgins AM, Burns K, Chunilal S, Dunstan T, Haysom HE, Kaplan Z, Rushford K, Saxby K, Tahiri R, Waters N, Wood EM. The cost of blood: a study of the total cost of red blood cell transfusion in patients with beta-thalassemia using time-driven activity-based costing. Transfusion 2019;59(11):3386-3395.
62. Burns KE, Haysom HE, Higgins AM, Waters N, Tahiri R, Rushford K, Dunstan T, Saxby K, Kaplan Z, Chunilal S, McQuilten ZK, Wood EM. A time-driven, activity-based costing methodology for determining the costs of red blood cell transfusion in patients with beta thalassaemia major. Transfus Med 2019;29(1):33-40.
63. Modell B, Darlison M. Global epidemiology of haemoglobin disorders and derived service indicators. Bull World Health Organ 2008;86(6):480-487.
64. Udeze C, Evans KA, Yang Y, Lillehaugen T, Manjelievskaia J, Mujumdar U, Li N, Andemariam B. Economic and clinical burden of managing transfusion-dependent β-thalassemia in the United States. J Med Econ 2023;26(1):924-932.
65. Tang CH, Furnback W, Wang BCM, Tang J, Tang D, Lu MY, Huang VW, Musallam KM. Relationship between transfusion burden, healthcare resource utilization, and complications in patients with beta-thalassemia in Taiwan: A real-world analysis. Transfusion 2021;61(10):2906-2917.
66. Weiss M, Parisi Jun M, Sheth S. Clinical and economic burden of regularly transfused adult patients with beta-thalassemia in the United States: A retrospective cohort study using payer claims. Am J Hematol 2019;94(5):E129-E132.

03 IRON OVERLOAD AND CHELATION

Authors: **John B. Porter[1], John C. Wood[2], Thomas D. Coates[3]**

[1.] Department of Haematology, University College London Hospitals, London, United Kingdom

[2.] Division of Cardiology, Children's Hospital Los Angeles, University of Southern California Keck School of Medicine, Los Angeles, CA, USA

[3.] Hematology Section, Cancer and Blood Disease Institute, Children's Hospital Los Angeles, University of Southern California Keck School of Medicine, Los Angeles, CA, USA

Competing interest: JBP reports honoraria from Agios Pharmaceuticals, bluebird bio, Celgene (Bristol Myers Squibb), La Jolla Pharmaceuticals, Protagonism, Silence Therapeutics, and Vifor; and is a consultant for Agios Pharmaceuticals, bluebird bio, and Celgene (Bristol Myers Squibb). JCW reports research funding from the National Heart, Lung, and Blood Institute and National Institute of Diabetes and Digestive and Kidney Diseases of the National Institutes of Health; research support-in-kind from Philips Healthcare; consultancy for Celgene (Bristol Myers Squibb), WorldCare Clinical, Agios Pharmaceuticals, Pharmacosmos, and Hillhurst; advisory boards for Vifor, Regeneron; and educational lectures for Chiesi and bluebird bio. TDC reports advisory support to Agios Pharmaceuticals, Bristol Myers Squibb, and Chiesi. All competing interests are outside the present work.

1. INTRODUCTION

Iron overload occurs when iron intake is increased over a sustained period, either as a result of red blood cell (RBC) transfusions or from increased iron absorption through the gastrointestinal tract. Both occur in β-thalassaemia syndromes, with blood transfusion being the major cause in transfusion-dependent β-thalassaemia (TDT) whereas increased gastrointestinal absorption is more important in non-transfusion dependent β-thalassaemia (NTDT) [1]. Iron overload is inevitable with repeated transfusions because humans lack mechanisms to excrete excess iron. Iron accumulation can damage many tissues, causing heart failure, cirrhosis, liver cancer, growth retardation and multiple endocrine abnormalities [2].

Chelation therapy aims to balance the rate of iron accumulation by increasing iron excretion in urine and/or faeces. If previous chelation has been delayed or inadequate, the goal would be for excretion to exceed rather than balance the current rate of iron accumulation. However, because iron is also essential for physiological purposes, excessive chelation rates must be avoided. This requires careful dose adjustment as iron levels fall. Optimal chelation not only aims to balance iron input with output from chelation but to detoxify highly reactive iron species like non-transferrin-bound iron (NTBI) by being present in plasma and tissues continuously [3, 4]. In fact, the most important role of chelation is to prevent iron toxicity. Continuous exposure to adequate levels of chelator can dramatically reduce reactive iron within hours [5] and can significantly correct organ dysfunction years before iron stored in tissue has been eliminated [4, 6].

Achieving regular adherence to iron chelation therapy throughout life is key to preventing iron toxicity as shown by early data that survival in TDT is related to the number of days chelator is taken per year [7]. Even short periods of treatment interruption can be damaging [4]. While the convenience and tolerability of chelators is important, factors such as psychological well-being, support of the family and the treatment centre are also key (see Chapter 12).

2. THE RATE OF IRON LOADING

In transfused patients, a unit processed from 420 mL of donor blood contains approximately 200 mg of iron (0.48 mg/mL of whole donor blood). For RBC preparations with variable haematocrits, the iron in mg/mL of blood can be estimated from 1.08 x the haematocrit of donated product (see Chapter 2 for further details) [8]. The iron content is the same for units that are packed, semi-packed, or diluted in additive solution.

In TDT patients receiving transfusions as per these Guidelines, the equivalent of 100-200 mL of pure RBC per kg body weight per year are transfused, equivalent to 108-216 mg of iron/kg body weight, or 0.3-0.6 mg/kg/day. This increases body iron stores to many times the norm, unless chelation treatment is provided.

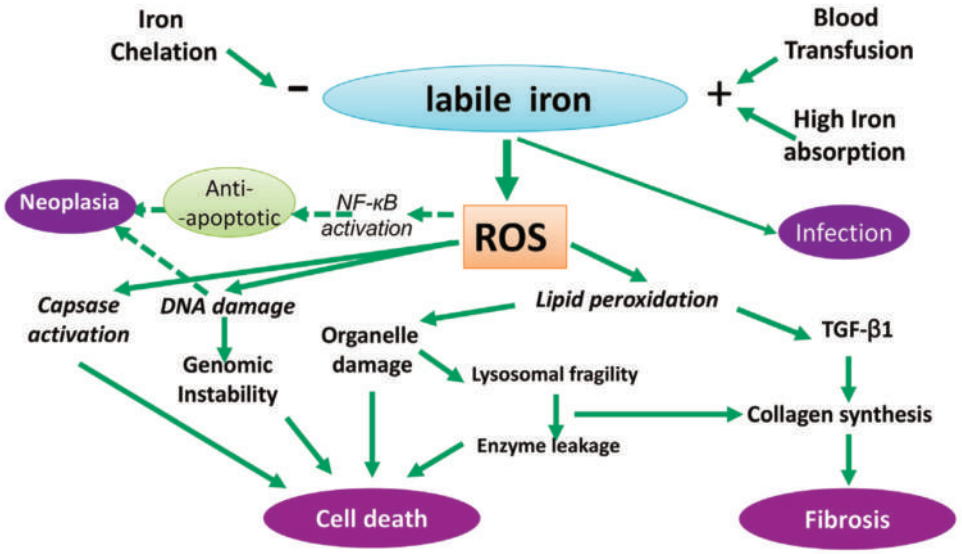
In TDT patients, the rate of dietary iron absorption is small relative to iron accumulated from blood transfusion. Whereas normal intestinal iron absorption is about 1-2 mg/day, this increases in β-thalassaemia secondary to expansion of red cell precursors and consequent inhibition of hepcidin synthesis in the liver [9]. Increased iron absorption becomes significant when bone marrow expansion exceeds five times that of healthy individuals. Such expansion can be prevented by using transfusion regimens that keep the pretransfusion haemoglobin above 9 g/dL [10] (a threshold of ≥9.5 g/dL is recommended in the Guidelines, see Chapter 2). When such haemoglobin values are not achieved, absorption can rise to 3-5 mg/day, representing an additional 1-2 g of iron loading per year.

3. TOXICITY FROM IRON OVERLOAD

3.1. Iron toxicity mechanisms

In health, iron is 'kept safe' by binding to molecules such as transferrin, but in iron overload the capacity to bind iron is exceeded both within cells and in plasma. The iron not bound to transferrin is iron is highly reactive, alternating between two states, Fe^{3+} and Fe^{2+}, with the latter leading to generation of harmful free radicals (atoms or molecules with unpaired electrons) damaging many tissues unless eliminated or buffered by chelator. These free radicals can damage lipid membranes, organelles and DNA, causing cell death and the generation of fibrosis. Free iron also increases the risk of infections and neoplasia (Figure 1) [4, 11].

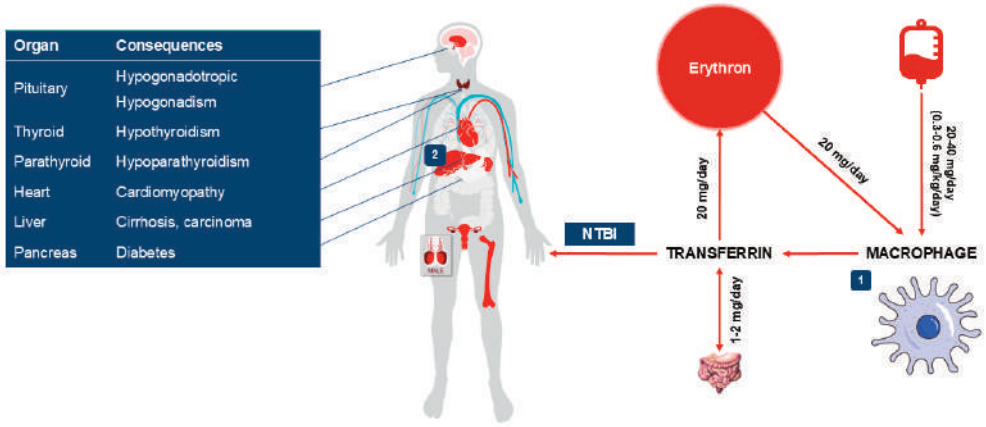
Figure 1. *Pathological mechanisms and consequences of iron overload. In iron overload resulting from repeated blood transfusions or long-term increased iron absorption, iron that is not bound to naturally occurring molecules, such as transferrin or ferritin, or to therapeutic iron chelators, generates a variety of ROS, most notably hydroxyl radicals. This occurs in cells where labile plasma iron is taken up and accumulates as storage iron (ferritin and haemosiderin). ROS generate lipid peroxidation and organelle and DNA damage and dysregulate mechanisms involved in apoptotic cell death, increasing the risk of neoplasia such as hepatoma. Labile iron is also more available to microorganisms than iron bound to transferrin or ferritin, thereby increasing the risk of infection. Abbreviations: ROS, reactive oxygen species; TGF-β1, transforming growth factor β1; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells. Reproduced with permission from [11].*



3.2. Distribution and consequences of transfusional iron overload

In the absence of iron overload, iron uptake into cells is controlled by the interaction of transferrin with its receptors – mainly on red cell precursors, hepatocytes and dividing cells. In iron overload, transferrin iron binding becomes saturated, and iron species appear in plasma that are not bound to transferrin (plasma NTBI). The distribution and mechanisms of NTBI uptake into cells is fundamentally different from transferrin-mediated uptake (Figure 2) [11].

Figure 2. *The main routes of iron turnover and uptake are shown by solid red arrows on the right panel: 20 mg of iron is delivered daily to the erythron in health. This increases several fold in untransfused β-thalassaemias but can be inhibited by transfusion. NTBI is generated when transferrin (which is about 30% saturated in healthy adults) becomes saturated. Transferrin saturation occurs following iron overload of the macrophage system, but also as a result of decreased clearance of transferrin iron in transfused patients. The organs in which NTBI is taken up and retained as storage iron are shown on the left, with >80% cleared by hepatocytes. Despite variable and lower quantities of iron taken into other tissues, serious and often irreversible iron-mediated damage may occur. Iron excretion by chelation therapy acts mainly at sites (1) the interception of iron released from macrophages after red cell catabolism, and (2) iron released by the catabolism of ferritin within hepatocytes. Abbreviations: NTBI, non-transferrin-bond iron.*



Macrophages in the liver (Kupffer cells) also load with iron by phagocytosis of senescent autologous or transfused RBCs. Breakdown of the resulting haemoglobin in the hepatic reticuloendothelial macrophages produces intracellular reactive Fe^{2+} which stimulates production of ferritin. Ferritin internalises reactive Fe^{2+} and converts it to non-toxic Fe^{3+} for storage. The stored Fe^{3+} is converted back to Fe^{2+} for release from liver macrophages, so there is toxic exposure on the way into and out of storage but the stored Fe^{3+} is not reactive. Liver iron concentration (LIC) reflects total body iron and increases linearly with number of transfusions in the absence of effective chelation [12].

Organ damage in transfusional iron overload reflects the pattern of NTBI uptake into tissues. Some tissues are spared, while others such as myocardial muscle, endocrine tissue, and hepatocytes take up NTBI rapidly. This iron is then stored as ferritin or haemosiderin which are visible by magnetic resonance imaging (MRI), often at different rates [13]. Without chelation therapy, myocardial iron overload can induce heart failure, as early as the second decade (see Chapter 4). Iron overload also damages the anterior pituitary leading to hypogonadism, growth retardation, and delayed puberty. Endocrine complications, namely diabetes mellitus, hypothyroidism and hypoparathyroidism also occur (see Chapter 6). Liver disease with fibrosis and eventually cirrhosis are also serious complications. Hepatocellular carcinoma, particularly if concomitant chronic viral hepatitis is present can also occur (See Chapter 5) [14].

4. MONITORING OF IRON OVERLOAD

Monitoring is essential in establishing effective iron chelation, tailored to individuals' specific needs. Some general principles of monitoring iron overload apply to all.

4.1. Serum ferritin

Serum ferritin (SF) levels generally reflect body iron stores and are relatively easy and inexpensive to determine sequentially. SF is most useful in identifying trends, with a decreasing SF trend indicating decreasing body iron stores. Lack of long-term SF control can increase the risk of iron-mediated complications and prognosis [7, 15-17]. In one study from the US, there was a lower risk of cardiac disease over a median follow up of 12 years in at least two-thirds of TDT patients receiving deferoxamine (DFO), when SF had been maintained below 2500 ng/mL [16]. Similar findings were observed in cohorts from the United Kingdom and Italy [17, 18]. Levels higher than 1700-2200 ng/mL were also reported to predict overall mortality in more recent studies [19, 20]. Maintenance of an even lower SF level (<1000 ng/mL) may be associated with additional advantages for survival, cardiac, and endocrine disease [17, 21, 22] (Table 1).

Table 1. *Use of serum ferritin for monitoring iron chelation treatment.*

Advantages	Disadvantages
Easy to assess repeatedly	Indirect estimate of iron burden
Inexpensive	Increased by inflammation
Trend identification possible with repeat samples	Cannot determine iron balance directly
Long-term control linked to outcome	Non-linear response to iron load at high levels
Reflects in part exposure to non-transferrin-bound iron	Absence of decrease does not exclude response
	Relationship to iron load varies with chelator type and chelation duration
	Relationship to liver iron concentration differs in different diseases

SF can be increased by factors other than iron overload, such as inflammation or tissue damage. SF production is also stimulated by the inflammatory cytokine, interleukin 6. In TDT, variation in body iron stores accounts for only 57% of SF variability [23]. A sudden increase in SF should prompt a search for hepatitis, other infections, or inflammatory conditions. The absence of SF decrements with chelation intensification does not necessarily exclude falling iron burdens. Indeed, at high SF levels >4000 ng/mL, SF does not correlate significantly with LIC. Longitudinally, a discordance between response to iron chelation therapy as measured by changes in SF vs LIC is noted in up to 30% of patients [24-26] and the trend in SF can be opposite to that of LIC about 23% of the time [26]. Below 4000 ng/mL SF values are mainly influenced by iron stores in macrophages, whereas above 4000 ng/mL they are determined increasingly by SF leakage from hepatocytes [15, 24, 27].

The relationship between SF and body iron stores may also vary with the type of chelator used [28, 29] and by duration of chelation therapy [30], although other studies with smaller patient numbers did not observe such variation [31]. Coefficients for the correlation between SF and myocardial iron values (on cross-sectional or longitudinal assessment) vary considerably between studies, with general agreement of poor correlation [20, 32].

4.2. Liver iron concentration

Inadequate control of LIC is linked to the risk of hepatic as well as extrahepatic damage. Normal LIC values of up to 1.8 mg/g dry weight (dw) and LIC values of up to 7 mg/g dw can be seen without apparent adverse effects on the liver, while levels >7 mg/g dw were associated with poor outcomes [20]. Sustained high LIC values (above 15-20 mg/g dw) have been linked to liver fibrosis progression [33], liver function abnormalities [34], as well as increased risk of myocardial iron and cardiovascular disease [18]. With inadequate LIC control, iron accumulates initially in the liver and later in the heart, so that high LIC can predict the risk of myocardial iron accumulation. Conversely, a falling LIC with chelation usually precedes improvement in myocardial iron [6, 35]. Thus, with chelation therapy, whilst high LIC increases the risk of cardiac iron overload, the measurement of LIC will not reliably predict concomitant myocardial iron and hence cardiac risk. Secondly, myocardial iron is removed more slowly by chelation than liver iron and so iron may be found in some patients despite currently well controlled LIC.

LIC is the most reliable indicator of body iron load, which can be derived from the following formula: **Total body iron stores in mg iron/kg body weight = 10.6 x LIC (in mg/g dw)** [12]. Sequential measurement of LIC is the best way to determine whether body iron is increasing or decreasing with time (iron balance). While SF measurement is simple, relatively inexpensive, and can be repeated frequently, LIC determination should be considered for those patients whose SF levels deviate from expected trends (i.e., those with suspected co-existing hepatitis or patients on chelation regimens with variable or uncertain responses), as this may reduce the risk of giving either inadequate or excessive doses of chelation therapy. Since the relationship of SF to iron overload and iron balance has not yet been established, assessment of LIC may be particularly useful when new chelating regimes are being used. As mentioned earlier, at high levels of SF (>4000 ng/mL), the relationship to LIC is not linear and patients may show a fall in LIC (negative iron balance) without a clear trend in SF in the first 6-12 months. When a patient fails to show a fall in SF over several months the change in LIC can identify whether the current regimen is adequate or need to be modified. Missing the equivalent of one or two days of chelation a week with attendant return of NTBI can affect SF and extrahepatic iron loading, so close attention to adherence is critical [4].

4.2.1. Methods of measuring liver iron concentration

Biopsy: LIC was initially estimated by chemical determination of a liver biopsy sample (fresh, fixed or from dewaxing of paraffin-embedded material) (Table 2). Biopsy is invasive, but in experienced hands, has a low complication rate [36]. Inadequate sample size (less than 4 mg dw or less than about a 2.5 cm core length) or uneven distribution of iron, particularly in the presence of cirrhosis [37], may give misleading results. Biopsy also allows the evaluation of liver histology which cannot yet be reliably estimated by non-invasive means. Laboratory standardisation is challenging and differences between laboratories, for example in wet to dry weight ratios, make standardisation demanding.

Table 2. *Rationale, advantages and disadvantages of liver iron concentration measurement by magnetic resonance imaging and biopsy.*

Advantages	Disadvantages
Gives the most reliable estimate of body iron	Expensive (either by biopsy or MRI)
Allows calculation of iron balance (LIC change)	Cannot be repeated as frequently as SF (cost with MRI or inconvenience with biopsy)
Long-term LIC control linked to prognosis	LIC unreliable as predictor of cardiac iron in chelated patients
LIC not affected by inflammation (unlike SF)	Biopsy complication rates at least 0.5%
Biopsy shows degree of liver damage	Biopsy method affected by sampling variation
MRI non-invasive with good patient acceptance	MRI determination less reliable above LIC of 30 mg/g dw
MRI method can readily be set up and standardised across different centres	

Abbreviations: LIC, liver iron concentration; MRI, magnetic resonance imaging; SF, serum ferritin; dw, dry weight.

Superconducting quantum interference device (SQUID): magnetic biosusceptometry by SQUID determines the paramagnetism of the liver which is proportional to LIC [23]. Current methodology requires liquid helium which is very expensive. Furthermore, the SQUID apparatus needs to be in an environment away from paramagnetic forces (e.g., lifts, cars) which is often impractical. For these reasons, the current generation of SQUID devices are unlikely to be used outside a small number of well-resourced centres.

MRI: MRI is the most widely used approach for LIC determination (Table 2). It has been shown to be superior to liver biopsy (on simulated study) for serial LIC observations [38]. The principle shared by all MRI techniques is that when a radiofrequency (rf) magnetic field pulse is applied (e.g., to liver or myocardium), protons take up energy, altering their spin orientation, ultimately returning to their original state. With spin echo, the time taken to return to the relaxed state is referred to as T1 in the longitudinal plane, and T2 in the transverse plane. Values may also be expressed as relaxation rates, the R1 rate (the same as 1/T1) and the R2 rate (the same as 1/T2). A variation of this principle are gradient echo techniques, achieved by applying a strong graded magnetic field to the pulse used for spin echo. This relies on multiple echoes over a shorter acquisition time than spin echo techniques. This shorter acquisition time may improve sensitivity and can be measured as T2* (in ms), where $1/T2^* = 1/T2 + 1/T2'$, and T2 is the tissue relaxation time and T2′ is the magnetic inhomogeneity of the tissue. Importantly, tissue iron concentration is not linearly related to T2* or T2, but is linearly related to 1/T2* or 1/T2 (R2* or R2). T2* (or R2*) can be achieved with a single breath hold, while T2 or R2 take a little longer to acquire data [39]. Both gradient and spin echo techniques have been used clinically and validated against liver biopsy, and can accurately measure LIC throughout the clinically relevant range with appropriate calibration and MRI acquisition techniques [40-43]. They are also equally effective in evaluating chronic response to iron chelation [44].

The strength of the magnetic field applied by these scanners is measured in Tesla (T) units. Iron imaging is preferably performed on 1.5T machines. 3T machines give a better signal to noise ratio but have greater susceptibility to artefacts, and the maximum detectable iron level is too low for many overloaded patients [45, 46]. Calibrations of R2 and R2* to iron concentration are different at 3T to 1.5T. However, some sites may only have access to 3T machines, which require faster gradients and dual radiofrequency transmit systems for suitable measurements [45, 46].

Manufacturers of suitable MRI scanners include Siemens (Erlangen, Germany), GE Healthcare (Milwaukee, WI, USA), and Philips Healthcare (Amsterdam, Netherlands). Liver packages (including standard sequences and analysis of the data) are included in the software provided with these MRI machines. Specialised LIC analysis software can also be bought separately.

A note of caution is that acquisition and analysis of MRI images for iron quantification requires training and experience. While well-performed R2 and R2* techniques perform comparably in clinical practice, the calibration to convert R2 or R2* results to LIC units depends on the field strength as well as the acquisition and post processing methods [47]. Although most imaging sites worldwide have the intrinsic capability for reliable R2 and R2* measurements, radiologists who fail to perform important quality control metrics may provide erroneous results. As a result, some practitioners may choose to outsource the quality control and analysis to a 3rd party vendor. The R2 based FerriScan® technique has been registered in the European Union and USA as a clinical service and it can be applied with little training, at any centre with a reasonably up-to-date MRI machine (see Table 2) [48]. An artificial intelligence (AI)-empowered solution is now also available (FerriSmart®).

4.3. Myocardial iron estimation: T2* and other tools

The physical principles of heart iron measurement by MRI are the same as for the liver, with the additional challenge of measuring a moving object – the myocardium. The T2* (or R2*) techniques have the advantage over T2 or R2 in that they require shorter acquisition times and can be achieved with a single breath hold [48]. The utility of myocardial T2* (mT2*) MRI was originally identified on the basis of shortened T2* values <20 ms in patients with decreased left ventricular ejection fraction (LVEF) [49]. More recently, the relationship between biochemically measured myocardial iron concentration (MIC) and mT2* has been shown using post-mortem myocardial material [50].

The mean MIC at post-mortem associated with prior heart failure was 5.98 mg/g dw (ranging from 3.2 to 9.5); levels that in the liver would not be regarded as harmful. The relationship of MIC to mT2* is: **MIC in mg/g dw = 45 x (mT2* in ms)$^{-1.22}$** [50]. This relationship is non-linear so small changes in mT2* at values <10 ms indicate relatively large changes in MIC. The risk of developing heart failure increases with mT2* values <10 ms, which are associated with a 160-fold increased risk of heart failure in the next 12 months [51].

This risk further increases progressively with mT2* values <10 ms, so that the proportion of patients developing heart failure in the next 12 months at mT2* of 8-10 ms, 6-8 ms and <6 ms was 18%, 31%, and 52% respectively. These risks were derived from patients whose chelation therapy and adherence was not reported, so this risk may be less in patients taking regular chelation [51]. For example, in a prospective study in patients with severe myocardial iron loading (mT2* values <10 ms), no patients developed heart failure over a 2 years period while taking deferasirox (DFX) and DFO combination therapy [52].

When locally validated, mT2* values may have predictive value in identifying patients at high risk of LVEF deterioration, thus allowing targeted intensification of treatment before heart failure develops. Prompt identification of patients at risk by MRI, timely therapeutic intervention and improved chelation options have contributed to decreasing number of cardiac deaths [53, 54]. mT2* monitoring has now been established and validated internationally [48], and recommended as part of yearly monitoring of multi-transfused patients at risk of developing myocardial iron loading. However, it is very important that a given centre undertakes procedures to independently validate and calibrate measurements of the method adopted, otherwise inappropriate assessment of heart failure prognosis may result. Table 3 summarises advantages and disadvantages of using mT2* MRI for monitoring cardiac iron overload.

Table 3. *Myocardial magnetic resonance imaging T2* method to assess myocardial iron.*

Advantages	Disadvantages
Rapidly assesses iron content in myocardial septum	Requires a validated centre with dedicated methods
Reproducible method	Requires experience with cardiac MRI
Linked to heart iron (reciprocal relationship)	
Potential to measure heart function at same visit	
Potential to measure LIC at same visit	
Linked to LVEF at time of measurement	
Linked to risk of heart failure	

Abbreviations: LIC, liver iron concentration; MRI, magnetic resonance imaging; LVEF, left ventricular ejection fraction.

Cardiac T1 and T2 mapping can also be used to identify cardiac iron loading and are becoming increasing available in dedicated cardiac imaging centres. Both can be collected in a single breath hold, similar to mT2*. Cardiac T1 is more sensitive to early cardiac iron deposition than mT2*, making it a useful adjunct to identify preclinical siderosis. However, cardiac T1 varies across imaging platforms and is not as well clinically validated as mT2*, making it less suitable as a single technique for assessing cardiac risk [55].

It is important to note that MRI only detects stored non-reactive Fe^{3+}. However, iron enters and leaves storage as reactive Fe^{2+}, so there is a relation between MRI detected Fe^{3+} and organ damage. With iron chelation, organ function may improve long before iron detected by MRI decreases [4, 6].

4.4. Cardiac function

Sequential monitoring of LVEF can identify patients at high risk of developing heart failure [15]. When LVEF fell below reference values, there was a 35-fold increased risk of clinical heart failure and death, with a median interval to progression of 3.5 years, allowing time for intensification

of chelation therapy. This approach required a reproducible method for determination of LVEF such as multigated acquisition (MUGA) scan or MRI, while echocardiography was generally too operator-dependent for this purpose. Furthermore, there is a clear need to identify high risk patients before there is a decline in LVEF. mT2* by MRI can achieve this and has additional predictive value (see above). However, as only a subset of patients with T2* values between 10 and 20 ms, or even with T2* less than 10 ms have abnormal heart function, sequential measurement of LVEF can identify the subset of patients who have developed decompensation of left ventricular function and are therefore at exceptionally high risk and require very intensive chelation therapy. Further discussion of monitoring of cardiac disease is discussed in Chapter 4.

4.5. Monitoring of other organ function and iron-mediated damage

This is discussed more fully in other Chapters. In general, by the time diabetes, hypothyroidism, hypoparathyroidism, or hypogonadotropic hypogonadism have been identified, irreversible damage has set in, and the focus then becomes replacing hormones. Individuals with significant iron loading can also have partial adrenal insufficiency [56, 57] and empiric stress dosing of corticosteroids should be considered in the face of clinical decompensation of any kind.

These are late effects whereas the primary aim of chelation is to prevent such damage. Iron overloaded patients should be monitored for evidence of endocrinopathies (see Chapter 6). There has been interest in using MRI to identify iron-mediated damage to the endocrine system. A correlation between MRI findings (loss of pituitary volume) and biochemical markers of pituitary damage was initially shown [58]. With improved MRI imaging, other endocrine organs have also been evaluated [59], with correlations established with clinical or subclinical disease [60-62]. There is generally a close correlation between iron deposition in the heart and deposition in endocrine tissues, such as the pituitary and pancreas [63, 64]. This is consistent with shared uptake mechanisms for NTBI in the heart and endocrine tissue as well as shared risks of damage to cardiac and endocrine systems once iron begins to escape from the liver. As control of NTBI is critical for protection of extrahepatic organs and NTBI levels rise as soon as chelator is absent [5], continual levels of chelator need to be maintained and thus daily adherence is of upmost importance [4].

4.6. 24-hour urinary iron estimation

This was initially used when assessing iron excretion by DFO (about half of total iron excreted in urine) [65] or deferiprone (DFP) (over 80% of iron excreted in urine), but is not useful with DFX, as nearly all the iron is excreted in faeces. The inherent variability in daily iron excretion necessitates repeated determinations and this is not widely used in routine monitoring. Urine iron has also been used in comparing combination or monotherapy regimes containing DFP [66-68].

4.7. Plasma non-transferrin-bound iron and labile plasma iron

As plasma iron that is not bound to transferrin, NTBI is considered the main route through which iron is distributed to liver and extrahepatic targets in TDT. NTBI levels might be expected to correlate with damage to these tissues. Assays may estimate NTBI directly using a chelation capture method followed by high performance liquid chromatography (HPLC) [69] or by colorimetric analysis [70], or indirectly by exploiting the impact of labile iron species to oxidised fluorochrome, such as in the labile plasma iron (LPI) assay [71, 72]. A potential advantage of the

LPI assay is that it is better suited to measurements when iron chelators are present in the plasma [71]. Whilst some loose associations of NTBI [73] or LPI [74] with some markers of cardiac iron or response to chelation have been reported, measurements have not been sufficiently strongly predictive of cardiac risk. This is partly because NTBI and LPI are highly labile, rapidly returning or even rebounding [5] after an iron chelator has been cleared [71]. Although NTBI correlates loosely with iron overload, it is affected by other factors such as ineffective erythropoiesis, the phase of transfusion cycle, and the rate of blood transfusion [75] adding to the complexity of interpreting levels [76]. It is also not clear which methods identify the iron species that are most strongly inked to myocardial iron uptake. Therefore, although the measurement of NTBI (or LPI) has proved a useful tool for evaluating how chelators interact with plasma iron pools, its value as a guide to routine treatment or prognosis has yet to be clearly demonstrated. While measured levels of NTBI are of limited value as an indication of risk from NTBI, the protection time from NTBI though the presence of chelator is of vital importance. NTBI can be cleared or decreased in the presence of chelator in plasma but reappears or even rebounds once the chelator is cleared [5, 72]. Therefore, chelator regimens where chelators are constantly present in plasma (and/or tissues) are to be preferred [4].

5. AIMS OF IRON CHELATION THERAPY

The aims of iron chelation therapy can vary based on clinical context and individual patient profile:

- **Prevention therapy:** the primary goal of chelation therapy is to prevent toxicity from iron. This means that adequate levels of chelator must me present all the to time keep reactive Fe^{2+} species near zero all the time. The toxicity from iron is related to sum of toxic Fe^{2+} exposure and the duration of time of the exposure. The secondary goal is to maintain safe levels of body iron at all times, by balancing iron intake from blood transfusions with iron excretion by chelation (iron balance).
- **Rescue therapy:** once iron overload has occurred, iron excretion rate with chelation must exceed that accumulated from transfusion. Removal of storage iron is slow and inefficient, because only a small proportion of body iron is available for chelation at any moment. Once iron has been deposited in some tissues, damage is often irreversible. Prevention is therefore preferable to rescue. Chelation therapy should therefore be initiated before toxic levels of iron have accumulated. Constant exposure to chelator can protect and reverse organ dysfunction by buffering reactive iron even though non-reactive iron is visible by MRI [6], although functional reversal is not guaranteed, especially with endocrine organs [77].
- **Emergency therapy**: if clinical heart failure develops, urgent action is required, which usually requires changing and/or intensifying the treatment.
- **Dose adjustment of therapy:** regimens require adjustment to changing circumstances. Reports that refer only to whether a patient is 'on' or 'off' a particular chelation regimen miss the critical importance of tailoring dosing and frequency to the patient needs. These can be identified by careful monitoring of body iron and its distribution. Monitoring is important to avoid a) under-chelation with increased iron toxicity or b) over-chelation and increased chelator toxicity. The dosing and regimen must be adjusted periodically to take these factors into account.

- **Adherence to therapy:** chelation must be taken regularly for it to work. This requires good adherence. Intermittent high dose chelation can induce negative iron balance but does not provide continuous protection from labile iron and also risks increased toxicity from the chelator. Poor adherence can result from practical issues such as difficulty with DFO infusions, intolerance of a particular chelator, psychological/psychosocial issues (see Chapter 12), or limited accessibility. A key role of the treating centre is the monitoring and encouragement of adherence, alongside support from the patient's family. Regular interaction with experienced thalassaemia centre providers to review the treatment plan and provide encouragement that good adherence to chelation can bring about excellent outcomes is critical.

6. SOURCES OF CHELATABLE IRON

Only a small fraction of body iron is available for iron chelation at any moment. This is because although iron is made available for chelation continuously, the processes involved liberate only small quantities of chelatable iron at any moment. Labile iron is constantly being generated, so that the efficiency of chelation and protective effects are better when a chelator is also continuously available (chelator present 24 hours a day). Chelatable iron is derived from two major sources: from the breakdown of red cells in macrophages (about 20 mg/day in healthy adults) and from the catabolism of stored ferritin within cells. In iron overload, most of the storage iron in the body is in hepatocytes, and the ferritin in these cells is turned over less frequently than in the absence of iron overload (every few days). Iron chelated within the liver is excreted through the biliary system, or circulates back into plasma and is excreted in the urine. The proportion of excreted iron in urine and faeces differs with each chelator. Although other cells, such as cardiomyocytes, contain about one tenth of the storage iron concentration of hepatocytes, iron from these cells is cleared more slowly by chelators [11, 78].

7. CHEMICAL AND PHARMACOLOGICAL PROPERTIES OF LICENSED IRON CHELATORS

Three chelators are licensed for clinical use: DFO (deferoxamine or desferrioxamine, branded as Desferal®) available as a parenteral formulation for subcutaneous, intramuscular, or intravenous injection; DFP (deferiprone, branded as Ferriprox®, Kelfer®, or GPO-L-ONE®) available as a tablet or oral solution; and DFX (deferasirox) available originally as dispersible tablets (DT, branded as EXJADE® or Asunra®), and later substituted with film-coated tablets (FCT) or sprinkle granules (both branded as EXJADE® in Europe or Jadenu® in the USA).

Their iron binding properties, routes of absorption, elimination, and metabolism differ (Table 4) [4, 68, 79-87]. DFO binds iron in a 1:1 ratio, which results in a very stable chelator-iron complex but is a large molecule that cannot be absorbed from the gut. DFX binds iron in a 2:1 chelator to iron ratio, and is small enough for oral absorption. DFP is even smaller and is suitable for oral absorption, but requires three molecules to bind iron, resulting in a less stable iron complex and a lower efficiency of iron binding at low chelator concentrations (low pM). The patterns of elimination of the chelator-iron complexes are shown in Table 4 and discussed as relevant for individual chelators in later sections.

Table 4. *Chemical and pharmacological properties of licensed chelators [4, 68, 79-87].*

Parameter	DFO	DFP	DFX
Molecular weight (Daltons)	560	139	373
Log iron binding affinity (pM)	26.6	19.9	22.5
Delivery	Parenteral, 8-12 hours, 5-7 days/week	Oral, 2-3 daily	Oral, once daily
Half-life of iron-free drug	20-30 minutes	3-4 hours	12-16 hours (high variability among patients)
Lipid solubility	Low	Intermediate	High
Route of iron excretion	Urinary and faecal	Urinary	Faecal
Max plasma levels (μM) of iron-free drug	7-10	90-450	80
Concentration of iron complex	Complex remains similar (about 7 μM) with ascending doses but the iron-free drug and metabolites increase	Complex correlates with urine iron excretion and predicts response to therapy	Complex accounts for about 10% of plasma drug in steady state
Minimum plasma level (μM) with daily dosing	0	0	20
Elimination of iron complex	Urine + faeces, iron complex removed more slowly than free drug	Urine	Faeces
Metabolism	Intrahepatic to metabolite B which binds iron	Glucuronide formed in liver does not bind iron	>90% eliminated in faeces, 60% unmetabolised; metabolism mainly in liver to glucuronides; oxidative metabolism by cytochrome 450 accounts for <10%; most metabolites bind iron
Chelation efficiency (% of drug excreted iron)	13	7	27

Abbreviations: DFO, deferoxamine; DFP, deferiprone; DFX, deferasirox.

8. PRACTICAL PRESCRIBING OF INDIVIDUAL CHELATORS

The potential benefits of chelation therapy must be balanced against unwanted adverse effects that are generally more likely when doses are high relative to the level of iron overload. These typically take time to develop, so that careful monitoring should reduce these risks. Table 5 summarises licensed indications (by age group), dosing, contraindications, and interactions for the three iron chelators in TDT [88-93]. Table 6 summarises key evidence on unwanted adverse effects and their monitoring requirements [56, 57, 88-117]. Unwanted effects are generally more likely at high chelator doses and at low levels of iron overload, and possibly in association with high rates of reduction in body iron. There is more information about the relationship of these variables to dose with DFO than with oral chelators. Among adverse effects listed in Table 6, diligent monitoring for sepsis risk in patients on DFP and for renal dysfunction for patients on DFX is critical.

Table 5. *Licensed indications, dosing, contraindications, and interactions for iron chelators in transfusion-dependent β-thalassaemia [88-93].*

Parameter	DFO	DFP	DFX
Indication in TDT by age	First line in ≥2 years	**USA:** first line in ≥3 years (oral solution) or ≥8 years (tablets) **Europe:** second line when current chelation therapy is contraindicated or inadequate in ≥6 years	**USA:** first line in ≥2 years **Europe:** second line when DFO is contraindicated in 2-5 years, first line in ≥6 years
Administration	Parenteral (sc, IM, or IV)	Oral, tablets or oral solution	Oral, FCT or sprinkle granules (previously DT)
Dosage and frequency	20-60 mg/kg, 5-7 day/week (50 mg/kg in Europe), children's dose up to 40 mg/kg	75 -99 (USA) / 75-100 (Europe) mg/kg/day in 2-3 divided doses daily	14-28 mg/kg/day once daily for FCT and granules (20-40 mg/kg/day for DT)
Contraindications	• Pregnancy/breast feeding (no data, but has been used in 3rd trimester) • Hypersensitivity • Severe renal disease or anuria	• Pregnancy/breast feeding • History of agranulocytosis or recurrent neutropaenia • Hypersensitivity (Henoch-Schönlein purpura. Urticaria, and periorbital oedema with skin rash)	• Pregnancy/breast feeding • Hypersensitivity • Estimated GFR <40 mL/min/1.73 m^2 (USA) or creatinine clearance <60 mL/min (Europe) • Platelet count <50 x10^9/L (USA) • Severe hepatic impairment (Child-Pugh Class C)

Table 5. *Continued*

Parameter	DFO	DFP	DFX
Key drug-drug interactions	• Co-administration with prochlorperazine may lead to temporary impairment of consciousness • Imaging results may be distorted by rapid urinary excretion of deferoxamine-bound Gallium-67 • Discontinuation 48 hours prior to scintigraphy is advisable	• Theoretical interactions with UGT1A6 inhibitors (e.g., diclofenac, probenecid, or silymarin [milk thistle]) • Avoid concomitant use with drugs associated with neutropaenia • Gallium-67 as with DFO • With oral preparations containing polyvalent cations (e.g., aluminium containing antacids and zinc), allow at least a 4-hour interval	• Theoretical interactions with drugs metabolised by CYP3A4 (e.g., midazolam) • Theoretical interactions with drugs metabolised by CYP1A2 (e.g., theophylline) • Gallium-67 as with DFO • Oral preparations containing polyvalent cations as with DFP

Abbreviations: DFO, deferoxamine; DFP, deferiprone; DFX, deferasirox; TDT, transfusion-dependent β-thalassaemia; sc, subcutaneous; IM, intramuscular; IV, intravenous; FCT, film-coated tablets; DT, dispersible tablets; GFR, glomerular filtration rate.

Table 6. *Key unwanted adverse effects and monitoring requirements [56, 57, 88-117].*

System*	DFO	DFP	DFX
Vision	• Retinopathy, typically only at high absolute doses or with continuous IV infusions • Symptoms may include: night blindness, reduced colour vision, impaired visual fields, or reduced acuity • Eye exam (visual acuity tests, slit-lamp examinations, funduscopy) annually • Electroretinography may detect early changes before symptoms are obvious, recommended in continuous or high dose treatment	• Retinopathy not generally an issue • Isolated reports of loss of vision (central scotoma)	• Retinopathy not clearly related to DFX • Diffuse retinal pigmentation in one case report • Electroretinographic effects previously seen with DFO have not been described • Eye exam before initiating treatment and annually thereafter
Hearing	• Bilateral sensorineural hearing loss at high doses relative to iron overload • Tinnitus bilaterally in severe cases • Audiometry annually, especially if SF <1000 ng/mL	• One study reported continued audiometric deterioration after switching from DFO to DFP, significance uncertain	• Very rare, significance uncertain, not clearly related to DFX • Audiometry before initiating treatment and annually thereafter
Hepatic	• Generally, well tolerated by liver • Improvement of deranged liver enzymes in severe iron overload with DFO use • ALT before initiating treatment and monthly thereafter	• Raised ALT reports variable: rates 7.5% (of 642 cases) to 65% • Liver histology fibrosis reports variable: stability or fibrosis progression • ALT before initiating treatment and monthly thereafter • Interrupt therapy if persistent increase in ALT	• Low incidence of abnormal LFT in adults, in EPIC 0.6% of 1115 TDT patients showed AST >10x ULN • Improvement in liver pathology in 219 TDT patients on DFX for ≥3 years • Contraindicated in severe (Child–Pugh C) hepatic impairment • Reduce starting dose by 50% in patients with moderate (Child–Pugh B) hepatic impairment • LFT and bilirubin before initiating treatment, every 2 weeks during the first month and monthly thereafter • If there is a persistent and progressive increase in LFT that cannot be attributed to other causes, treatment should be interrupted

Table 6. *Continued*

System*	DFO	DFP	DFX
			• Once the cause of the LFT abnormalities has been clarified or after return to normal levels, cautious re-initiation of treatment at a lower dose followed by gradual dose escalation may be considered
Renal	• Generally well tolerated, • except at very high doses • Iron complex eliminated in bile and urine, but iron complex trapped in plasma in renal failure • Reversible, dose-dependent increases in serum creatinine within the normal range observed in 14% of patients • Fluctuating proteinuria can occur irrespective of chelation in 25% of TDT, and elevation of urine calcium and cystatin C were observed in DFX or DFP plus DFO • Cases of acute renal failure requiring dialysis have been reported after high doses of DFO • Contraindicated in severe renal disease or anuria • Serum creatinine before initiating treatment then monthly	• Generally well tolerated, not nephrotoxic and can be used in the face of decreased renal function • Fluctuating proteinuria can occur irrespective of chelation in 25% of TDT, and elevation of urine calcium and cystatin C were observed in DFX or DFP plus DFO • Serum creatinine before initiating treatment then monthly	• Reversible, dose-dependent increases in serum creatinine within the normal range observed in 38% of patients, mainly at high doses • Fluctuating proteinuria can occur irrespective of chelation in 25% of TDT, and elevation of urine calcium and cystatin C were observed in DFX or DFP plus DFO • Proteinuria may occur occasionally with renal tubular acidosis • Severe dose-related renal dysfunction including some cases of fatal renal Fanconi syndrome have been reported • Prolonged exposure, possibly decades, to even low-level nephrotoxic agents may result in renal failure • The relative risk of acute kidney increases by 1.5 for each 250 ng/mL decrease in SF below 1250 • USA: contraindicated if estimated GFR <40 mL/min 1.73/m^2, reduce stating dose by 50% if GFR 40-60; and Europe: contraindicated if creatinine clearance <60 mL/min • Serum creatinine (duplicate) before initiating treatment, weekly in the first month of treatment initiation or modification, and monthly thereafter • Urinary protein before initiating treatment and monthly thereafter • Treatment should be interrupted or reduced by 7 mg/kg (FCT or sprinkle granules, 10 mg/kg if

Table 6. *Continued*

System*	DFO	DFP	DFX
			DT) if serum creatinine increase is >33% above pre-treatment average at two consecutive visits and not attributed to other causes (>age-appropriate ULN in paediatric patients) • Interrupt therapy if serum creatinine remains >33% despite dose reduction • Treatment may be reintroduced depending on individual clinical circumstances • Proteinuria can fluctuate and is not likely to require discontinuation of treatment but if there is a clear upward trend in the protein/creatinine ratio or >1 mg/g, consider interruption or dose reduction
Gastrointestinal	• Given parenterally without gastrointestinal effects	• Gastrointestinal symptoms such as nausea, vomiting, and abdominal pain were the most frequent adverse reactions reported by patients participating in clinical trials and led to discontinuation in 1.6% of patients • Starting at a lower dose and escalating to full dose over a few months can reduce gastrointestinal issues	• Mild and reported in 15-26% of patients, rarely require dose adjustment or discontinuation • Special attention should be given to patients taking concomitant medications that can increase the possibility of gastric ulceration and bleeding • FCT and sprinkle granules can be given with food and this seems to improve gastrointestinal disturbances (DT 12.8% versus FCT 4.6%)
Haematologic	• Blood counts before initiating treatment and regularly thereafter	• Agranulocytosis (ANC <0.5 × 10^9/L) may occur in 1-2% of patients, may be preceded by neutropaenia (6%), and is more likely in patients without iron overload • Blood counts with ANC before initiating treatment and every 1-2 weeks thereafter (longer frequency every 2-4 weeks may be considered after 12 months of therapy)	• Neutropaenia, agranulocytosis, worsening anaemia, and thrombocytopaenia have been reported in patients treated with DFX, mainly those with preexisting haematologic disorders like myelodysplastic syndromes • Contraindicated in patients with platelet counts below 50 x10^9/L (USA) • Blood counts before initiating treatment and regularly thereafter

Table 6. *Continued*

System*	DFO	DFP	DFX
		• Advise patients to stop treatment and seek emergent medical attention with significant fever. They should be seen in the emergency room and a standard fever-neutropaenia protocol followed based on ANC • Interrupt if neutropaenia (ANC<1.5 × 10^9/L) develops and consider granulocyte colony-stimulating factor and hospitalisation in case of agranulocytosis • In the event of neutropenia, rechallenge may be considered; in the event of agranulocytosis, rechallenge is contraindicated	
Musculoskeletal	• Observations of growth retardation and skeletal deformation with the use of high doses in young children (<3 years) with low SF levels • Regular monitoring of growth and development	• Arthralgia reported in 10% of patients • Regular monitoring of growth and development	• Generally well tolerated • No impact on growth and development in children • Regular monitoring of growth and development
Dermatologic	• Local induration and inflammation at site of infusion • Worse at concentrations >10%	• Generally well tolerated	• About 8-14% of patients develop skin rash • For rashes of mild to moderate severity, no dose adjustment is needed since the rash often resolves spontaneously. In severe cases, interrupt treatment with reintroduction at a lower dose with escalation after resolution of the rash • Severe cutaneous adverse reactions, including Stevens-Johnson syndrome, toxic epidermal necrolysis and drug reaction with eosinophilia and systemic symptoms have been reported in some cases

Table 6. *Continued*

System*	DFO	DFP	DFX
Hypersensitivity	• Very rare, can be treated by careful desensitisation, under close supervision	• Generally not an issue, occasional cases described	• Generally not an issue, occasional cases described
Infections	• Some bacterial infections are facilitated by the iron complex of DFO (e.g., *Yersinia* spp., *Klebsiella* spp. as the organisms can use the chelate-iron complex for growth	• No evidence	• No evidence
Nutritional	• Fever suggestive of septicaemia should prompt investigation and management • Decreased plasma zinc concentrations have been observed in a minority of patients • Zinc levels annually • Supplementation in the event of a deficiency	• Decreased plasma zinc concentrations have been observed in a minority of patients • Zinc levels annually • Supplementation in the event of a deficiency	• Low affinity for zinc and lower urinary excretion, does not cause constant low serum levels of these metals • Zinc levels annually • Supplementation in the event of a deficiency

**Refer to local prescribing information for full details on adverse event rates, monitoring recommendations, and adverse events management including dose modifications and interruptions.*

Abbreviations: DFO, deferoxamine; DFP, deferiprone; DFX, deferasirox; IV, intravenous; SF, serum ferritin; ALT, alanine transaminase; LFT, liver function tests; AST; aspartate transaminase; ULN, upper limit of normal; FCT, film-coated tablet; DT, dispersible tablet; GFR, glomerular filtration rate; ANC, absolute neutrophil count.

The following sections summarise evidence of beneficial effects and practical application of iron chelation strategies in monotherapy or combination. Practitioners are advised to refer to local prescribing information in their country and follow appropriate indications, dosing and dose modifications, precautions, and adverse events monitoring and management standards.

8.1. Deferoxamine monotherapy

DFO is licensed for the treatment of transfusional iron overload worldwide for affected patients above the age of 2 years, reflecting its long-standing clinical use. There are some small differences in age of treatment, commencement, and maximum doses recommended in different countries (Table 5).

8.1.1. Evidence of beneficial effects

DFO was first introduced in the late 1960s and gradually reduced the complications of iron overload and, when taken sufficiently regularly and at sufficient doses, improved survival progressively. Age of commencing chelation is a key factor affecting survival [17, 21, 23] as well as comorbidities such as hypogonadism [118] and other endocrine disturbances, including diabetes mellitus [16, 17, 23]. However, chronic treatment is costly and inconvenient, requiring subcutaneous or intravenous infusion over at least 8 hours a day at least 5 days a week in regularly transfused patients. Adherence to therapy has been the main limiting factor to successful outcomes [7]. Over-chelation can also be a problem, particularly in children or when doses are not modified for age or level of iron overload. Because of such concerns, guidelines have been conservative, generally recommending that therapy not be started until SF levels reach 1000 ng/mL, and with downward adjustment of dosing over chelation below this SF value.

8.1.2. Effects on serum ferritin

Long-term control of SF with DFO has been linked to protection from heart disease and to improved survival if levels are consistently less than 2500 ng/mL [16] with even better outcomes at levels <1000 ng/mL [17]. Control of SF is dependent on dose and frequency of use compared with transfusional iron loading rate [8]. For example, a mean daily dose of 42 mg/kg resulted in a small mean decrease in SF over one year, whereas 51 mg/kg resulted in a decrease of approximately 1000 ng/mL [106].

8.1.3. Effects on liver iron

A mean dose of 37 mg/kg stabilised LIC for baseline LIC values of between 3 and 7 mg/g dw [106]. At LIC values >14 mg/g dw, a mean dose of 51 mg/kg resulted in LIC decreases of 6.4 mg/g dw. Thus, a dose of 50 mg/kg at least 5 days a week is recommended, if a decrease to optimal LIC levels is required once growth has ceased. It should be emphasised that these are average changes and that the dose required varies with the transfusional iron rate [8].

8.1.4. Effects on cardiac iron

Myocardial iron, as estimated by mT2*, can improve with either subcutaneous or intravenous DFO, provided that treatment is given at adequate doses, frequency, and duration. Improvement in mild to moderate cardiac iron, even at low intermittent doses (5 days a week), has been confirmed [119, 120].

8.1.5. Effects on heart function

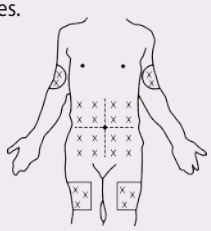
Subcutaneous DFO has long been known to prevent [121] or improve asymptomatic cardiac disease in TDT [122, 123]. After the introduction of DFO, the incidence of iron-induced heart disease fell progressively – with a key factor being the age of starting treatment [17, 124]. Symptomatic heart disease can be reversed by high dose intravenous DFO [125-127]. The same results can be obtained with excellent long-term prognosis at lower doses (50-60 mg/kg/day), and consequently less drug toxicity using continuous dosing [15, 125]. Continuous intravenous doses of 50-60 mg/kg/day can normalise LVEF over three months [6], significantly earlier than normalisation of liver or cardiac iron stores. However, if advanced heart failure has developed before treatment is intensified, the chances of successful rescue are reduced. Early intervention for decreased left ventricular function is therefore recommended. Once heart function has improved, sustained compliance is critical to improve outcomes, especially while myocardial iron remains increased [15]. See Chapter 4 for further information on treatment of heart failure.

8.1.6. Recommended treatment regimen: standard therapy

Starting DFO therapy: provided that treatment is (1) begun within 2-3 years of beginning transfusion therapy, (2) administered regularly (at least 5 times a week, preferably 7 days a week), and (3) administered in adequate doses, DFO has a well-established impact on survival and on cardiac and other complications of iron overload. In TDT, this should start before transfusions have deposited enough iron to cause tissue damage. Current practice is to start after the first 10-20 transfusions, or when SF level approaches 1000 ng/mL. If chelation therapy begins before 3 years of age, particularly careful monitoring of growth and bone development is advised, along with reduced dosage.

Standard dosing and frequency: slow subcutaneous infusion of a 10% DFO solution over 8-12 hours is recommended, a minimum of 5 days per week (see Table 7) for practical details on DFO infusion). In countries where pre-filled balloon infusers are available, this eases adherence to DFO. In general, average daily doses should not exceed 40 mg/kg until skeletal growth has been completed: 20-40 mg/kg for children and up to 50-60 mg/kg for adults, as an 8-12-hour subcutaneous infusion. To achieve *negative* iron balance in patients with average transfusion rates, 50 mg/kg/day at least 5 days a week is required. However, daily administration is preferable to maximise reduction in NTBI and thus reactive iron exposure. It is important that patients with high iron loading and those at increased risk of cardiac complications, receive adequate doses and frequency, are advised about compliance, or are considered for alternative chelator regimens.

Table 7. *Practical aspects of deferoxamine infusion.*

Parameter	Practical aspects
Strength of infusion	• The manufacturers of DFO recommend that each 500 mg vial of the drug is dissolved in at least 5 mL of water, giving a 10% solution. • Concentrations in excess of this may increase the risk of local reactions at the site of infusion.
Site of infusion	• Avoid inserting needles near important vessels, nerves or organs. • The abdomen is generally the best place. • It is often necessary to 'rotate' the sites. • The skin over the deltoid or the lateral aspect of the thigh provides alternative sites.  
Types of infuser	• For patients who find dissolving, mixing and drawing up DFO a problem, pre-filled syringes or balloons may be useful. • Balloon pumps, are smaller, lighter, and quieter than their mechanical predecessors.
Local reactions	• Persistent local reactions may be reduced by varying injection sites, lowering the strength of infusion. • Application of topical low potency corticosteroid cream after injection can reduce local reactions.
Intravenous infusions	• 10% solutions of DFO given to peripheral veins will damage and sclerose the vein. • If infused (as an emergency) into a peripheral vein, the solution must be diluted – for example in 200-500 mL of saline.
Indwelling intravenous lines	• Infection and thrombosis of catheters may occur. • Careful aseptic procedures must be followed in order to prevent possible infection. • The risk of thrombosis and infection is likely to be greater in centres that do not have regular experience in the use of long-term in-dwelling lines. • Use of prophylactic anticoagulation should be considered, as line-thrombosis is relatively common in TDT. • As development of a thrombus can occur at the tip of the catheter, it is advisable, if possible, to avoid placing the tip in the right atrium.
Intravenous DFO with blood transfusions	• Contribution to iron balance is very limited and not recommended. • Special attention must be given to avoiding accidental boluses due to DFO collecting in the dead space of the infusion line.

Abbreviations: DFO, deferoxamine; TDT, transfusion-dependent β-thalassaemia.

Dose adjustment to avoid DFO toxicity: at low SF levels (500-1000 ng/mL), the DFO dose needs to be reduced, and DFO-related toxicities monitored particularly carefully. Dose reductions can be guided using the **therapeutic index calculated as: mean daily dose (mg/kg)/SF ng/mL,** and kept at <0.025 [95]. This index is not a substitute for careful clinical monitoring. LIC may be a more reliable alternative to SF in monitoring response.

Use with vitamin C: vitamin C increases iron excretion by increasing the availability of chelatable iron but if given in excessive doses, may increase the toxicity of iron. It is recommended not to give more than 2-3 mg/kg/day as a supplement, taken at the time of the DFO infusion, so that the liberated iron is rapidly chelated.

8.1.7. Recommended treatment regimen: rescue therapy

Rescue to achieve negative iron balance: if iron has already accumulated to harmful levels, negative iron balance is necessary. With rescue therapy, dose adjustment is critical to success which may include adjustments to frequency, duration, as well as dose. Decreased dosing is required as body iron becomes better controlled. Table 8 shows how the dose can be adjusted to achieve negative iron balance, depending on the transfusion rate [8]. At transfusion rates >0.5 mg/kg/day only about half of patients will be in negative iron balance at doses of 35-50 mg/kg/day, while >50 mg/kg/day are required to achieve negative iron balance. While these recommendations serve as good starting points, ultimately, the chelation regimen must be modified based on effect on serial LIC measures and drop in SF.

Table 8. *Percentage of responders (% in negative iron balance) by deferoxamine dose and transfusion rate. Adapted with permission from [8].*

DFO dose (mg/kg)	Low transfusion rate (<0.3 mg/kg/day)	Intermediate transfusion rate (0.3-0.5 mg/kg/day)	High transfusion rate (>0.5 mg/kg/day)
35 to <50	76	75	52
≥50	100	86	89

Abbreviations: DFO, deferoxamine.

Rescue to remove cardiac iron: with mild to moderate myocardial iron (mT2* 10-20 ms), increasing the mean dose to 50-60 mg/kg/day may be sufficient to improve the mT2* provided adherence can be maintained. For patients with higher cardiac iron (mT2* 6-10 ms), other chelation regimes have been effective, such as combination of DFP with DFO or DFX monotherapy (see below). For severe cases of cardiac iron (mT2* <6 ms), more intensive regimes need to be considered (see below). For patients with abnormal LVEF, emergency therapy is recommended.

Intensive therapy for other reasons: prior to pregnancy (see Chapter 7) or haematopoietic stem cell transplantation (see Chapter 14), avoidance of high levels of iron overload is desirable, and intensification of therapy may be helpful. The optimal regimen has not been systematically studied but may include dose adjustment with attention to adherence through goal setting.

8.1.8. Recommended treatment regimen: emergency therapy

In high-risk cases with decreased LVEF, continuous infusion is potentially more beneficial than periodic infusions because it reduces exposure to toxic free iron (NTBI) [5]. Intensification through continuous, 24-hour intravenous DFO via an implanted intravenous delivery system (e.g., Port-a-cath) [125], or subcutaneously [15] has been shown to normalise heart function, reverse heart failure, improve mT2* [6, 128], and lead to long-term survival, provided treatment is maintained. In emergency before a central line can be inserted, DFO can be given through a peripheral vein, provided it is diluted in at least 100 mL of saline to avoid damage to the veins where the drug is infused. At least 50 mg/kg/day (not exceeding 60 mg/kg/day) is recommended as a 24-hour infusion [15, 125]. Higher doses have been occasionally used but DFO is not licensed at such doses and the risk of retinopathy increases. Vitamin C is recommended only when acute heart dysfunction has settled, which usually occurs by three months of continuous treatment [6]. Addition of DFP to DFO has been advocated [129] based mainly on a randomised study in patients without heart failure whose LVEF improved more on the combination arm [130]. Although another randomised study (DFO monotherapy vs same regimen with added DFP) in patients with more severe decrements in LVEF showed no difference in rate of improvement in T2* or LVEF [128]; as there were no additional side effects in the combination arm, the addition of DFP would appear reasonable here.

8.2. Deferiprone monotherapy

DFP is an orally absorbed chelator that began clinical trials in the United Kingdom in the 1980s. DFP was licensed in several countries and the European Union from the 1990s and in the USA in October 2011 [131]. The accepted indications for treatment differ slightly in different countries (Table 5).

8.2.1 Effects on serum ferritin

DFP will maintain SF at desirable levels in about one third of TDT patients. The effect on SF is generally greater when starting SF levels are high (>2500 ng/mL). Thus at 75 mg/kg/day (in three divided doses), the decreasing effect on SF was greater in patients with high baseline SF values >2500 ng/mL [132-135] but there was no decrease, when baseline values were <2500 ng/mL [99, 133, 136]. In paediatric Thai patients (age >2 years), about 45% had significant reduction of SF after 1 year at mean doses of over 79 mg/kg/day [132]. The US Food and Drug Administration (FDA) licensing agreement in 2011 concluded that with DFP monotherapy, a minimum 20% reduction in SF was met in only 50% of patients. Comparisons with DFO at various doses in pooled analyses suggested no difference between the two drugs at 12 months [100, 120, 137-140].

8.2.2. Effects on liver iron

Change in LIC with DFP has been compared with DFO [100, 102, 120, 137, 141]. After long-term monotherapy, variable levels of high residual LIC values (>15 mg/g dw) have been found (11% [142], 18% [101], and 58% [99]. Overall negative iron balance (decrease in LIC) at standard transfusion rates is achieved in only about a third of patients receiving 75 mg/kg/day [30].

8.2.3. Effects on cardiac iron

In a randomised study, higher dose DFP (92 mg/kg/day) was compared with DFO at 43 mg/kg 5-7 days/week (mean daily dose 35 mg/kg/day). The increase in mT2* was greater than in the DFO group, although relatively low DFO doses were used as the comparator [120].

8.2.4. Effects on heart function

LVEF showed a better improvement compared to patients treated with DFO [120]. In another trial, however, the results were similar with the two approaches [100], while 3 years DFP monotherapy was associated with significant increase in LVEF in patients with normal LVEF at baseline [143]. A retrospective analysis suggested DFP monotherapy offered superior cardiac protection to DFO [144].

8.2.5. Compliance with deferiprone

This varies considerably [132]. For example, compliance with DFP and DFO in one study showed rates of 95% and 72%, respectively, while another study reported rates of 94% and 93%, respectively [120]. As with other oral chelators, (i) compliance tends to be higher in the context of clinical studies than in routine use, and (ii) with respect to compliance, the importance of constant supervision and patient support should not be overlooked.

8.2.6. Long-term benefits of deferiprone monotherapy

A survival advantage of DFP either alone [144] or with DFO [145] (see below) over DFO alone has been reported in some retrospective studies. Other retrospective or observational studies used surrogate markers, such as SF, mT2*, or LVEF (though not liver iron) [143, 146, 147]. However, two *systematic* analyses have *not* found clear evidence of survival advantages of this or of any particular chelator regimen [148, 149]. A more recent retrospective analysis of 663 TDT patients with mild-moderate iron overload receiving different iron chelators in monotherapy over ten years, survival was comparable between DFO, DFP, and DFX [150].

8.2.7. Recommended treatment regimen

Starting DFP therapy: most deferiprone data came from studies that enrolled patients ≥6 years of age, and only few local trials included patients <6 years [132, 151, 152]. More recently, the independent consortium Deferiprone Evaluation in Paediatrics (DEEP), investigated safety and efficacy of DFP in young children. DEEP-3 was a multicentre, retro- and prospective, non-interventional which included TDT patients with iron overload who started DFP between the ages of one month to <18 years. Among 297 patients enrolled, 112 were <6 years of age. The study did not find any unexpected adverse drug reactions with incidences in patients <6 years of age being similar to older patients. However, agranulocytosis was also present in this paediatric population, supporting the need for regular monitoring of blood counts. Agranulocytosis from DFP using a cut-off <0.5 x10^9/L has an incidence of 2.15% and 1.23% if an agranulocytosis cut-off level of <0.2 x 10^9/L which is more strongly related to sepsis risk is used [153]. DFP agranulocytosis is not dose related nor predictable. It is critical that patients on DFP stop the drug and immediately seek medical attention if they have significant fever. They should tell emergency room staff they are on a drug that can cause low neutrophils as they will be unfamiliar with DFP, and the local fever-neutropaenia protocol should be followed. Arthropathy was also present which can have significant impact on quality of life in children and needs to be closely monitored [154] The approved dosing regimens for DFP yielded exposure in children comparable to that observed in adults and adolescents [155]. The phase 3, DEEP-2 trial compared an oral solution of DFP (75-100 mg/kg/day) to DFX DT (20-40 mg/kg/day) in transfusion-dependent patients with haemoglobinopathies who were between 1 month and 18 years of age. Around 90% of patients had TDT and 30% were <6 years of age in

both arms. Non-inferiority of DFP versus DFX was reported. No significant difference between the groups was shown in the occurrence of serious and drug-related adverse events, , but with higher study discontinuation rate in the DFP arm. Three cases of reversible agranulocytosis occurred in the DFP group [156]. DFP is now approved as first-line treatment in TDT patients ≥3 years of age (oral solution) and ≥8 years of age (tablets) in the USA 'based on a reduction in serum ferritin levels', and as second-line treatment when current chelation therapy is contraindicated or inadequate in patients ≥6 years in Europe [90-92, 157]. In Thailand and many Asian countries, DFP was registered for similar indications and is licensed for use from the age of 6 years.

Standard dosing and frequency: the dose most thoroughly evaluated is 75 mg/kg/day, in 2-3 divided doses and is recommended in adults as the starting dose. The drug is licensed up to 100 mg/kg/day (in Europe, up to 99 mg/kg/day in USA) but formal safety studies are limited. Labelling includes charts stating how many tablets and half tablets to use per dose for patient weights ranging from 20 to 90 kg. Each 500 mg tablet is scored to facilitate tablet splitting. An oral solution is also available for paediatric use. There is no guidance on dose adjustment at low SF for DFP but the label states that temporary interruption of therapy should be considered if SF falls below 500 ng/mL [90-92].

Dose escalation: while the relationship of dose to iron balance or SF trends has not been reported within a single study, the presumption is that higher doses will increase iron excretion and response rate. Iron excretion is related to DFP dose and at 100 mg/kg/day total iron excretion is similar to 60 mg/kg/day of DFO and about 1.8-fold higher than at 75 mg/kg/day of DFP [158], and is non-inferior to DFO with respect to LIC or SF when studied prospectively [159]. Doses of 100 mg/kg/day have been given in at least one small prospective study, with no reported increase in side effects [120] and the drug is licensed up to this dose. Patients without iron overload may be more likely to develop agranulocytosis [160].

Use with vitamin C: the effect of vitamin C on iron excretion with DFP is not clear and is thus not recommended.

8.3. Deferasirox monotherapy

DFX was developed as a once-daily oral monotherapy for the treatment of iron overload. The drug is licensed as first-line monotherapy for TDT in over 100 countries worldwide, although the earliest age at which deferasirox qualifies as first-line treatment differs between the USA and Europe (Table 5) [88, 89].

8.3.1. Chemistry and pharmacology

DFX is an orally absorbed chelator binding iron in a 3:1 ratio. The chemical properties and pharmacology are summarised in Table 4. The original DT formulation has been replaced in many countries by a FCT (or sprinkle granules) formulation that is better absorbed, and preferred by patients [109, 161]. Due to enhanced absorption, doses of the FCT (or sprinkle granules) formulation need to be decreased to 0.7 times of those previously recommended with DT. Dosing in studies published mostly before 2018 therefore need to be converted to the equivalent dosing of FCT (or sprinkle granules). Importantly, absorption and clearance kinetics are not the same in all patients. Plasma levels are low in 60% and very low in 30% of individuals 12 hours after a single dose [84]. This would result in inadequate NTBI removal equivalent to not chelating a third of the time. Some studies suggested that splitting the dose twice a day

may significantly increase the area-under-the-curve exposure [162] and improves chelation efficiency [85, 86, 163].

8.3.2. Effects on serum ferritin

There is a dose-dependent effect on SF [106, 164, 165]. A randomised study found that 20 mg/kg/day of the original DT formulation stabilised SF close to 2000 ng/mL. At 30 mg/kg/day, mean SF was reduced by 1249 ng/mL over 1 year [106]. Longer-term, the proportion of patients with SF <1000 ng/mL and less than 2500 ng/mL decreased progressively. At 4-5 years follow up, median SF fell to <1500 ng/mL. Overall, 73% of patients attained SF levels ≤2500 ng/mL and 41% of patients achieved SF ≤1000 ng/mL, compared with 64% and 12% at baseline [108]. The interaction between dose and SF response was examined in over 1000 TDT patients in the EPIC study [103]. The initial dose (original DT) was 20 mg/kg/day for patients receiving 2-4 packed red blood cell units/month, and 10 or 30 mg/kg/ day for patients receiving less or more frequent transfusions. Dose adjustments were made on the basis of SF trends at 3 monthly intervals. A significant though modest SF fall was seen at 1 year. The largest SF decrease of 1496 ng/mL/year was noted in patients with the highest baseline SF (baseline median SF 6230 ng/mL) [166] but who were treated at high dosages (35-40 mg/kg/day). These doses or equivalent FCT (or sprinkle granules) doses are now recommended for heavily iron overloaded patients. Interestingly, SF levels that had not dropped after six months on once daily dosing, steadily decreased when the same daily dose was changed to every 12-hour dosing [163], consistent with a 12-25% increase in DFX exposure [162] and increased chelation efficiency with twice daily dosing [85, 86]. This suggests better NTBI control throughout the day, less exposure to reactive Fe^{2+} and less stimulation of SF production.

8.3.3. Dose effects on iron balance

The dose effects below refer to the original DT formulation, unless otherwise stated. Short-term formal balance studies demonstrated the effect of dose on iron balance [167]. In a longer-term large, randomised study, iron balance was determined by changes in LIC over 1 year [106]. In TDT, negative iron balance was achieved at 30 mg/kg/day, with a mean LIC decrease of 8.9 mg/g dw. The blood transfusion rate influenced the response to treatment (Table 9) [8].

Table 9. *Percentage of responders (% in negative iron balance shown by decreases in liver iron concentration) by deferasirox dispersible tablet dose and transfusion rate. *The equivalent dose of the new film-coated tablet formulation is provided in brackets for ease of reference. Adapted with permission from [8].*

DFX dose (mg/kg)	Low transfusion rate (<0.3 mg/kg/day)	Intermediate transfusion rate (0.3-0.5 mg/kg/day)	High transfusion rate (>0.5 mg/kg/day)
10 (7*)	29	14	0
20 (14*)	76	55	47
30 (21*)	96	83	82

Abbreviations: DFX, deferasirox.

8.3.4. Effect on cardiac iron

Improvement in mT2* was first reported in a retrospective analysis after 1 and 2 years [168, 169]. Prospective data demonstrated the efficacy of DFX in improving mT2* over 1 year with a baseline mT2* range of 5-20 ms [170], while, a steady, significant mT2* improvement was observed, from a mean of 12.0 ms at baseline to 17.1 ms after 3 years, despite a high baseline LIC (mean 28 mg/g dw) [171]. Here, 68% of patients with baseline mT2* between 10 to <20 ms normalised mT2*, and 50% of patients with mT2* >5 to <10 ms at baseline improved it to 10 to <20 ms. There was no significant variation in LVEF and no deaths. A randomised 1 year comparison of subcutaneous DFO (50-60 mg/kg/day for 5-7 days/week) with DFX DT (target 40 mg/kg/day) for patients with mT2* of 6-20 ms [119] and high baseline LIC (mean 29.8 mg/g dw) found similar improvements in mT2* with both drug regimens, with sustained improvements over 2 years [172]. Taken together, these studies show that DFX is effective for patients with mT2* from >5-20 ms. As with other chelation regimes, high baseline heart iron (mT2* <10 ms) will typically take several years to clear.

8.3.5. Effects on heart function

In the above studies, LVEF remained stable despite low mT2* values, and there were neither deaths nor episodes of symptomatic heart failure. Only one case of atrial fibrillation and one case of cardiomyopathy were reported. As previously mentioned, according to risk analysis of heart failure and mT2* in TDT from other cohorts, the risk of developing cardiac failure was expected to be substantial, with a relative risk 160-fold higher for patients with mT2* <10 ms [51]. The stability of LVEF and the absence of heart failure in this high-risk group of patients suggests that DFX renders effective prophylaxis for heart failure, even in patients with mT2* values of 5-10 ms. This may be related to the 24-hour 'protection time' against labile iron that results from the long plasma half-life [173]. Other smaller studies show similar stability over 5 years [174]. A 12-year real-world follow up study also showed no cases of heart failure on treatment, and for the first time, also showed progressive improvement in LVEF [175]. However, DFX has not been formally evaluated in patients with symptomatic heart failure or LVEF <56%, and other chelation options are recommended for these.

8.3.6. Convenience and impact on quality of life

Convenience and quality of life, as with other oral chelation regimes, are expected to impact on adherence and hence survival. This is likely to have a greater impact outside formal clinical studies. In the large-scale EPIC study, patients reported improved quality of life (estimated by SF36 scores) and greater adherence to chelation therapy compared with baseline before starting DFX.

8.3.7. Film-coated tablet and sprinkle granule formulations

The FCT was introduced in 2017, although the DT continues to be used in some countries. Data on the FCT are not as extensive as in the registration studies. Over a 2-year period in the randomised ECLIPSE trial, TDT patients (≥10 years old) transitioning to the FCT had a reduction in SF relative to prior treatment with DT. There were no new safety concerns with the FCT preparation, and no major concerns associated with hepatic or renal safety, or haematological abnormalities (thrombocytopaenia/neutropaenia) [109, 151, 176]. Studies showed further improvements in adherence and patient reported acceptability as well as better gastrointestinal

tolerability (FCT can be taken with food) [161, 177]. The MIMAS study evaluated the DFX FCT formulation in younger patients (2 to <6 years) at doses of 14-28 mg/kg/day. The full daily dose had to be crushed and administered by sprinkling on soft food to be consumed immediately. Similar tolerability was noted as with adults, with an overall favorable patient satisfaction and decreased concerns regarding palatability and gastrointestinal symptoms and improved treatment adherence [178]. The JUPITER trial recruiting TDT patients ≥2 years of age also confirmed distinct patient preference upon switching from DFX DT to FCT [179]. More recently, the randomised CALYPSO trial included TDT patients with a median age of 5 years (range 2-16 years) and showed comparable compliance, safety, and efficacy of a new DFX sprinkle granules formulation and DFX DT [157, 180].

8.3.8. Recommended treatment regimen: standard therapy

Starting DFX therapy: the criteria for starting treatment (SF level and number of transfusions) are similar to those of DFO. The labelling for age of commencement differs in countries following USA licensing (first line for ≥2 years) from those that adhere to European Union licensing (first line for ≥6 years, second line for 2-5 years) [88, 89]. A fall in SF and LIC has been seen across all age groups ≥2 years, with no age-related adverse effects on growth, sexual development or bones [103, 108, 165, 181-184].

Standard dosing and frequency: the original DT formulation was taken as a suspension in water once daily. The FCT is recommended to be swallowed whole before food or with a light meal. it may also be crushed and mixed with soft foods (e.g., apple sauce or yogurt) immediately before taking it orally. Granules can be sprinkled on soft food immediately before taking it orally. Due to increased absorption of the FCT and sprinkle granules formulations, they should be taken at 0.7 times the DT dose shown to be effective and safe. The starting dose for FCT (or sprinkle granules) is 14 mg/kg/day for TDT patients who have received 10-20 transfusion episodes. Optimal maintenance doses will depend on the transfusional iron-loading rate (Table 9). For patients with high transfusional iron loading rates (>21 mL/kg/month of packed RBC) a higher dose of 21 mg/kg day will be necessary. For patients with already severe iron overload, doses of 21-28 mg/kg/day may be required. Due to the rapid fall in SF that can occur with improved adherence using the FCT (or sprinkle granules), careful monitoring of SF trends and serum creatinine, with downward adjustment of dose when SF values fall below 1000 ng/mL is advisable [185]. A target of 500-1000 ng/mL appears to be achievable with DFX without additional toxicity, provided that doses are adjusted downwards as SF falls towards 500 ng/mL.

8.3.9. Recommended treatment regimen: rescue therapy

Patients with mild to moderate cardiac iron (mT2* 5-20 ms): in prospective studies, DFX resulted in preservation and stabilisation of left ventricular function. Doses of up to 40 mg/kg of the original DT formulation (equivalent to 28 mg/kg FCT or sprinkle granules) have been used and are advisable in patients with very high levels of liver iron or SF [186].

Patients with severe cardiac iron (mT2* <6 ms): prospective trials have been confined to patients with mT2* values ≥6 ms. For patients with mT2* <6 ms, other alternative chelation regimes are recommended.

Patients with reduced LVEF or symptomatic heart failure (emergency therapy): DFX has not been formally evaluated for this indication and is therefore not recommended.

9. COMBINATION THERAPIES

The term 'combination therapy' has been used to cover approaches to improve outcomes if monotherapy is inadequate. These are given when either there is dose limiting toxicity with a monotherapy (for example renal toxicity with DFX) or when it is hoped that by combining two chelators, we may improve chelation efficacy (for example DFO with DFP for siderotic heart failure). Most combinations have not been subjected to large, randomised trials and drug labelling typically describes the benefits and risks of monotherapies. Guidelines and expert reviews of combinations may fill these gaps [187] but the level of evidence is typically less than with monotherapies.

9.1. Pharmacology of combination therapies

In principle, two chelators can be given at the same time (simultaneously, true combination) or one after the other (sequentially). If the drugs are given simultaneously, they may interact in a process that involves the 'shuttling' of iron, which may lead to additional chelation of iron from cells or plasma NTBI [188] and so improved efficiency of iron chelation in plasma [189]. Simultaneous exposure to two chelators may in principle cause synergistic removal of cellular iron, as was demonstrated in cell culture with combinations of all three chelators [190]. The most commonly used regimes have included DFP daily at standard divided doses, combined with varying frequency and dosing of DFO. More recently, combinations of DFX with DFO or DFX with DFP have been evaluated. Available trails have been mostly small and/or investigator-led rather than by pharmaceutical companies.

9.2. Combined deferoxamine and deferiprone

Various combinations of DFP and DFO have been used, either in the context of a formal trial or on an *ad hoc* basis, usually when monotherapy with DFO or DFP has failed to control iron overload or its effects. If compliance with DFO is poor, the use of DFP can improve overall exposure to chelation. Alternatively, if control of SF or LIC is inadequate with DFP monotherapy, DFO can be added to increase iron excretion.

9.2.1. Effects of on serum ferritin

In a randomised study [130], SF decreased more with combined treatment (DFO five days a week plus DFP seven days a week) than with standard DFO monotherapy (40 mg/kg five days a week). However, in another randomised study [66], SF decreased more with combination therapy than DFP monotherapy but similarly to DFO monotherapy 5 nights a week. Alternating 5 days of DFP with 2 days of DFO/week showed comparable efficacy to DFO monotherapy 5-7 days/week [191]. On the other hand, a 5-year randomised clinical trial also showed a greater SF reduction with sequential DFP (75 mg/kg/day for 4 days/week) and DFO (50 mg/kg/day for 3 days/week) compared with DFP alone, but with no clear survival benefit [192]. While much of the variability between trials may relate to variable compliance in the DFO monotherapy group, taken together, these studies show that SF can be controlled with a relatively low frequency of DFO, for example, twice a week when combined with DFP at standard doses (75 mg/kg/day), while sequential therapy may benefit some patients.

9.2.2. Effects on liver iron

Here there is less evidence available and results are variable. Overall, results support combination therapy being more effective than DFP monotherapy so that DFO monotherapy or combination

therapy with DFP plus DFO (two times weekly) was more effective than DFP monotherapy at controlling LIC [66]. Another randomised study showed that combination was more effective than DFO monotherapy [130]. Similarly, in patients with heart failure who received DFO with or without DFP, there was decrease in LIC and SF in the combination arm but not with DFO monotherapy [128].

9.2.3. Effects on cardiac iron and heart function

In a randomised study, LVEF increased within the normal range by approximately 2.5% in a combination arm vs 0.5% in the DFO monotherapy arm [130]. For high-risk patients with decreased LVEF or with symptomatic heart disease, a randomised study showed equivalent improvement in LVEF and mT2* in patients receiving either DFO intensification or DFO intensification plus DFP [128]. Observational studies have also reported improvements in heart function within the normal range using combined treatment over 12-57 months [193] or 3 years [194]. If the imperative is to have improvement in mT2* as rapidly as possible (for example in preparation for or prior to haematopoietic stem cell transplantation), then continuous intravenous DFO is an option, or the use of high-dose DFP monotherapy (>90 mg/kg) or regular doses of DFP in combination with standard DFO (5 days a week).

9.2.4. Long-term effects on survival

Some retrospective analyses reported survival benefit compared with the previously prescribed DFO alone [145, 195]. However, 75 of 344 patients discontinued chronic combination therapy because of agranulocytosis (5%), recurrent neutropaenia (2.9%), gastrointestinal disturbances (5.6%), arthralgia (1.6%), allergic reactions (0.7%), weight gain (0.7%), increased liver enzymes (0.3%), non-adherence to DFO (3.3%), pregnancy (2.6%) and other reasons (2%). The very low SF using a 'flexible' approach to combination therapy has also been reported to reverse organ dysfunction without adverse effects [77]. However, controlled trials and meta-analysis demonstrating improvement in disease-related symptoms, organ function, or increased survival are lacking [149].

9.2.5. Safety

Formal safety data on combined DFO and DFP treatment are limited. The side effects described above are largely consistent with the known effects of the individual chelating agents, with the possible exception of cerebellar syndrome in a single case [145, 195]. Tolerability of simultaneous combinations may differ from sequential use, but has not been formally studied.

9.2.6. Possible treatment regimens

Combinations of DFP and DFO are useful, especially when various monotherapy regimes have not been adhered to and/or have failed to control either liver or cardiac iron. If a patient is not doing well with DFP monotherapy, combined treatment offers an additional option to improve iron balance. For patients not doing well with DFO monotherapy for reasons of compliance, and where dose intensification has failed, combined treatment has been used as a way of decreasing the frequency at which DFO is needed to maintain SF and iron balance. For patients with very high levels of cardiac iron (<8 ms) and/or cardiac dysfunction without frank heart failure, 24-hour treatment with DFO and daily therapy with DFP should be strongly considered.

9.3. Combined deferasirox and deferoxamine

Prospective studies have shown rapid removal of liver iron and improvement in cardiac iron in patients receiving DFX plus DFO combinations [52, 189]. In 60 patients with severe liver and heart iron overload (mT2* 5-10 ms) treated with DFX DT 20-40 mg/kg/day for 7 days per week plus DFO 40 mg/kg/day for 5 days per week, SF and LIC decreased by 44% and 52%, respectively, with increase in mT2* of 33%. Improvements in mT2* were greater in patients with baseline LIC <30 those with LIC >30 mg/g dw. LVEF remained stable during the study. Tolerability was consistent with monotherapy regimes [52].

9.4. Combined deferasirox and deferiprone

This combination synergistically mobilises cardiomyocytic iron in cell culture [190] consistent with clinical case reports of effectiveness [196]. For example, in 16 patients with TDT, SF, LIC, and mT2* improved over 2 years without adverse effects [197] with reversal of cardiac dysfunction in 2/4 patients. In a larger 1 year randomised trial [198] of 96 paediatric patients, two combination regimes were compared: DFP 75 mg/kg in two divided doses was given in both regimes and combined with either DFX DT 20 mg/kg once daily, or with overnight DFO at 40 mg/kg. SF, LIC, and mT2* improved significantly in both groups with greater improvement in mT2* and in quality of life in the oral combination group with no serious adverse events. In another study of 49 multi-transfused children in India, combination of DFP in 3 divided doses with DFX DT 30 mg/kg/day was well tolerated and more effective at decreasing SF than DFX monotherapy [199]. In a single arm study of 36 young patients previously treated with monotherapies, the SF was reduced by 3275 ng/mL at 1 year while about 20% of patients experienced mild gastrointestinal side effects, joint symptoms, raised aspartate transaminase, or had 25% raised creatinine >33% above baseline [200]. In a smaller study, common adverse events included arthralgia and gastrointestinal symptoms, while no episodes of neutropaenia/agranulocytosis occurred [201].

10. WHICH CHELATION REGIMEN, WHEN, AND HOW MUCH?

10.1. Standard therapy for obtaining iron balance

The licensing of individual chelators, specified in the country where the treatment is prescribed, should act as an initial guide on when to start the therapy and at what dose. Standard first-line doses have been discussed above and depend in part on the rate of transfusional iron loading. Starting chelation before overload has built up, or irreversible damage has occurred, is critical to success. With DFO, chelation is often withheld until the SF had reached 1000 ng/mL because of fears toxicity would have on growth, ears and eyes at low levels of body iron. If a patient is failing on first-line therapy, dose adjustment and attention to adherence (practical as well as psychological support) are the next steps. If this fails, then regimen adjustment can be considered, depending on the circumstances – some of which are described below.

10.2. Iron load too high or increasing: rescue therapy to achieve negative iron balance

If body iron load builds up because of delays in starting chelation therapy, inadequate dosing, and/or poor adherence, or because of poor response to an individual monotherapy, rescue therapy is required by one or more of the following:

- **Increasing the daily dose of chelation.**
  - DFO monotherapy is effective at producing negative iron balance if it is given in sufficient doses and sufficient frequency, but adherence is often a problem.
  - Dose escalation of DFX is effective at producing negative iron balance. Doses of DFX DT >35 mg/kg and up to 40 mg/kg (up to 28 mg/kg for FCT or sprinkle granules) are effective in patients with high LIC or SF.
  - DFP monotherapy is likely to achieve iron balance at 75 mg/kg in only about one third of patients, with average transfusion rates. It may be increased up to 100 mg/kg with close monitoring. DFO is often added.
- **Increasing the frequency of the chelator (improving adherence or increasing prescription advice)**
  - If adherence is the major reason for a regimen not working, every effort should be made by the healthcare team to support the patient and their family in achieving better adherence.
- **Switching chelator regimen.**
- **Rotating or combining chelators.**
  - Rotation of individual monotherapies (sequential chelation) can be helpful in managing individual patients, often for reasons of adherence as much as for specific complications.
  - True 'combination therapy' (where two chelators are combined with some degree of overlap pharmacologically) is widely practiced, although not specifically licensed. This can be useful when monotherapy is inadequate, either to control iron balance or to control iron distribution, particularly in the heart.

10.3. Mild increase in cardiac iron (myocardial T2* 10-20 ms): rescue therapy to remove cardiac iron

DFO, DFP, and DFX monotherapy can all be effective at decreasing cardiac iron, but need to be given without interruption for optimal effects and at adequate doses. The immediate risk of heart failure is low, provided that the patient remains on chelation therapy without interruption [51]. Regular daily monotherapy at optimal doses (often an increase from current dose or frequency) will usually improve heart iron but can take several years of consistent therapy to normalise. Monotherapy with DFX is usually effective at improving mT2* across a full range of LIC concentrations [119, 171]. If the imperative is to do this as rapidly as possible (for example in preparation for pregnancy or prior to haematopoietic stem cell transplantation), then high dose DFP monotherapy (>90 mg/kg) or regular doses of DFP in combination with standard DFO 5 days a week should be used, rather than DFO alone when given subcutaneously 5 days a week. DFX has not been compared directly with DFP either alone or in combination with DFO. If there is no trend of improvement in mT2* with DFO, DFP, or DFX monotherapy, then combined DFP and DFO should be considered.

10.4. Myocardial T2* <10 ms: rescue therapy for cardiac iron overload

The risk of developing heart failure increases with lower mT2*, especially when mT2* values drop below 10 ms (i.e., higher cardiac iron). However, if continuous chelation therapy is given, heart failure may be prevented even before the mT2* is corrected. As mentioned earlier, this has been

shown for continuous 24-hour DFO, with high dose DFX in a population where mT2* was 6-10 ms, and in patients treated with different combination regimes (DFO+DFP, DFO+DFX). Patients with mT2* ≤6 ms, are a very high-risk group for developing heart failure. This group has not been evaluated extensively with interventional studies (except people with heart failure). There is some experience of treating these patients with DFO+DFP, but randomised trials did not include patients with mT2* values <8 ms [130]. In the absence of formal comparisons with other regimens, the combination of DFO (given as often and as continuously as possible) with DFP at standard doses is recommended. DFX monotherapy at doses >30 mg/kg/day (>21 mg/kg of FCT or sprinkle granules) has also been shown to be effective for patients with T2* >5 ms and normal heart function. If patients also have high levels of body iron (high LIC) as well as heart iron, it is important that the regimen also reduces total body iron.

10.5. Patients with heart failure: reverse heart failure

If chelation therapy is taken regularly, clinical heart failure is now rare. Reversal of heart failure requires continuous DFO therapy and can occur within a few weeks of starting treatment. This will not succeed in all cases, but if started early in the development of heart failure, is usually effective. The addition of DFP in these circumstances may be beneficial, although a small, randomised comparison did not show a difference with or without DFP [128]. Once reversal of heart failure has been demonstrated both clinically and with myocardial MRI or echocardiography, continuation of the same therapy is recommended until the mT2* improves to >8 ms, which may take many years, depending on the starting mT2*. The key to success is the timely introduction of intensification and the maintenance of intensive treatment after the heart failure has been corrected.

10.6. Downward adjustment of chelator dose if body iron falls rapidly or reaches low levels

An increasingly common challenge for patients who respond well to a chelation regimen is that the clinician does not recognise this and/or does not adjust the dose downward soon enough to prevent toxicity from over-chelation. This is more likely in centres without long-term or regular experience in monitoring and prescribing chelation. Regular monitoring for SF trends (every 1-3 months) and for the known toxicities of each chelator are minimum requirements. The general principle of downward dose adjustment with rapidly falling body iron loads is clear, but the specifics as regards how much and when are less so. In general, the risk of over chelation with DFO increases when the SF is low relative to the dose. This has not been analysed systematically with other chelators. With DFX, low levels of SF, even below 500 ng/mL can be safely achieved, even in patients not receiving transfusion, provided that the doses of DFX are low (5-10 mg/kg of DT formulation) [202]. Cases of toxicity from over-chelation have been observed even at SF >500 ng/mL or if the rate of decrease is rapid. DFX dose adjustment should be made at the first sign of increasing serum creatinine values. With DFP it is not clear whether to or how to adjust dosing at low levels of SF or with rapid decrements in SF.

KEY POINTS AND RECOMMENDATIONS

1. Uncontrolled transfusional iron overload increases the risks of heart failure, endocrine damage, liver cirrhosis and hepatocellular carcinoma in transfusion-dependent β-thalassaemia (TDT) (**Grade C**).

2. The absolute change in total body iron in response to transfusion and chelation can be calculated from change in liver iron concentration (LIC). The direction of change in body iron in response to transfusion and chelation can usually but not always be estimated from the trend in serum ferritin (SF). Cardiac storage iron concentration is directly related to the risk of heart failure, which can be reliably estimated by myocardial T2* (mT2*). Cardiac iron accumulates later than liver iron, and is rare before the age of 8 years or 5 years of transfusion on those beginning regular transfusion later in life, affecting a subset of patients; while chelation of storage iron from the liver tends to be faster than from the myocardium (**Grade B, Class I**).

3. Serial SF measurement is indicated in all TDT patients, to be conducted regularly at least every 3 months or at shorter/longer frequencies as needed based on iron overload level and iron chelation modification needs (**Grade B, Class I**).

4. Hepatic and cardiac magnetic resonance imagining (MRI) for the assessment of LIC and mT2* should be performed annually starting the age of 8-10 years (or earlier if feasible without sedation need). Shorter/longer frequencies can be adopted as needed based on iron overload level and iron chelation modification needs. Reading and interpretation should be done by trained staff or outsourced third party vendors, using a validated method with appropriate calibration and MRI acquisition techniques (**Grade B, Class IIa**).

5. LIC determination should be considered in patients whose SF levels are high (>4000 ng/mL) or deviate from expected trends, or when new chelating regimes are being used. LIC assessment cannot predict (or replace) mT2* assessment (**Grade C, Class IIb**). An algorithm for prioritisation of MRI assessment when resources are limited is provided in Chapter 4, Figure 15.

6. TDT patients should undergo regular assessment for growth, development, and organ function (including the heart, liver, and endocrine glands) as per recommendations in respective Chapters in these Guidelines.

7. Chelation therapy is an effective treatment modality in improving survival, decreasing the risk of heart failure, and decreasing morbidities from transfusion-induced iron overload (**Grade C, Class I**).

8. Chelation therapy at the correct doses and frequency can balance iron excretion with iron accumulation from transfusions (**Grade A, Class I**).

9. Prevention of iron accumulation using chelation therapy is preferable to rescue treatment because iron-mediated damage is often irreversible, and removal of storage iron by chelation is slow - particularly after it has escaped the liver (**Grade B, Class I**).

10. Response to chelation is dependent on the dose applied and the duration of exposure (**Grade A, Class I**).

11. Response to chelation is affected by the rate of blood transfusion (**Grade B, Class I**).

12. Chelation therapy removes myocardial storage iron slowly (months or years) (**Grade A, Class I**).

13. Chelation can reverse iron-mediated cardiac dysfunction rapidly (within weeks) by rapid chelation of labile iron, if 24-hour chelation cover is achieved (**Grade B, Class IIa**).

14. The optimal chelation regimen and dosing depend on approved local indications (Table 5) and prescribing information of individual chelators, must be tailored for the individual, and will vary based on current clinical situation and iron overload profile (refer to Section 10 in this Chapter) (**Grade A, Class I**).

15. Over-chelation increases side effects from chelation therapy, and doses should therefore be decreased as serum ferritin or liver iron levels fall (demonstrated most clearly with deferoxamine) (**Grade B, Class I**).

16. Patients receiving iron chelation should be closely monitored for unwanted adverse effects and their management including dose modifications/interruptions according to local prescribing information (**Grade A, Class I**).

17. Chelation therapy will not be effective if it is not taken regularly – a key aspect of chelation management is to work with patients and their families to optimise adherence (**Grade B, Class I**).

REFERENCES

1. Kattamis A, Kwiatkowski JL, Aydinok Y. Thalassaemia. *Lancet* 2022;399(10343):2310-2324.
2. Taher AT, Musallam KM, Cappellini MD. beta-Thalassemias. *N Engl J Med* 2021;384(8):727-743.
3. Porter JB. Iron Through the Prism of Haematology. *Br J Haematol* 2020;191(4):587-592.
4. Coates TD. Management of iron overload: lessons from transfusion-dependent hemoglobinopathies. *Blood* 2024. Online ahead of print.
5. Porter JB, Abeysinghe RD, Marshall L, Hider RC, Singh S. Kinetics of removal and reappearance of non-transferrin-bound plasma iron with deferoxamine therapy. *Blood* 1996;88(2):705-713.
6. Anderson LJ, Westwood MA, Holden S, Davis B, Prescott E, Wonke B, Porter JB, Walker JM, Pennell DJ. Myocardial iron clearance during reversal of siderotic cardiomyopathy with intravenous desferrioxamine: a prospective study using T2* cardiovascular magnetic resonance. *Br J Haematol* 2004;127(3):348-355.
7. Gabutti V, Piga A. Results of long-term iron-chelating therapy. *Acta Haematol* 1996;95(1):26-36.
8. Cohen AR, Glimm E, Porter JB. Effect of transfusional iron intake on response to chelation therapy in beta-thalassemia major. *Blood* 2008;111(2):583-587.
9. Guerra A, Parhiz H, Rivella S. Novel potential therapeutics to modify iron metabolism and red cell synthesis in diseases associated with defective erythropoiesis. *Haematologica* 2023;108(10): 2582-2593.
10. Cazzola M, Borgna-Pignatti C, Locatelli F, Ponchio L, Beguin Y, De Stefano P. A moderate transfusion regimen may reduce iron loading in beta-thalassemia major without producing excessive expansion of erythropoiesis. *Transfusion* 1997;37(2):135-140.
11. Porter JB, Garbowski M. The pathophysiology of transfusional iron overload. *Hematol Oncol Clin North Am* 2014;28(4):683-701, vi.
12. Angelucci E, Brittenham GM, McLaren CE, Ripalti M, Baronciani D, Giardini C, Galimberti M, Polchi P, Lucarelli G. Hepatic iron concentration and total body iron stores in thalassemia major. *N Engl J Med* 2000;343(5):327-331.
13. Taher AT, Saliba AN. Iron overload in thalassemia: different organs at different rates. *Hematology Am Soc Hematol Educ Program* 2017;2017(1):265-271.
14. Shah FT, Porter JB, Sadasivam N, Kaya B, Moon JC, Velangi M, Ako E, Pancham S. Guidelines for the monitoring and management of iron overload in patients with haemoglobinopathies and rare anaemias. *Br J Haematol* 2022;196(2):336-350.
15. Davis BA, O'Sullivan C, Jarritt PH, Porter JB. Value of sequential monitoring of left ventricular ejection fraction in the management of thalassemia major. *Blood* 2004;104(1):263-269.
16. Olivieri NF, Nathan DG, MacMillan JH, Wayne AS, Liu PP, McGee A, Martin M, Koren G, Cohen AR. Survival in medically treated patients with homozygous beta-thalassemia. *N Engl J Med* 1994;331(9):574-578.
17. Borgna-Pignatti C, Rugolotto S, De Stefano P, Zhao H, Cappellini MD, Del Vecchio GC, Romeo MA, Forni GL, Gamberini MR, Ghilardi R, Piga A, Cnaan A. Survival and complications in patients with thalassemia major treated with transfusion and deferoxamine. *Haematologica* 2004;89(10): 1187-1193.
18. Telfer PT, Prestcott E, Holden S, Walker M, Hoffbrand AV, Wonke B. Hepatic iron concentration combined with long-term monitoring of serum ferritin to predict complications of iron overload in thalassaemia major. *Br J Haematol* 2000;110(4):971-977.
19. Kamperidis V, Vlachou M, Pappa Z, Pantelidou D, Karamitsos T, Papadopoulou D, Kartas A, Boutou A, Ventoulis I, Vlachaki E, Giannakoulas G, Karvounis H. Prediction of long-term survival in patients

with transfusion-dependent hemoglobinopathies: Insights from cardiac imaging and ferritin. *Hellenic J Cardiol* 2021;62(6):429-438.
20. Musallam KM, Barella S, Origa R, Ferrero GB, Lisi R, Pasanisi A, Longo F, Gianesin B, Forni GL, Webthal p. Revisiting iron overload status and change thresholds as predictors of mortality in transfusion-dependent beta-thalassemia: a 10-year cohort study. *Ann Hematol* 2024;103(7):2283-2297.
21. Forni GL, Gianesin B, Musallam KM, Longo F, Rosso R, Lisi R, Gamberini MR, Pinto VM, Graziadei G, Vitucci A, Bonetti F, Musto P, Piga A, Cappellini MD, Borgna-Pignatti C, Webthal p. Overall and complication-free survival in a large cohort of patients with beta-thalassemia major followed over 50 years. *Am J Hematol* 2023;98(3):381-387.
22. Belhoul KM, Bakir ML, Saned MS, Kadhim AM, Musallam KM, Taher AT. Serum ferritin levels and endocrinopathy in medically treated patients with beta thalassemia major. *Ann Hematol* 2012;91(7):1107-1114.
23. Brittenham GM, Cohen AR, McLaren CE, Martin MB, Griffith PM, Nienhuis AW, Young NS, Allen CJ, Farrell DE, Harris JW. Hepatic iron stores and plasma ferritin concentration in patients with sickle cell anemia and thalassemia major. *Am J Hematol* 1993;42(1):81-85.
24. Porter JB, Elalfy M, Taher A, Aydinok Y, Lee SH, Sutcharitchan P, El-Ali A, Han J, El-Beshlawy A. Limitations of serum ferritin to predict liver iron concentration responses to deferasirox therapy in patients with transfusion-dependent thalassaemia. *Eur J Haematol* 2017;98(3):280-288.
25. Bayraktaroglu S, Karadas N, Onen S, Karapinar DY, Aydinok Y. Modern management of iron overload in thalassemia major patients guided by MRI techniques: real-world data from a long-term cohort study. *Ann Hematol* 2022;101(3):521-529.
26. Puliyel M, Sposto R, Berdoukas VA, Hofstra TC, Nord A, Carson S, Wood J, Coates TD. Ferritin trends do not predict changes in total body iron in patients with transfusional iron overload. *Am J Hematol* 2014;89(4):391-394.
27. Worwood M, Cragg SJ, Jacobs A, McLaren C, Ricketts C, Economidou J. Binding of serum ferritin to concanavalin A: patients with homozygous beta thalassaemia and transfusional iron overload. *Br J Haematol* 1980;46(3):409-416.
28. Ang AL, Shah FT, Davis BA, Thomas A, Murugachandran G, Kumuradevan J, Garbowksi MW, Porter JB. Deferiprone Is Associated with Lower Serum Ferritin (SF) Relative to Liver Iron Concentration (LIC) Than Deferoxamine and Deferasirox- Implications for Clinical Practice [abstract]. *Blood* 2010;116(21):4246.
29. Danjou F, Origa R, Anni F, Saba L, Cossa S, Podda G, Galanello R. Longitudinal analysis of heart and liver iron in thalassemia major patients according to chelation treatment. *Blood Cells Mol Dis* 2013;51(3):142-145.
30. Fischer R, Longo F, Nielsen P, Engelhardt R, Hider RC, Piga A. Monitoring long-term efficacy of iron chelation therapy by deferiprone and desferrioxamine in patients with beta-thalassaemia major: application of SQUID biomagnetic liver susceptometry. *Br J Haematol* 2003;121(6):938-948.
31. Vitrano A, Sacco M, Rosso R, Quota A, Fiorino D, Oliva E, Gerardi C, Roccamo G, Spadola V, Filosa A, Tese L, Calvaruso G, Pitrolo L, Mistretta L, Cassara F, Di Maggio R, Maggio A. Longitudinal changes in LIC and other parameters in patients receiving different chelation regimens: Data from LICNET. *Eur J Haematol* 2018;100(2):124-130.
32. Krittayaphong R, Viprakasit V, Saiviroonporn P, Wangworatrakul W, Wood JC. Serum ferritin in the diagnosis of cardiac and liver iron overload in thalassaemia patients real-world practice: a multicentre study. *Br J Haematol* 2018;182(2):301-305.

33. Angelucci E, Muretto P, Nicolucci A, Baronciani D, Erer B, Gaziev J, Ripalti M, Sodani P, Tomassoni S, Visani G, Lucarelli G. Effects of iron overload and hepatitis C virus positivity in determining progression of liver fibrosis in thalassemia following bone marrow transplantation. *Blood* 2002;100(1):17-21.
34. Jensen PD, Jensen FT, Christensen T, Eiskjaer H, Baandrup U, Nielsen JL. Evaluation of myocardial iron by magnetic resonance imaging during iron chelation therapy with deferrioxamine: indication of close relation between myocardial iron content and chelatable iron pool. *Blood* 2003;101(11):4632-4639.
35. Noetzli LJ, Carson SM, Nord AS, Coates TD, Wood JC. Longitudinal analysis of heart and liver iron in thalassemia major. *Blood* 2008;112(7):2973-2978.
36. Angelucci E, Giovagnoni A, Valeri G, Paci E, Ripalti M, Muretto P, McLaren C, Brittenham GM, Lucarelli G. Limitations of magnetic resonance imaging in measurement of hepatic iron. *Blood* 1997;90(12):4736-4742.
37. Villeneuve JP, Bilodeau M, Lepage R, Cote J, Lefebvre M. Variability in hepatic iron concentration measurement from needle-biopsy specimens. *J Hepatol* 1996;25(2):172-177.
38. Wood JC, Zhang P, Rienhoff H, Abi-Saab W, Neufeld EJ. Liver MRI is more precise than liver biopsy for assessing total body iron balance: a comparison of MRI relaxometry with simulated liver biopsy results. *Magn Reson Imaging* 2015;33(6):761-767.
39. Wood JC. Estimating tissue iron burden: current status and future prospects. *Br J Haematol* 2015;170(1):15-28.
40. St Pierre TG, Clark PR, Chua-anusorn W, Fleming AJ, Jeffrey GP, Olynyk JK, Pootrakul P, Robins E, Lindeman R. Noninvasive measurement and imaging of liver iron concentrations using proton magnetic resonance. *Blood* 2005;105(2):855-861.
41. Hankins JS, McCarville MB, Loeffler RB, Smeltzer MP, Onciu M, Hoffer FA, Li CS, Wang WC, Ware RE, Hillenbrand CM. R2* magnetic resonance imaging of the liver in patients with iron overload. *Blood* 2009;113(20):4853-4855.
42. Garbowski MW, Carpenter JP, Smith G, Roughton M, Alam MH, He T, Pennell DJ, Porter JB. Biopsy-based calibration of T2* magnetic resonance for estimation of liver iron concentration and comparison with R2 Ferriscan. *J Cardiovasc Magn Reson* 2014;16(1):40.
43. Wood JC, Enriquez C, Ghugre N, Tyzka JM, Carson S, Nelson MD, Coates TD. MRI R2 and R2* mapping accurately estimates hepatic iron concentration in transfusion-dependent thalassemia and sickle cell disease patients. *Blood* 2005;106(4):1460-1465.
44. Wood JC, Zhang P, Rienhoff H, Abi-Saab W, Neufeld E. R2 and R2* are equally effective in evaluating chronic response to iron chelation. *Am J Hematol* 2014;89(5):505-508.
45. Wood JC, Ghugre N. Magnetic resonance imaging assessment of excess iron in thalassemia, sickle cell disease and other iron overload diseases. *Hemoglobin* 2008;32(1-2):85-96.
46. Storey P, Thompson AA, Carqueville CL, Wood JC, de Freitas RA, Rigsby CK. R2* imaging of transfusional iron burden at 3T and comparison with 1.5T. *J Magn Reson Imaging* 2007;25(3): 540-547.
47. Bacigalupo L, Paparo F, Zefiro D, Viberti CM, Cevasco L, Gianesin B, Pinto VM, Rollandi GA, Wood JC, Forni GL. Comparison between different software programs and post-processing techniques for the MRI quantification of liver iron concentration in thalassemia patients. *La Radiologia medica* 2016;121(10):751-762.
48. Kirk P, He T, Anderson LJ, Roughton M, Tanner MA, Lam WW, Au WY, Chu WC, Chan G, Galanello R, Matta G, Fogel M, Cohen AR, Tan RS, Chen K, Ng I, Lai A, Fucharoen S, Laothamata J, Chuncharunee S, Jongjirasiri S, Firmin DN, Smith GC, Pennell DJ. International reproducibility of single breathhold T2* MR for cardiac and liver iron assessment among five thalassemia centers. *J Magn Reson Imaging* 2010;32(2):315-319.

49. Anderson LJ, Holden S, Davis B, Prescott E, Charrier CC, Bunce NH, Firmin DN, Wonke B, Porter J, Walker JM, Pennell DJ. Cardiovascular T2-star (T2*) magnetic resonance for the early diagnosis of myocardial iron overload. *Eur Heart J* 2001;22(23):2171-2179.
50. Carpenter JP, He T, Kirk P, Roughton M, Anderson LJ, de Noronha SV, Sheppard MN, Porter JB, Walker JM, Wood JC, Galanello R, Forni G, Catani G, Matta G, Fucharoen S, Fleming A, House MJ, Black G, Firmin DN, St Pierre TG, Pennell DJ. On T2* magnetic resonance and cardiac iron. *Circulation* 2011;123(14):1519-1528.
51. Kirk P, Roughton M, Porter JB, Walker JM, Tanner MA, Patel J, Wu D, Taylor J, Westwood MA, Anderson LJ, Pennell DJ. Cardiac T2* magnetic resonance for prediction of cardiac complications in thalassemia major. *Circulation* 2009;120(20):1961-1968.
52. Aydinok Y, Kattamis A, Cappellini MD, El-Beshlawy A, Origa R, Elalfy M, Kilinc Y, Perrotta S, Karakas Z, Viprakasit V, Habr D, Constantinovici N, Shen J, Porter JB, Investigators H. Effects of deferasirox-deferoxamine on myocardial and liver iron in patients with severe transfusional iron overload. *Blood* 2015;125(25):3868-3877.
53. Voskaridou E, Kattamis A, Fragodimitri C, Kourakli A, Chalkia P, Diamantidis M, Vlachaki E, Drosou M, Lafioniatis S, Maragkos K, Petropoulou F, Eftihiadis E, Economou M, Klironomos E, Koutsouka F, Nestora K, Tzoumari I, Papageorgiou O, Basileiadi A, Lafiatis I, Dimitriadou E, Kalpaka A, Kalkana C, Xanthopoulidis G, Adamopoulos I, Kaiafas P, Mpitzioni A, Goula A, Kontonis I, Alepi C, Anastasiadis A, Papadopoulou M, Maili P, Dionisopoulou D, Tsirka A, Makis A, Kostaridou S, Politou M, Papassotiriou I, Greek Haemoglobinopathies Study G. National registry of hemoglobinopathies in Greece: updated demographics, current trends in affected births, and causes of mortality. *Ann Hematol* 2019;98(1):55-66.
54. Thomas AS, Garbowski M, Ang AL, Shah FT, Walker JM, Moon JC, Pennell DJ, Porter JB. A Decade Follow-up of a Thalassemia Major (TM) Cohort Monitored by Cardiac Magnetic Resonance Imaging (CMR): Significant Reduction In Patients with Cardiac Iron and In Total Mortality [abstract]. *Blood* 2010;116(21):1011.
55. Alam MH, Auger D, Smith GC, He T, Vassiliou V, Baksi AJ, Wage R, Drivas P, Feng Y, Firmin DN, Pennell DJ. T1 at 1.5T and 3T compared with conventional T2* at 1.5T for cardiac siderosis. *J Cardiovasc Magn Reson* 2015;17:102.
56. Nakavachara P, Viprakasit V. Adrenal insufficiency is prevalent in HbE/beta-thalassaemia paediatric patients irrespective of their clinical severity and transfusion requirement. *Clin Endocrinol (Oxf)* 2013;79(6):776-783.
57. Huang KE, Mittelman SD, Coates TD, Geffner ME, Wood JC. A significant proportion of thalassemia major patients have adrenal insufficiency detectable on provocative testing. *J Pediatr Hematol Oncol* 2015;37(1):54-59.
58. Chatterjee R, Katz M, Oatridge A, Bydder GM, Porter JB. Selective loss of anterior pituitary volume with severe pituitary-gonadal insufficiency in poorly compliant male thalassemic patients with pubertal arrest. *Ann N Y Acad Sci* 1998;850:479-482.
59. Wood JC. Diagnosis and management of transfusion iron overload: the role of imaging. *Am J Hematol* 2007;82(12 Suppl):1132-1135.
60. Noetzli LJ, Panigrahy A, Mittelman SD, Hyderi A, Dongelyan A, Coates TD, Wood JC. Pituitary iron and volume predict hypogonadism in transfusional iron overload. *Am J Hematol* 2012;87(2):167-171.
61. Wood JC, Noetzl L, Hyderi A, Joukar M, Coates T, Mittelman S. Predicting pituitary iron and endocrine dysfunction. *Ann N Y Acad Sci* 2010;1202:123-128.
62. Noetzli LJ, Mittelman SD, Watanabe RM, Coates TD, Wood JC. Pancreatic iron and glucose dysregulation in thalassemia major. *Am J Hematol* 2012;87(2):155-160.

63. Noetzli LJ, Papudesi J, Coates TD, Wood JC. Pancreatic iron loading predicts cardiac iron loading in thalassemia major. *Blood* 2009;114(19):4021-4026.
64. Au WY, Lam WW, Chu WW, Yuen HL, Ling AS, Li RC, Chan HM, Lee HK, Law MF, Liu HS, Liang R, Ha SY. A cross-sectional magnetic resonance imaging assessment of organ specific hemosiderosis in 180 thalassemia major patients in Hong Kong. *Haematologica* 2008;93(5):784-786.
65. Pippard MJ, Callender ST, Finch CA. Ferrioxamine excretion in iron-loaded man. *Blood* 1982;60(2):288-294.
66. Aydinok Y, Ulger Z, Nart D, Terzi A, Cetiner N, Ellis G, Zimmermann A, Manz C. A randomized controlled 1-year study of daily deferiprone plus twice weekly desferrioxamine compared with daily deferiprone monotherapy in patients with thalassemia major. *Haematologica* 2007;92(12):1599-1606.
67. Mourad FH, Hoffbrand AV, Sheikh-Taha M, Koussa S, Khoriaty AI, Taher A. Comparison between desferrioxamine and combined therapy with desferrioxamine and deferiprone in iron overloaded thalassaemia patients. *Br J Haematol* 2003;121(1):187-189.
68. Aydinok Y, Evans P, Terzi M, Cetiner N, Porter JB. Randomised Prospective Evaluation of Iron Balance, Chelation Efficiency, Urine Excretion and NTBI Progression with Deferiprone (DFP) or Deferoxamine (DFO) Monotherapy or with Combined DFP Plus DFO [abstract]. *Blood* 2005;106(11):2698.
69. Singh S, Hider RC, Porter JB. A direct method for quantification of non-transferrin-bound iron. *Analytical biochemistry* 1990;186(2):320-323.
70. Gosriwatana I, Loreal O, Lu S, Brissot P, Porter J, Hider RC. Quantification of non-transferrin-bound iron in the presence of unsaturated transferrin. *Anal Biochem* 1999;273(2):212-220.
71. Zanninelli G, Breuer W, Cabantchik ZI. Daily labile plasma iron as an indicator of chelator activity in Thalassaemia major patients. *Br J Haematol* 2009;147(5):744-751.
72. Cabantchik ZI, Breuer W, Zanninelli G, Cianciulli P. LPI-labile plasma iron in iron overload. *Best Pract Res Clin Haematol* 2005;18(2):277-287.
73. Piga A, Longo F, Duca L, Roggero S, Vinciguerra T, Calabrese R, Hershko C, Cappellini MD. High nontransferrin bound iron levels and heart disease in thalassemia major. *Am J Hematol* 2009;84(1):29-33.
74. Wood JC, Glynos T, Thompson A, Giardina P, Harmatz P, Kang BP, Paley C, Coates TD. Relationship between labile plasma iron, liver iron concentration and cardiac response in a deferasirox monotherapy trial. *Haematologica* 2011;96(7):1055-1058.
75. Porter JB, Lin KH, Beris P, Forni GL, Taher A, Habr D, Domokos G, Roubert B, Thein SL, investigators Es. Response of iron overload to deferasirox in rare transfusion-dependent anaemias: equivalent effects on serum ferritin and labile plasma iron for haemolytic or production anaemias. *Eur J Haematol* 2011;87(4):338-348.
76. Hod EA, Zhang N, Sokol SA, Wojczyk BS, Francis RO, Ansaldi D, Francis KP, Della-Latta P, Whittier S, Sheth S, Hendrickson JE, Zimring JC, Brittenham GM, Spitalnik SL. Transfusion of red blood cells after prolonged storage produces harmful effects that are mediated by iron and inflammation. *Blood* 2010;115(21):4284-4292.
77. Farmaki K, Tzoumari I, Pappa C, Chouliaras G, Berdoukas V. Normalisation of total body iron load with very intensive combined chelation reverses cardiac and endocrine complications of thalassaemia major. *Br J Haematol* 2010;148(3):466-475.
78. Porter JB, Garbowski MW. Interaction of Transfusion and Iron Chelation in Thalassemias. *Hematol Oncol Clin North Am* 2018;32(2):247-259.
79. Porter JB, Rafique R, Srichairatanakool S, Davis BA, Shah FT, Hair T, Evans P. Recent insights into interactions of deferoxamine with cellular and plasma iron pools: Implications for clinical use. *Ann N Y Acad Sci* 2005;1054:155-168.

80. Galanello R, Piga A, Alberti D, Rouan MC, Bigler H, Sechaud R. Safety, tolerability, and pharmacokinetics of ICL670, a new orally active iron-chelating agent in patients with transfusion-dependent iron overload due to beta-thalassemia. *J Clin Pharmacol* 2003;43(6):565-572.
81. Kontoghiorghes GJ, Goddard JG, Bartlett AN, Sheppard L. Pharmacokinetic studies in humans with the oral iron chelator 1,2-dimethyl-3-hydroxypyrid-4-one. *Clin Pharmacol Therap* 1990;48(3):255-261.
82. Waldmeier F, Bruin GJ, Glaenzel U, Hazell K, Sechaud R, Warrington S, Porter JB. Pharmacokinetics, metabolism, and disposition of deferasirox in beta-thalassemic patients with transfusion-dependent iron overload who are at pharmacokinetic steady state. *Drug Metab Dispos* 2010;38(5):808-816.
83. Porter JB, Faherty A, Stallibrass L, Brookman L, Hassan I, Howes C. A trial to investigate the relationship between DFO pharmacokinetics and metabolism and DFO-related toxicity. *Ann N Y Acad Sci* 1998;850:483-487.
84. Chirnomas D, Smith AL, Braunstein J, Finkelstein Y, Pereira L, Bergmann AK, Grant FD, Paley C, Shannon M, Neufeld EJ. Deferasirox pharmacokinetics in patients with adequate versus inadequate response. *Blood* 2009;114(19):4009-4013.
85. Babu T, Panachiyil GM, Sebastian J, Ravi MD. Effectiveness and tolerability of twice daily dosing of deferasirox in unresponsive and intolerant transfusion-dependent beta-thalassemia patients: A narrative review. *Indian J Pharmacol* 2020;52(6):514-519.
86. Pongtanakul B, Viprakasit V. Twice daily deferasirox significantly improves clinical efficacy in transfusion dependent thalassaemias who were inadequate responders to standard once daily dose. *Blood Cells Mol Dis* 2013;51(2):96-97.
87. Chang HH, Lu MY, Liao YM, Lin PC, Yang YL, Lin DT, Chiou SS, Jou ST, Lin KH, Chang TT. Improved efficacy and tolerability of oral deferasirox by twice-daily dosing for patients with transfusion-dependent beta-thalassemia. *Pediatr Blood Cancer* 2010.
88. JADENU® (deferasirox) tablets and JADENU® Sprinkle (deferasirox) granules [US Prescribing Information]. July 2020. Available at: https://www.novartis.com/us-en/sites/novartis_us/files/jadenu.pdf. Accessed: 13 Jan 2025.
89. EXJADE® (deferasirox) film-coated tablets [EU Summary of Product Characteristics]. September 2023. Available at: https://www.ema.europa.eu/en/documents/product-information/exjade-epar-product-information_en.pdf. Accessed: 13 Jan 2025.
90. FERRIPROX® (deferirpone) film-coated tablets [EU Summary of Product Characteristics]. December 2022. Available at: https://www.ema.europa.eu/en/documents/product-information/ferriprox-epar-product-information_en.pdf. Accessed: 13 Jan 2025.
91. FERRIPROX® (deferiprone) oral solution [US Prescribing Information]. November 2021. Available at: https://resources.chiesiusa.com/Ferriprox/FERRIPROX_OS_PI_MG_IFU_US.pdf. Accessed: 13 Jan 2025.
92. FERRIPROX® (deferiprone) tablets [US Prescribing Information]. November 2021. Available at: https://resources.chiesiusa.com/Ferriprox/FERRIPROX_PI_US.pdf. Accessed: 13 Jan 2025.
93. DESFERAL® (deferoxamine mesylate) for injection, for intramuscular, intravenous, or subcutaneous use [US Prescribing Information]. September 2022. Available at: https://www.accessdata.fda.gov/drugsatfda_docs/label/2022/016267s062lbl.pdf. Accessed: 13 Jan 2025.
94. Vichinsky E. Clinical application of deferasirox: practical patient management. *Am J Hematol* 2008;83(5):398-402.
95. Porter JB, Jaswon MS, Huehns ER, East CA, Hazell JW. Desferrioxamine ototoxicity: evaluation of risk factors in thalassaemic patients and guidelines for safe dosage. *Br J Haematol* 1989;73(3):403-409.
96. Chiodo AA, Alberti PW, Sher GD, Francombe WH, Tyler B. Desferrioxamine ototoxicity in an adult transfusion-dependent population. *J Otolaryngol* 1997;26(2):116-122.

97. Olivieri NF, Sabouhanian A, Gallie BL. Single-center retrospective study of the effectiveness and toxicity of the oral iron chelating drugs deferiprone and deferasirox. *PLoS One* 2019;14(2):e0211942.
98. Wanless IR, Sweeney G, Dhillon AP, Guido M, Piga A, Galanello R, Gamberini MR, Schwartz E, Cohen AR. Lack of progressive hepatic fibrosis during long-term therapy with deferiprone in subjects with transfusion-dependent beta-thalassemia. *Blood* 2002;100(5):1566-1569.
99. Hoffbrand AV, F AL-R, Davis B, Siritanakatkul N, Jackson BF, Cochrane J, Prescott E, Wonke B. Long-term trial of deferiprone in 51 transfusion-dependent iron overloaded patients. *Blood* 1998;91(1):295-300.
100. Maggio A, D'Amico G, Morabito A, Capra M, Ciaccio C, Cianciulli P, Di Gregorio F, Garozzo G, Malizia R, Magnano C, Mangiagli A, Quarta G, Rizzo M, D'Ascola DG, Rizzo A, Midiri M. Deferiprone versus deferoxamine in patients with thalassemia major: a randomized clinical trial. *Blood Cells Mol Dis* 2002;28(2):196-208.
101. Tondury P, Zimmermann A, Nielsen P, Hirt A. Liver iron and fibrosis during long-term treatment with deferiprone in Swiss thalassaemic patients. *Br J Haematol* 1998;101(3):413-415.
102. Olivieri NF, Brittenham GM, McLaren CE, Templeton DM, Cameron RG, McClelland RA, Burt AD, Fleming KA. Long-term safety and effectiveness of iron-chelation therapy with deferiprone for thalassemia major. *N Engl J Med* 1998;339(7):417-423.
103. Cappellini MD, Porter J, El-Beshlawy A, Li CK, Seymour JF, Elalfy M, Gattermann N, Giraudier S, Lee JW, Chan LL, Lin KH, Rose C, Taher A, Thein SL, Viprakasit V, Habr D, Domokos G, Roubert B, Kattamis A, Investigators ES. Tailoring iron chelation by iron intake and serum ferritin: the prospective EPIC study of deferasirox in 1744 patients with transfusion-dependent anemias. *Haematologica* 2010;95(4):557-566.
104. Deugnier Y, Turlin B, Ropert M, Cappellini MD, Porter JB, Giannone V, Zhang Y, Griffel L, Brissot P. Improvement in liver pathology of patients with beta-thalassemia treated with deferasirox for at least 3 years. *Gastroenterology* 2011;141(4):1202-1211, 1211 e1201-1203.
105. Adramerina A, Printza N, Hatzipantelis E, Symeonidis S, Tarazi L, Teli A, Economou M. Use of Deferasirox Film-Coated Tablets in Pediatric Patients with Transfusion Dependent Thalassemia: A Single Center Experience. *Biology (Basel)* 2022;11(2).
106. Cappellini MD, Cohen A, Piga A, Bejaoui M, Perrotta S, Agaoglu L, Aydinok Y, Kattamis A, Kilinc Y, Porter J, Capra M, Galanello R, Fattoum S, Drelichman G, Magnano C, Verissimo M, Athanassiou-Metaxa M, Giardina P, Kourakli-Symeonidis A, Janka-Schaub G, Coates T, Vermylen C, Olivieri N, Thuret I, Opitz H, Ressayre-Djaffer C, Marks P, Alberti D. A phase 3 study of deferasirox (ICL670), a once-daily oral iron chelator, in patients with beta-thalassemia. *Blood* 2006;107(9):3455-3462.
107. Economou M, Printza N, Teli A, Tzimouli V, Tsatra I, Papachristou F, Athanassiou-Metaxa M. Renal dysfunction in patients with beta-thalassemia major receiving iron chelation therapy either with deferoxamine and deferiprone or with deferasirox. *Acta Haematol* 2010;123(3):148-152.
108. Cappellini MD, Bejaoui M, Agaoglu L, Canatan D, Capra M, Cohen A, Drelichman G, Economou M, Fattoum S, Kattamis A, Kilinc Y, Perrotta S, Piga A, Porter JB, Griffel L, Dong V, Clark J, Aydinok Y. Iron chelation with deferasirox in adult and pediatric patients with thalassemia major: efficacy and safety during 5 years' follow-up. *Blood* 2011;118(4):884-893.
109. Taher AT, Origa R, Perrotta S, Kourakli A, Ruffo GB, Kattamis A, Goh AS, Cortoos A, Huang V, Weill M, Merino Herranz R, Porter JB. New film-coated tablet formulation of deferasirox is well tolerated in patients with thalassemia or lower-risk MDS: Results of the randomized, phase II ECLIPSE study. *Am J Hematol* 2017;92(5):420-428.
110. Shah NR. Advances in iron chelation therapy: transitioning to a new oral formulation. *Drugs Context* 2017;6:212502.

111. Bousquet J, Navarro M, Robert G, Aye P, Michel FB. Rapid desensitisation for desferrioxamine anaphylactoid reaction. *Lancet* 1983;2(8354):859-860.
112. Miller KB, Rosenwasser LJ, Bessette JA, Beer DJ, Rocklin RE. Rapid desensitisation for desferrioxamine anaphylactic reaction. *Lancet* 1981;1(8228):1059.
113. Erdogan E, Canatan D, Ormeci AR, Vural H, Aylak F. The effects of chelators on zinc levels in patients with thalassemia major. *J Trace Elem Med Biol* 2013;27(2):109-111.
114. Dee CM, Cheuk DK, Ha SY, Chiang AK, Chan GC. Incidence of deferasirox-associated renal tubular dysfunction in children and young adults with beta-thalassaemia. *Br J Haematol* 2014;167(3):434-436.
115. Bhandari S, Galanello R. Renal aspects of thalassaemia a changing paradigm. *Eur J Haematol* 2012;89(3):187-197.
116. Bird ST, Swain RS, Tian F, Okusanya OO, Waldron P, Khurana M, Durmowicz EL, Ma Y, Major JM, Gelperin K. Effects of deferasirox dose and decreasing serum ferritin concentrations on kidney function in paediatric patients: an analysis of clinical laboratory data from pooled clinical studies. *Lancet Child Adolesc Health* 2019;3(1):15-22.
117. Huang WF, Chou HC, Tsai YW, Hsiao FY. Safety of deferasirox: a retrospective cohort study on the risks of gastrointestinal, liver and renal events. *Pharmacoepidemiol Drug Saf* 2014;23(11):1176-1182.
118. Bronspiegel-Weintrob N, Olivieri NF, Tyler B, Andrews DF, Freedman MH, Holland FJ. Effect of age at the start of iron chelation therapy on gonadal function in beta-thalassemia major. *N Engl J Med* 1990;323(11):713-719.
119. Pennell DJ, Porter JB, Piga A, Lai Y, El-Beshlawy A, Belhoul KM, Elalfy M, Yesilipek A, Kilinc Y, Lawniczek T, Habr D, Weisskopf M, Zhang Y, Aydinok Y, investigators Cs. A 1-year randomized controlled trial of deferasirox vs deferoxamine for myocardial iron removal in beta-thalassemia major (CORDELIA). *Blood* 2014;123(10):1447-1454.
120. Pennell DJ, Berdoukas V, Karagiorga M, Ladis V, Piga A, Aessopos A, Gotsis ED, Tanner MA, Smith GC, Westwood MA, Wonke B, Galanello R. Randomized controlled trial of deferiprone or deferoxamine in beta-thalassemia major patients with asymptomatic myocardial siderosis. *Blood* 2006;107(9):3738-3744.
121. Wolfe L, Olivieri N, Sallan D, Colan S, Rose V, Propper R, Freedman MH, Nathan DG. Prevention of cardiac disease by subcutaneous deferoxamine in patients with thalassemia major. *N Engl J Med* 1985;312(25):1600-1603.
122. Aldouri MA, Wonke B, Hoffbrand AV, Flynn DM, Ward SE, Agnew JE, Hilson AJ. High incidence of cardiomyopathy in beta-thalassaemia patients receiving regular transfusion and iron chelation: reversal by intensified chelation. *Acta Haematol* 1990;84(3):113-117.
123. Freeman AP, Giles RW, Berdoukas VA, Walsh WF, Choy D, Murray PC. Early left ventricular dysfunction and chelation therapy in thalassemia major. *Ann Intern Med* 1983;99(4):450-454.
124. Brittenham GM, Griffith PM, Nienhuis AW, McLaren CE, Young NS, Tucker EE, Allen CJ, Farrell DE, Harris JW. Efficacy of deferoxamine in preventing complications of iron overload in patients with thalassemia major. *N Engl J Med* 1994;331(9):567-573.
125. Davis BA, Porter JB. Long-term outcome of continuous 24-hour deferoxamine infusion via indwelling intravenous catheters in high-risk beta-thalassemia. *Blood* 2000;95(4):1229-1236.
126. Cohen AR, Mizanin J, Schwartz E. Rapid removal of excessive iron with daily, high-dose intravenous chelation therapy. *J Pediatr* 1989;115(1):151-155.
127. Marcus RE, Davies SC, Bantock HM, Underwood SR, Walton S, Huehns ER. Desferrioxamine to improve cardiac function in iron-overloaded patients with thalassemia major. *Lancet* 1984;1(8373):392-393.

128. Porter JB, Wood J, Olivieri N, Vichinsky EP, Taher A, Neufeld E, Giardina P, Thompson A, Moore B, Evans P, Kim HY, Macklin EA, Trachtenberg F. Treatment of heart failure in adults with thalassemia major: response in patients randomised to deferoxamine with or without deferiprone. *J Cardiovasc Magn Reson* 2013;15(1):38.
129. Pennell DJ, Udelson JE, Arai AE, Bozkurt B, Cohen AR, Galanello R, Hoffman TM, Kiernan MS, Lerakis S, Piga A, Porter JB, Walker JM, Wood J, American Heart Association Committee on Heart F, Transplantation of the Council on Clinical C, Council on Cardiovascular R, Imaging. Cardiovascular function and treatment in beta-thalassemia major: a consensus statement from the American Heart Association. *Circulation* 2013;128(3):281-308.
130. Tanner MA, Galanello R, Dessi C, Smith GC, Westwood MA, Agus A, Roughton M, Assomull R, Nair SV, Walker JM, Pennell DJ. A randomized, placebo-controlled, double-blind trial of the effect of combined therapy with deferoxamine and deferiprone on myocardial iron in thalassemia major using cardiovascular magnetic resonance. *Circulation* 2007;115(14):1876-1884.
131. Traynor K. Deferiprone approved for iron overload. *Am J Health Syst Pharm* 2011;68(22):2106.
132. Viprakasit V, Nuchprayoon I, Chuansumrit A, Torcharus K, Pongtanakul B, Laothamatas J, Srichairatanakool S, Pooliam J, Supajitkasem S, Suriyaphol P, Tanphaichitr VS, Tuchinda S. Deferiprone (GPO-L-ONE((R))) monotherapy reduces iron overload in transfusion-dependent thalassemias: 1-year results from a multicenter prospective, single arm, open label, dose escalating phase III pediatric study (GPO-L-ONE; A001) from Thailand. *Am J Hematol* 2013;88(4):251-260.
133. Olivieri NF, Brittenham GM, Matsui D, Berkovitch M, Blendis LM, Cameron RG, McClelland RA, Liu PP, Templeton DM, Koren G. Iron-chelation therapy with oral deferiprone in patients with thalassemia major. *N Engl J Med* 1995;332(14):918-922.
134. Agarwal MB, Gupte SS, Viswanathan C, Vasandani D, Ramanathan J, Desai N, Puniyani RR, Chhablani AT. Long-term assessment of efficacy and safety of L1, an oral iron chelator, in transfusion dependent thalassaemia: Indian trial. *Br J Haematol* 1992;82(2):460-466.
135. al-Refaie FN, Wonke B, Hoffbrand AV, Wickens DG, Nortey P, Kontoghiorghes GJ. Efficacy and possible adverse effects of the oral iron chelator 1,2-dimethyl-3-hydroxypyrid-4-one (L1) in thalassemia major. *Blood* 1992;80(3):593-599.
136. Cohen AR, Galanello R, Piga A, Dipalma A, Vullo C, Tricta F. Safety profile of the oral iron chelator deferiprone: a multicentre study. *Br J Haematol* 2000;108(2):305-312.
137. Ha SY, Chik KW, Ling SC, Lee AC, Luk CW, Lam CW, Ng IO, Chan GC. A randomized controlled study evaluating the safety and efficacy of deferiprone treatment in thalassemia major patients from Hong Kong. *Hemoglobin* 2006;30(2):263-274.
138. Gomber S, Saxena R, Madan N. Comparative efficacy of desferrioxamine, deferiprone and in combination on iron chelation in thalassemic children. *Indian Pediatr* 2004;41(1):21-27.
139. Olivieri NF, Brittenham GM. Iron-chelating therapy and the treatment of thalassemia. *Blood* 1997;89(3):739-761.
140. Olivieri NF, Brittenham GM. Final results of the randomized trial of deferiprone (L1) and deferoxamine (DFO) [abstract]. *Blood* 1997;90(10 Suppl 1):264a.
141. El-Beshlawy A, Manz C, Naja M, Eltagui M, Tarabishi C, Youssry I, Sobh H, Hamdy M, Sharaf I, Mostafa A, Shaker O, Hoffbrand AV, Taher A. Iron chelation in thalassemia: combined or monotherapy? The Egyptian experience. *Ann Hematol* 2008;87(7):545-550.
142. Del Vecchio GC, Schettini F, Piacente L, De Santis A, Giordano P, De Mattia D. Effects of deferiprone on immune status and cytokine pattern in thalassaemia major. *Acta Haematol* 2002;108(3):144-149.
143. Maggio A, Vitrano A, Lucania G, Capra M, Cuccia L, Gagliardotto F, Pitrolo L, Prossomariti L, Filosa A, Caruso V, Gerardi C, Campisi S, Cianciulli P, Rizzo M, D'Ascola G, Ciancio A, Di Maggio R, Calvaruso

G, Pantalone GR, Rigano P. Long-term use of deferiprone significantly enhances left-ventricular ejection function in thalassemia major patients. *Am J Hematol* 2012;87(7):732-733.

144. Borgna-Pignatti C, Cappellini MD, De Stefano P, Del Vecchio GC, Forni GL, Gamberini MR, Ghilardi R, Piga A, Romeo MA, Zhao H, Cnaan A. Cardiac morbidity and mortality in deferoxamine- or deferiprone-treated patients with thalassemia major. *Blood* 2006;107(9):3733-3737.
145. Telfer P, Coen PG, Christou S, Hadjigavriel M, Kolnakou A, Pangalou E, Pavlides N, Psiloines M, Simamonian K, Skordos G, Sitarou M, Angastiniotis M. Survival of medically treated thalassemia patients in Cyprus. Trends and risk factors over the period 1980-2004. *Haematologica* 2006;91(9):1187-1192.
146. Filosa A, Vitrano A, Rigano P, Calvaruso G, Barone R, Capra M, Cuccia L, Gagliardotto F, Pitrolo L, Prossomariti L, Casale M, Caruso V, Gerardi C, Campisi S, Cianciulli P, Rizzo M, D'Ascola G, Ciancio A, Maggio A. Long-term treatment with deferiprone enhances left ventricular ejection function when compared to deferoxamine in patients with thalassemia major. *Blood Cells Mol Dis* 2013;51(2):85-88.
147. Pepe A, Meloni A, Capra M, Cianciulli P, Prossomariti L, Malaventura C, Putti MC, Lippi A, Romeo MA, Bisconte MG, Filosa A, Caruso V, Quarta A, Pitrolo L, Missere M, Midiri M, Rossi G, Positano V, Lombardi M, Maggio A. Deferasirox, deferiprone and desferrioxamine treatment in thalassemia major patients: cardiac iron and function comparison determined by quantitative magnetic resonance imaging. *Haematologica* 2011;96(1):41-47.
148. Fisher SA, Brunskill SJ, Doree C, Gooding S, Chowdhury O, Roberts DJ. Desferrioxamine mesylate for managing transfusional iron overload in people with transfusion-dependent thalassaemia. *Cochrane Database Syst Rev* 2013;2013(8):CD004450.
149. Maggio A, Filosa A, Vitrano A, Aloj G, Kattamis A, Ceci A, Fucharoen S, Cianciulli P, Grady RW, Prossomariti L, Porter JB, Iacono A, Cappellini MD, Bonifazi F, Cassara F, Harmatz P, Wood J, Gluud C. Iron chelation therapy in thalassemia major: a systematic review with meta-analyses of 1520 patients included on randomized clinical trials. *Blood Cells Mol Dis* 2011;47(3):166-175.
150. Musallam KM, Barella S, Origa R, Ferrero GB, Lisi R, Pasanisi A, Longo F, Gianesin B, Forni GL, Webthal p. Differential effects of iron chelators on iron burden and long-term morbidity and mortality outcomes in a large cohort of transfusion-dependent beta-thalassemia patients who remained on the same monotherapy over 10 years. *Blood Cells Mol Dis* 2024;107:102859.
151. Ceci A, Baiardi P, Felisi M, Cappellini MD, Carnelli V, De Sanctis V, Galanello R, Maggio A, Masera G, Piga A, Schettini F, Stefano I, Tricta F. The safety and effectiveness of deferiprone in a large-scale, 3-year study in Italian patients. *Br J Haematol* 2002;118(1):330-336.
152. ElAlfy MS, Sari TT, Lee CL, Tricta F, El-Beshlawy A. The safety, tolerability, and efficacy of a liquid formulation of deferiprone in young children with transfusional iron overload. *J Pediatr Hematol Oncol* 2010;32(8):601-605.
153. Badawy SM, Palmblad J, Tricta F, Toiber Temin N, Fradette C, Lin L, Rozova A, Sheth S. Rates of severe neutropenia and infection risk in patients treated with deferiprone: 28 years of data. *Blood Adv* 2024;8(21):5641-5649.
154. Botzenhardt S, Felisi M, Bonifazi D, Del Vecchio GC, Putti MC, Kattamis A, Ceci A, Wong ICK, Neubert A, consortium D. Long-term safety of deferiprone treatment in children from the Mediterranean region with beta-thalassemia major: the DEEP-3 multi-center observational safety study. *Haematologica* 2018;103(1):e1-e4.
155. Bellanti F, Del Vecchio GC, Putti MC, Maggio A, Filosa A, Cosmi C, Mangiarini L, Spino M, Connelly J, Ceci A, Della Pasqua O, Consortium DEiP. Population pharmacokinetics and dosing recommendations for the use of deferiprone in children younger than 6 years. *British journal of Br J Clin Pharmacol* 2017;83(3):593-602.

156. Maggio A, Kattamis A, Felisi M, Reggiardo G, El-Beshlawy A, Bejaoui M, Sherief L, Christou S, Cosmi C, Della Pasqua O, Del Vecchio GC, Filosa A, Cuccia L, Hassab H, Kreka M, Origa R, Putti MC, Spino M, Telfer P, Tempesta B, Vitrano A, Tsang YC, Zaka A, Tricta F, Bonifazi D, Ceci A. Evaluation of the efficacy and safety of deferiprone compared with deferasirox in paediatric patients with transfusion-dependent haemoglobinopathies (DEEP-2): a multicentre, randomised, open-label, non-inferiority, phase 3 trial. *Lancet Haematol* 2020;7(6):e469-e478.
157. Forni GL, Kattamis A, Kuo KHM, Maggio A, Sheth S, Taher AT, Viprakasit V. Iron chelation therapy for children with transfusion-dependent beta-thalassemia: How young is too young? *Pediatr Blood Cancer* 2024;71(8):e31035.
158. Galanello R. Deferiprone in the treatment of transfusion-dependent thalassemia: a review and perspective. *Ther Clin Risk Manag* 2007;3(5):795-805.
159. Kwiatkowski JL, Hamdy M, El-Beshlawy A, Ebeid FSE, Badr M, Alshehri A, Kanter J, Inusa B, Adly AAM, Williams S, Kilinc Y, Lee D, Tricta F, Elalfy MS. Deferiprone vs deferoxamine for transfusional iron overload in SCD and other anemias: a randomized, open-label noninferiority study. *Blood Adv* 2022;6(4):1243-1254.
160. Sammaraiee Y, Banerjee G, Farmer S, Hylton B, Cowley P, Eleftheriou P, Porter J, Werring DJ. Risks associated with oral deferiprone in the treatment of infratentorial superficial siderosis. *J Neurol* 2020;267(1):239-243.
161. Taher AT, Origa R, Perrotta S, Kouraklis A, Ruffo GB, Kattamis A, Goh AS, Huang V, Zia A, Herranz RM, Porter JB. Patient-reported outcomes from a randomized phase II study of the deferasirox film-coated tablet in patients with transfusion-dependent anemias. *Health Qual Life Outcomes* 2018;16(1):216.
162. Panachiyil GM, Babu T, Sebastian J, Ravi MD. Efficacy and Tolerability of Twice-Daily Dosing Schedule of Deferasirox in Transfusion-Dependent Paediatric Beta-Thalassaemia Patients: A Randomized Controlled Study. *J Pharm Pract* 2023;36(4):749-755.
163. Chang HH, Lu MY, Liao YM, Lin PC, Yang YL, Lin DT, Chiou SS, Jou ST, Lin KH, Chang TT. Improved efficacy and tolerability of oral deferasirox by twice-daily dosing for patients with transfusion-dependent beta-thalassemia. *Pediatr Blood Cancer* 2011;56(3):420-424.
164. Porter J, Galanello R, Saglio G, Neufeld EJ, Vichinsky E, Cappellini MD, Olivieri N, Piga A, Cunningham MJ, Soulieres D, Gattermann N, Tchernia G, Maertens J, Giardina P, Kwiatkowski J, Quarta G, Jeng M, Forni GL, Stadler M, Cario H, Debusscher L, Della Porta M, Cazzola M, Greenberg P, Alimena G, Rabault B, Gathmann I, Ford JM, Alberti D, Rose C. Relative response of patients with myelodysplastic syndromes and other transfusion-dependent anaemias to deferasirox (ICL670): a 1-yr prospective study. *Eur J Haematol* 2008;80(2):168-176.
165. Piga A, Galanello R, Forni GL, Cappellini MD, Origa R, Zappu A, Donato G, Bordone E, Lavagetto A, Zanaboni L, Sechaud R, Hewson N, Ford JM, Opitz H, Alberti D. Randomized phase II trial of deferasirox (Exjade, ICL670), a once-daily, orally-administered iron chelator, in comparison to deferoxamine in thalassemia patients with transfusional iron overload. *Haematologica* 2006;91(7):873-880.
166. Porter JB, Elalfy MS, Taher AT, Aydinok Y, Chan LL, Lee SH, Sutcharitchan P, Habr D, Martin N, El-Beshlawy A. Efficacy and safety of deferasirox at low and high iron burdens: results from the EPIC magnetic resonance imaging substudy. *Ann Hematol* 2013;92(2):211-219.
167. Nisbet-Brown E, Olivieri NF, Giardina PJ, Grady RW, Neufeld EJ, Sechaud R, Krebs-Brown AJ, Anderson JR, Alberti D, Sizer KC, Nathan DG. Effectiveness and safety of ICL670 in iron-loaded patients with thalassaemia: a randomised, double-blind, placebo-controlled, dose-escalation trial. *Lancet* 2003;361(9369):1597-1602.
168. Porter JB, Shah FT. Iron overload in thalassemia and related conditions: therapeutic goals and assessment of response to chelation therapies. *Hematol Oncol Clin North Am* 2010;24(6):1109-1130.

169. Porter JB, Tanner MA, Pennell DJ, Eleftheriou P. Improved Myocardial T2* in Transfusion Dependent Anemias Receiving ICL670 (Deferasirox) [abstract]. *Blood* 2005;106(11):3600.
170. Pennell DJ, Porter JB, Cappellini MD, El-Beshlawy A, Chan LL, Aydinok Y, Elalfy MS, Sutcharitchan P, Li CK, Ibrahim H, Viprakasit V, Kattamis A, Smith G, Habr D, Domokos G, Roubert B, Taher A. Efficacy of deferasirox in reducing and preventing cardiac iron overload in beta-thalassemia. *Blood* 2010;115(12):2364-2371.
171. Pennell DJ, Porter JB, Cappellini MD, Chan LL, El-Beshlawy A, Aydinok Y, Ibrahim H, Li CK, Viprakasit V, Elalfy MS, Kattamis A, Smith G, Habr D, Domokos G, Roubert B, Taher A. Deferasirox for up to 3 years leads to continued improvement of myocardial T2* in patients with beta-thalassemia major. *Haematologica* 2012;97(6):842-848.
172. Pennell DJ, Porter JB, Piga A, Lai YR, El-Beshlawy A, Elalfy M, Yesilipek A, Kilinc Y, Habr D, Musallam KM, Shen J, Aydinok Y, investigators Cs. Sustained improvements in myocardial T2* over 2 years in severely iron-overloaded patients with beta thalassemia major treated with deferasirox or deferoxamine. *Am J Hematol* 2015;90(2):91-96.
173. Daar S, Pathare A, Nick H, Kriemler-Krahn U, Hmissi A, Habr D, Taher A. Reduction in labile plasma iron during treatment with deferasirox, a once-daily oral iron chelator, in heavily iron-overloaded patients with beta-thalassaemia. *Eur J Haematol* 2009;82(6):454-457.
174. Vlachaki E, Agapidou A, Spanos G, Klonizakis P, Vetsiou E, Mavroudi M, Boura P. Five Years of Deferasirox Therapy for Cardiac Iron in beta-Thalassemia Major. *Hemoglobin* 2015;39(5):299-304.
175. Casale M, Filosa A, Ragozzino A, Amendola G, Roberti D, Tartaglione I, De Michele E, Cozzolino D, Rispoli G, Palmieri F, Pugliese U, Scianguetta S, Signoriello G, Musallam KM, Perrotta S. Long-term improvement in cardiac magnetic resonance in beta-thalassemia major patients treated with deferasirox extends to patients with abnormal baseline cardiac function. *Am J Hematol* 2019;94(3):312-318.
176. Taher AT, Weber S, Han J, Bruederle A, Porter JB. Predicting serum ferritin levels in patients with iron overload treated with the film-coated tablet of deferasirox during the ECLIPSE study. *Am J Hematol* 2019;94(1):E15-E17.
177. Taher AT, Origa R, Perrotta S, Kouraklis A, Belhoul K, Huang V, Han J, Bruederle A, Bobbili P, Duh MS, Porter JB. Influence of patient-reported outcomes on the treatment effect of deferasirox film-coated and dispersible tablet formulations in the ECLIPSE trial: A post hoc mediation analysis. *Am J Hematol* 2019;94(4):E96-E99.
178. Wali Y, Hassan T, Charoenkwan P, Trompeter S, Tartaglione I, Origa R, Gamberini MR, Viprakasit V, Izquierdo M, Opio S, Roy T, Taher A. Crushed deferasirox film-coated tablets in pediatric patients with transfusional hemosiderosis: Results from a single-arm, interventional phase 4 study (MIMAS). *Am J Hematol* 2022;97(8):E292-E295.
179. Viprakasit V, Hamdy MM, Hassab HMA, Sherief LM, Al-Bagshi M, Khattab M, Chuncharunee S, Dung PC, Kupesiz A, Shekhawat A, Sonawane Y, Perez LT, Slader C, Taher AT. Patient preference for deferasirox film-coated versus dispersible tablet formulation: a sequential-design phase 2 study in patients with thalassemia. *Ann Hematol* 2023;102(8):2039-2049.
180. Taher AT, Wali Y, Cruz MC, Charoenkwan P, Aydinok Y, Werner O, Govindaraju S, Romen F, Viprakasit V. Compliance and clinical benefit of deferasirox granule and dispersible tablet formulation in pediatric patients with transfusional iron overload: in a randomized, open-label, multicenter, phase II study. *Haematologica* 2024;109(5):1413-1425.
181. Aydinok Y, Unal S, Oymak Y, Vergin C, Turker ZD, Yildiz D, Yesilipek A. Observational study comparing long-term safety and efficacy of Deferasirox with Desferrioxamine therapy in chelation-naive children with transfusional iron overload. *Eur J Haematol* 2012;88(5):431-438.

182. Aycicek A, Koc A, Abuhandan M. Efficacy of deferasirox in children with beta-thalassemia: single-center 3 year experience. *Pediatr Int* 2014;56(4):530-533.
183. Chang HH, Lu MY, Peng SS, Yang YL, Lin DT, Jou ST, Lin KH. The long-term efficacy and tolerability of oral deferasirox for patients with transfusion-dependent beta-thalassemia in Taiwan. *Ann Hematol* 2015;94(12):1945-1952.
184. Vichinsky E, El-Beshlawy A, Al Zoebie A, Kamdem A, Koussa S, Chotsampancharoen T, Bruederle A, Gilotti G, Han J, Elalfy M. Long-term safety and efficacy of deferasirox in young pediatric patients with transfusional hemosiderosis: Results from a 5-year observational study (ENTRUST). *Pediatr Blood Cancer* 2017;64(9).
185. Cheesman S, Shah R, Trompeter S, Eleftheriou P, Hylton B, Garbowski MW, Porter J. Real-World Experience of Switching from Deferasirox Dispersible to Film-Coated Tablets: Impact on Adherence to Chelation Therapy, Iron Overload and Renal Function [abstract]. *Blood* 2018;132(Suppl 1):1062.
186. Taher A, El-Beshlawy A, Elalfy MS, Al Zir K, Daar S, Habr D, Kriemler-Krahn U, Hmissi A, Al Jefri A. Efficacy and safety of deferasirox, an oral iron chelator, in heavily iron-overloaded patients with beta-thalassaemia: the ESCALATOR study. *Eur J Haematol* 2009;82(6):458-465.
187. Aydinok Y. Combination chelation therapy. *Ann N Y Acad Sci* 2023;1529(1):33-41.
188. Evans P, Kayyali R, Hider RC, Eccleston J, Porter JB. Mechanisms for the shuttling of plasma non-transferrin-bound iron (NTBI) onto deferoxamine by deferiprone. *Transl Res* 2010;156(2):55-67.
189. Lal A, Porter J, Sweeters N, Ng V, Evans P, Neumayr L, Kurio G, Harmatz P, Vichinsky E. Combined chelation therapy with deferasirox and deferoxamine in thalassemia. *Blood Cells Mol Dis* 2013;50(2):99-104.
190. Vlachodimitropoulou Koumoutsea E, Garbowski M, Porter J. Synergistic intracellular iron chelation combinations: mechanisms and conditions for optimizing iron mobilization. *Br J Haematol* 2015;170(6):874-883.
191. Galanello R, Kattamis A, Piga A, Fischer R, Leoni G, Ladis V, Voi V, Lund U, Tricta F. A prospective randomized controlled trial on the safety and efficacy of alternating deferoxamine and deferiprone in the treatment of iron overload in patients with thalassemia. *Haematologica* 2006;91(9):1241-1243.
192. Maggio A, Vitrano A, Capra M, Cuccia L, Gagliardotto F, Filosa A, Romeo MA, Magnano C, Caruso V, Argento C, Gerardi C, Campisi S, Violi P, Malizia R, Cianciulli P, Rizzo M, D'Ascola DG, Quota A, Prossomariti L, Fidone C, Rigano P, Pepe A, D'Amico G, Morabito A, Gluud C. Long-term sequential deferiprone-deferoxamine versus deferiprone alone for thalassaemia major patients: a randomized clinical trial. *Br J Haematol* 2009;145(2):245-254.
193. Origa R, Bina P, Agus A, Crobu G, Defraia E, Dessi C, Leoni G, Muroni PP, Galanello R. Combined therapy with deferiprone and desferrioxamine in thalassemia major. *Haematologica* 2005;90(10):1309-1314.
194. Kattamis A, Ladis V, Berdousi H, Kelekis NL, Alexopoulou E, Papasotiriou I, Drakaki K, Kaloumenou I, Galani A, Kattamis C. Iron chelation treatment with combined therapy with deferiprone and deferioxamine: a 12-month trial. *Blood Cells Mol Dis* 2006;36(1):21-25.
195. Telfer P. Update on survival in thalassemia major. *Hemoglobin* 2009;33 Suppl 1:S76-80.
196. Voskaridou E, Christoulas D, Terpos E. Successful chelation therapy with the combination of deferasirox and deferiprone in a patient with thalassaemia major and persisting severe iron overload after single-agent chelation therapies. *Br J Haematol* 2011;154(5):654-656.
197. Farmaki K, Tzoumari I, Pappa C. Oral chelators in transfusion-dependent thalassemia major patients may prevent or reverse iron overload complications. *Blood Cells Mol Dis* 2011;47(1):33-40.

198. Elalfy MS, Adly AM, Wali Y, Tony S, Samir A, Elhenawy YI. Efficacy and safety of a novel combination of two oral chelators deferasirox/deferiprone over deferoxamine/deferiprone in severely iron overloaded young beta thalassemia major patients. *Eur J Haematol* 2015;95(5):411-420.
199. Gomber S, Jain P, Sharma S, Narang M. Comparative Efficacy and Safety of Oral Iron Chelators and their Novel Combination in Children with Thalassemia. *Indian Pediatr* 2016;53(3):207-210.
200. Totadri S, Bansal D, Bhatia P, Attri SV, Trehan A, Marwaha RK. The deferiprone and deferasirox combination is efficacious in iron overloaded patients with beta-thalassemia major: A prospective, single center, open-label study. *Pediatr Blood Cancer* 2015;62(9):1592-1596.
201. Hammond J, Thompson AA, Fogel MA, Hammond K, Kokroko J, Kwiatkowski JL. Combination Oral Chelation in Adult Patients With Transfusion-dependent Thalassemia and High Iron Burden. *J Pediatr Hematol Oncol* 2019;41(1):e47-e50.
202. Taher AT, Porter JB, Viprakasit V, Kattamis A, Chuncharunee S, Sutcharitchan P, Siritanaratkul N, Galanello R, Karakas Z, Lawniczek T, Habr D, Ros J, Zhu Z, Cappellini MD. Deferasirox effectively reduces iron overload in non-transfusion-dependent thalassemia (NTDT) patients: 1-year extension results from the THALASSA study. *Ann Hematol* 2013;92(11):1485-1493.

04 CARDIOVASCULAR DISEASE

Authors: **J. Malcolm Walker[1], Dimitrios Farmakis[2]**

[1.] Institute of Cardiovascular Science, University College London; Hatter Cardiovascular Institute, London, United Kingdom

[2.] Department of Cardiology, Athens University Hospital Attikon, National and Kapodistrian University of Athens Medical School, Athens, Greece

Competing interest: JMW reports speaker honoraria and travel reimbursement from Chiesi Pharma. DF reports speaker honoraria, consultation fees, and/or grants from Abbott, AstraZeneca, Bayer, Boehringer Ingelheim, Leo, Myocardial Solutions, Roche, and Viatris. All competing interests are outside the present work.

1. INTRODUCTION

Despite the improvements in the outlook for β-thalassaemia patients over the past years [1, 2], cardiovascular complications still account for a significant proportion of mortality and morbidity, either because of limited access to or poor compliance with contemporary disease-specific therapies or because of ageing of patients treated properly with these therapies.

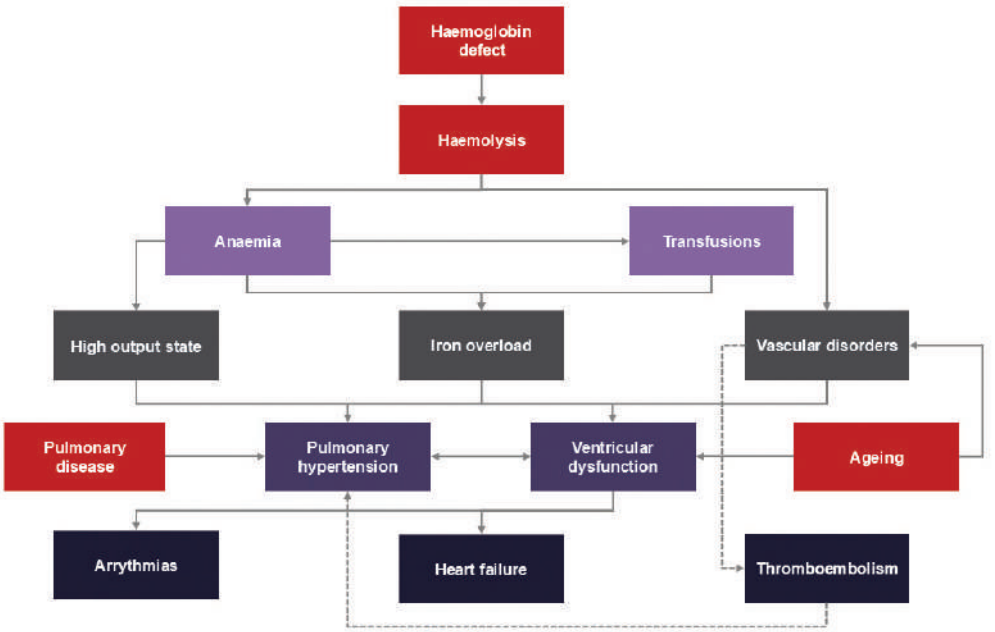
The improvement in survival has resulted from the effective implementation of modern diagnostic and therapeutic modalities, including magnetic resonance imaging (MRI)-guided chelation therapy [3] and the adoption of more intensive, sometimes combined chelation regimes [1, 4]. This progress is not however universal and β-thalassaemia populations living in low- and middle-income countries (LMICs) are often faced with limited access to optimal care and therefore the global burden of life-limiting complications, including cardiovascular disease remains high [5-7]. Adopting simplified, rapid diagnostic techniques using rapid MRI, applicable in LMICs should help improve access and thus holds the promise to improve the iron overload burden [8, 9]; but this cannot be achieved without significant concomitant improvements in the education of patients and physicians and better organisation of health service delivery systems [10-12]. On the other hand, patients living in richer nations are faced with ageing-related cardiovascular conditions, in particular, atrial fibrillation and heart failure with preserved ejection fraction (HFpEF) [13].

This Chapter provides an overview of cardiovascular complications that may arise in transfusion-dependent β-thalassaemia (TDT), focusing on the particularities of cardiovascular disease in these patients that need to be taken into consideration during their assessment and treatment.

2. PATHOPHYSIOLOGY

The pathophysiology of cardiovascular disease in β-thalassaemia has been reviewed in detail elsewhere (Figure 1) [14, 15]. In the pretransfusion era, the severe chronic anaemia dominated the picture, to be overtaken by excessive tissue iron loading after regular transfusion became the standard of care. Once chelation was adopted to remove iron, the dominant cause of mortality in young adults, cardiac iron overload, was addressed and survival improved. Today, most β-thalassaemia patients can expect to survive into old age, albeit with considerable cardiovascular issues to be managed, which include atrial fibrillation, restrictive ventricular physiology producing the syndrome of HFpEF and pulmonary hypertension.

Figure 1. *The effects anaemia on morbidity and the effect of transfusions given to mitigate chronic anaemia in patients with β-thalassaemia. (A) Regular transfusions increased the median survival in β-thalassaemia major from less than 10 years in the 1960s to about 18 years in the 1970s with subsequent further significant improvement due to advances in management of iron overload [3, 18]. (B) Survival in patients with NTDT placed on regular transfusion is much better than patients with NTDT only occasionally transfused, with 12.5% of deaths from non-iron-related cardiomyopathy [20]. (C) Number of morbidities in NTDT over 10 years is directly related to haemoglobin level with the probability of multiple morbidities about 3 times higher at 9 g/dL than at 10 g/dL [22]. (D) Morbidity-free survival is significantly better if the haemoglobin is >10 g/dL [21]. Abbreviations: NTDT, non-transfusion-dependent β-thalassaemia; Hb, haemoglobin. Reproduced with permission from [16].*



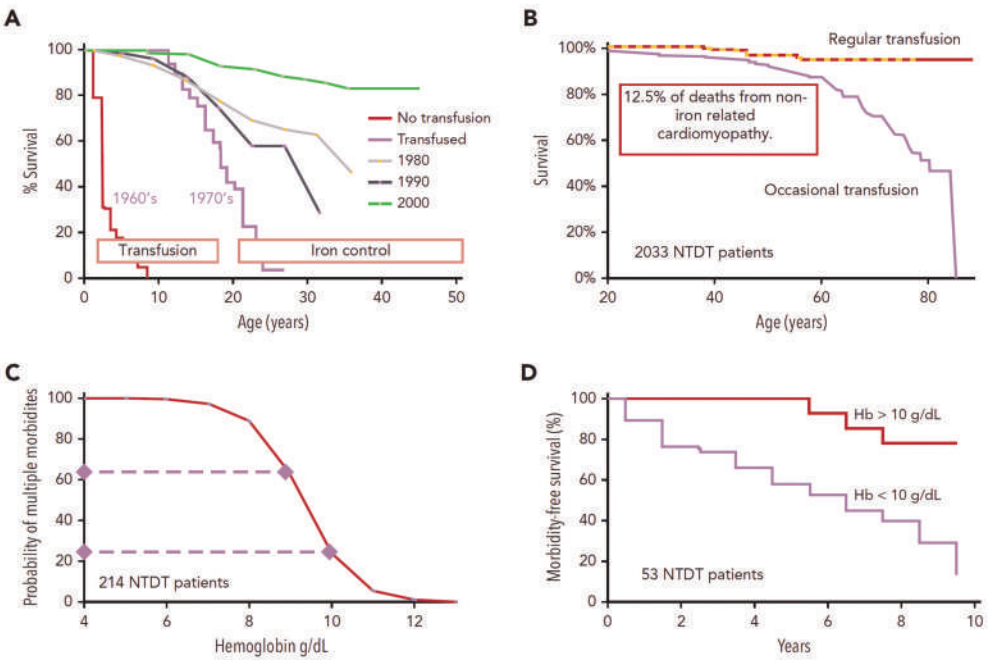
3. CARDIOVASCULAR CONSEQUENCES OF CHRONIC ANAEMIA

Recommendations for a target pretransfusion haemoglobin level of ≥9.0-9.5 g/dL have substantially improved the quality of life for TDT patients, with a demonstrable, additional associated survival benefit [3, 16-18]. Moreover, untreated anaemia (especially with haemoglobin levels <10 g/dL) has been consistently associated with increased risk of cardiovascular disease-related morbidity and mortality in patients with non-transfusion-dependent β-thalassaemia (Figure 2) [19-22]. Even the modest chronic anaemia that most, even transfused β-thalassaemia patients endure, has important acute and longer-term cardiovascular consequences. The immediate effect of anaemia is to cause a compensatory increase in cardiac output to maintain tissue oxygen delivery, which is further compromised in β-thalassaemia by the relative excess of foetal haemoglobin (HbF), which releases its oxygen less well than adult haemoglobin (HbA). β-Thalassaemia patients may therefore have larger cardiac chamber sizes for their body surface area than non-affected individuals [23] and the lower limits of normality for parameters such as the

ejection fraction are higher in β-thalassaemia than non-thalassaemic populations, at least in the older age groups, 63% being suggested as the probable lower limit of normality [24].

Awareness of these adaptations and differences in β-thalassaemia becomes important clinically when determining the cardiovascular status of individual patients. Chronic anaemia is also associated with a vasodilated circulation and it is no surprise that blood pressure is lower in healthy thalassaemic patients than in non-thalassaemic individuals [25]. Despite vasodilatation, studies have shown a reduced vascular compliance [26], with increased afterload on the heart [27]. Maintained for a lifetime, these haemodynamics will exacerbate and probably accelerate the ageing of the circulation leading to the development of restrictive ventricular physiology [24, 28], a scenario that might contribute to the development of atrial fibrillation and HFpEF. Low blood pressure may make it difficult to treat thalassaemic patients with cardiac medications which tend to lower blood pressure further.

Figure 2. *The effects anaemia on morbidity and the effect of transfusions given to mitigate chronic anaemia in patients with β-thalassaemia. (A) Regular transfusions increased the median survival in β-thalassaemia major from less than 10 years in the 1960s to about 18 years in the 1970s with subsequent further significant improvement due to advances in management of iron overload [3, 18]. (B) Survival in patients with NTDT placed on regular transfusion is much better than patients with NTDT only occasionally transfused, with 12.5% of deaths from non-iron-related cardiomyopathy [20]. (C) Number of morbidities in NTDT over 10 years is directly related to haemoglobin level with the probability of multiple morbidities about 3 times higher at 9 g/dL than at 10 g/dL [22]. (D) Morbidity-free survival is significantly better if the haemoglobin is >10 g/dL [21]. Abbreviations: NTDT, non-transfusion-dependent β-thalassaemia; Hb, haemoglobin. Reproduced with permission from [16].*



4. CARDIOVASCULAR COMPLICATIONS

A wide range of cardiovascular abnormalities are seen in patients with β-thalassaemia, involving an interaction between the adaptations to chronic anaemia plus a dominating influence of excess tissue iron loading and changes in coagulation in the context of endocrinopathies, including diabetes.

4.1. Cardiac dysfunction

Tissue iron loading begins in β-thalassaemia once transferrin saturation is exceeded, by a combination of dysregulated gastrointestinal iron absorption plus exogenous sources from transfused blood [29]. Non-transferrin-bound iron enters heart cells via a number of mechanisms (Figures 3 and 4) [30-32]. The importance of L and T-type calcium channels has been emphasised and has potential therapeutic implications for inhibiting myocyte iron loading, by calcium channel blocking drugs, which are in widespread global use for common conditions, such as hypertension.

Figure 3. *Iron homeostasis in cardiac myocytes. Iron in blood is normally bound to Tr and enters cells through specific receptors (TFR1 & TFR2β). The only method of exit from the cell is via FPN1. Unbound iron Fe^{2+} enters cells via DMT1, as well as calcium channels (LTCC and TTCC). Within the myocyte it is bound to ferritin, but also stored in mitochondria, excess iron appears in a labile pool. Abbreviations: ACO1, aconitase 1; ARE, AU-rich element; DMT1, divalent metal transporter 1; IRE, iron-responsive element; IRP, iron regulatory protein; FPN1, ferroportin-1; FTH, ferritin heavy chain; FTL, ferritin light chain; LTCC, L-type calcium channel; NDUSF1, NADH:ubiquinone oxidoreductase core subunit S1; TF, transferrin; TFR1, transferrin receptor 1; TFR2β, transferrin receptor 2β; TTCC, T-type calcium channel; TTP, tristetraprolin; UQCRFS1, ubiquinol-cytochrome c reductase, rieske iron-sulfur polypeptide 1; UTR, untranslated region; ZIP8, zinc transporter 8; and ZIP14, zinc transporter 14. Reproduced with permission from [30].*

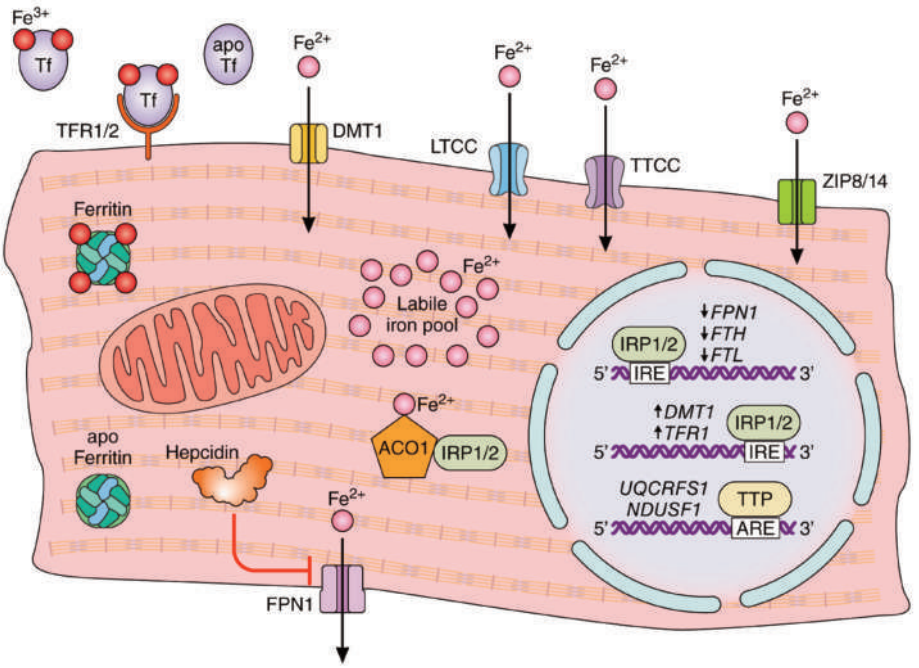
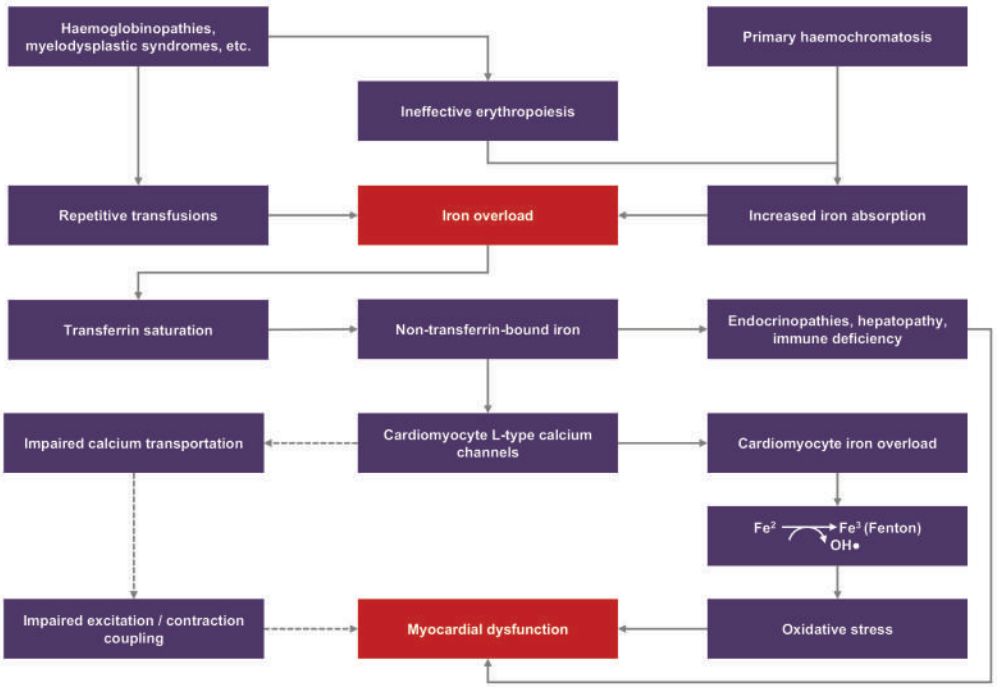
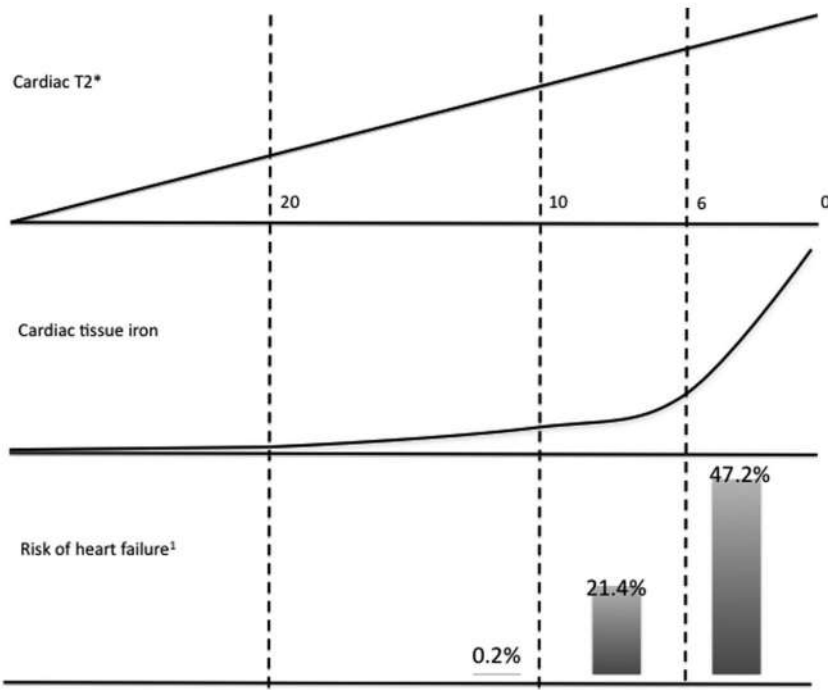


Figure 4. *Pathophysiology of iron overload cardiomyopathy. Lines indicate not well-defined mechanisms; double line, indirect effects. Redrawn with permission from [31].*



Once inside myocytes, iron binds to cytosolic ferritin, where it is safely sequestered, but if the storage capacity is exceeded, the release of free iron is highly toxic to cells [33]. Thus, patients may have significant iron overloading for long periods, even years, without major effects on cardiac function. However, rapid decompensation with acute myocyte contractile failure, producing acute heart failure, may occur at any time, precipitated by infection or other physiological or metabolic insults. The risk of decompensation rises with the degree of iron loading, as determined by the MRI relaxation parameter, T2*, to approach nearly 50% at one year for the most severely loaded patients (T2* <6 msec; Figure 5) [31, 34]. This observation underpins the critical importance of the detection of cardiac iron loading before symptoms, or clinical signs of cardiac failure are evident and, even more importantly, emphasises the crucial role of preventing iron loading in the at-risk populations.

Figure 5. *Schematic representation of the relationship of cardiac iron overload, as estimated by magnetic resonance imaging T2*, left ventricular ejection fraction, and the risk of heart failure in patients with β-thalassaemia.* [1]*Data extracted from [34]. Reproduced with permission from [31].*



4.1.1. Acute heart failure in iron overload

The dramatic presentation of acute decompensated heart failure has become a rarity in most centres dealing with β-thalassaemia. As a form of acute heart failure, it is unusual, by virtue of its capacity to be reversed by treatment with iron chelation, initially parenterally, but supported by oral chelators [35] and conventional techniques for acute heat failure treatment [36]. A strategy for managing acute iron-induced heart failure is presented in Table 1 [37].

4.1.2. Chronic ventricular dysfunction and heart failure with reduced ejection fraction (HFrEF)

A more frequently encountered phenotype in current clinical practice is the patient with impaired ventricular contractile function, often first detected during surveillance echocardiography, or MRI. Unless other causes for heart failure are definitively identified, treatment should be based around iron chelation, as this presentation is frequently associated with difficulties with adherence to chelation therapy.

Left ventricular systolic dysfunction, or a drop in left ventricular ejection fraction (LVEF) from previously documented values, generally indicates significant iron overload and requires increased chelation, after careful determination of possible causes for non-adherence to prescribed treatment.

Table 1. *Management of acute decompensated heart failure in transfusion-dependent β-thalassaemia. Adapted with permission from [37].*

The aim of treatment in acute heart failure is to keep the patient alive so that iron chelator treatment can detoxify the cardiac iron. The following management strategy is recommended:

1. Immediate commencement of 24-hour-per-day continuous (uninterrupted) intravenous iron chelation treatment with deferoxamine 50 mg/kg/day.
2. The patient should have continuous electrocardiographic and haemodynamic monitoring.
3. As soon as is practical, perform bedside echocardiography to confirm the diagnosis of heart failure and exclude other cardiovascular conditions, including pulmonary embolism.
4. Introduce deferiprone as soon as possible at a dose of 75 mg/kg/day in divided doses.
5. Supportive haemodynamic therapy should be geared to maintain cerebral and renal perfusion, avoiding aggressive inotropic therapy, which can be detrimental. Blood pressure is typically low in transfusion-dependent β-thalassaemia patients and should not attract specific therapy if renal and cerebral perfusion is maintained.
6. Only minimum diuretic treatment should be used because of the importance of maintaining preload. Consideration should be given to the alternatives such as venous ultrafiltration.
7. Cardiac arrhythmias are common and often respond to continuous iron chelation treatment. Meticulous attention should be given to normalization of electrolyte abnormalities, and consideration should be given to the use of magnesium infusion to stabilise ventricular arrhythmia. Nevertheless, amiodarone is the drug of choice to treat haemodynamically significant arrhythmias. β-Blockers can be used if the haemodynamic status allows.
8. Maintain meticulous glucose control with insulin/potassium infusion. This may also help with cardiac inotropic status.
9. Give hydrocortisone on the presumption of inadequate adrenal response to stress.
10. Check thyroid, liver, and renal function and calcium, magnesium, vitamin D, carnitine, and other metabolic parameters and correct these when necessary.
11. Maintain haemoglobin between 10 and 12 g/dL. This may require frequent small-volume transfusions.
12. Search for precipitating conditions such as infections and correct as necessary.
13. The introduction of β-blockers has the merit of reducing the propensity to arrhythmia and may take priority over renin-angiotensin-aldosterone system inhibitors.
14. Cardiac MRI T2* should be performed as soon as is practical. If cardiac T2* is >20 ms, then myocarditis should be considered as a cause of heart failure, using a standard cardiac MRI myocarditis protocol.

Additional notes:

- Clinical stabilization can occur within 14 days after commencement of continuous iron chelation treatment but can also take months.
- Patients with renal failure may require early dialysis to remove the iron chelator complex and, although experience with this is limited and may vary by chelator.
- Deferasirox has not been evaluated in acute heart failure and may be ill-advised in the presence of marginal renal perfusion.
- Consideration should be given to mechanical support devices to support both ventricles, bearing in mind the right ventricle is often compromised. There is no published evidence for this approach.
- Discussion with a cardiac transplantation centre should be undertaken early, so that a timely transfer can be considered if the initial response is inadequate.

Although there are no prospective trials specifically addressing TDT patients with impaired left ventricular function or heart failure, they need to be given the opportunity to benefit from current optimal heart failure treatment strategies, as indicated in published guidelines [38] Treatment is often complicated by the tendency for heart failure drugs to exacerbate pre-existing low blood pressure and dosage titration of heart failure medication needs to be individualised.

The length of time heart failure medication should be continued, once iron overload has been reversed, a situation that may take years of intensified chelation, is not established. In other forms of heart failure, in non-thalassaemic populations, cessation of heart failure medication once normal function has been restored, has been associated with recurrence of cardiac dysfunction, so that life-long continuation is recommended [39]. It is not clear whether such a recommendation is justified in β-thalassaemia, where cardiac dysfunction is related to a specified cardiac "toxin", excess iron.

Following the pragmatic principles of increasing chelation adherence, targeting the reduction of tissue iron overload and using conventional heart failure medication, where indicated, there has been a demonstrable reduction in mortality and in the development of heart failure [1, 40]. Combining chelators has been associated with improved rates of tissue iron removal and improvements in cardiac function [41-44] and, thus, would appear to be the treatment of choice when ventricular dysfunction is detected.

4.1.3. Restrictive physiology and heart failure with preserved ejection fraction

Cardiac restriction refers to the syndrome characterised by changes in compliance of the ventricles, increasing the resistance to filling in diastole. Clinically, the features seen on scanning include enlarged atria, decreased atrial systolic function and high ventricular filling pressures. Overall ventricular systolic function is often preserved, although parameters of longitudinal function are usually abnormal. Some of these features have been reported in small studies of thalassaemic patients [45] and there is a relationship with iron loading [46]. These changes in cardiac diastolic function are often insidious and exacerbated by ageing. In time, systolic dysfunction may also occur.

Increased left atrial filling pressures lead to increases in pulmonary arterial pressure which if severe may generate right heart failure through post-capillary pulmonary hypertension and right-sided heart failure, especially if the right ventricle is involved with iron overload.

In β-thalassaemia, iron loading of the tissues, chronic anaemia with the associated persistent high output state and abnormalities of vascular endothelial function [26], plus diabetes, all contribute to the development of restriction and subsequent potential progression to HFpEF.

For the patient, fluid congestion, oedema, hepatic congestion, exercise limitation and a high risk of atrial fibrillation are the consequences. Historically, HFpEF was treated purely symptomatically, largely with diuretics. However, sodium glucose co-transporter 2 inhibitors (SGLT2i) have shown a mortality benefit in all types of heart failure, including, for the first time, in HFpEF [47]. Thus, although there is no direct prospective data in β-thalassaemia so far, it appears rational to consider the introduction of SGLT2i early in β-thalassaemia patients with any form of heart failure (including HFrEF and HFpEF) [47].

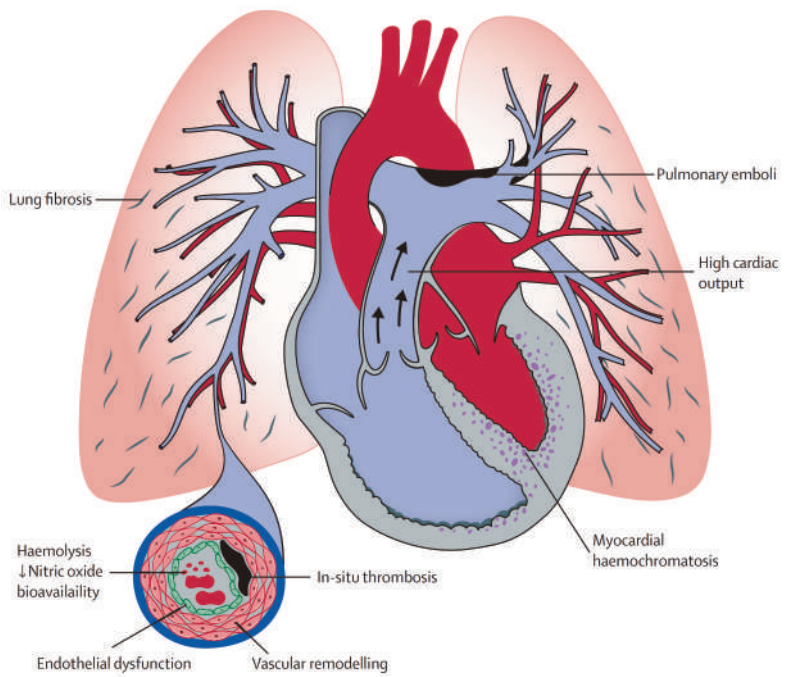
Otherwise, treatment is largely geared to prevention, by amelioration of the factors leading to the syndrome of HFpEF. This would include optimal pretransfusion haemoglobin, intensive chelation, and now, the early consideration of SGLT2i therapy in patients with diabetes and/or chronic kidney disease (CKD), as SGLT2i have been shown to prevent heart failure events in general patients with diabetes and CKD [47].

4.2. Pulmonary hypertension

Pulmonary hypertension is relatively uncommon in TDT patients, as evident from studies in Europe (9/1062, 0.8%) [1], and more frequently encountered in non-transfusion-dependent β-thalassaemia patients (4.8%), especially in splenectomised adults [48]. It is also likely more prevalent in LMICs [49, 50].

Pathophysiologically, pulmonary hypertension may be due to left ventricular disease, pulmonary disease, pulmonary arterial obstruction, or pulmonary arterial vascular disease (pulmonary arterial hypertension). β-Thalassaemia patients are likely to have a combination of several of these components in action generating the risk of pulmonary hypertension and leading to right-sided heart failure and premature mortality (Figure 6) [51].

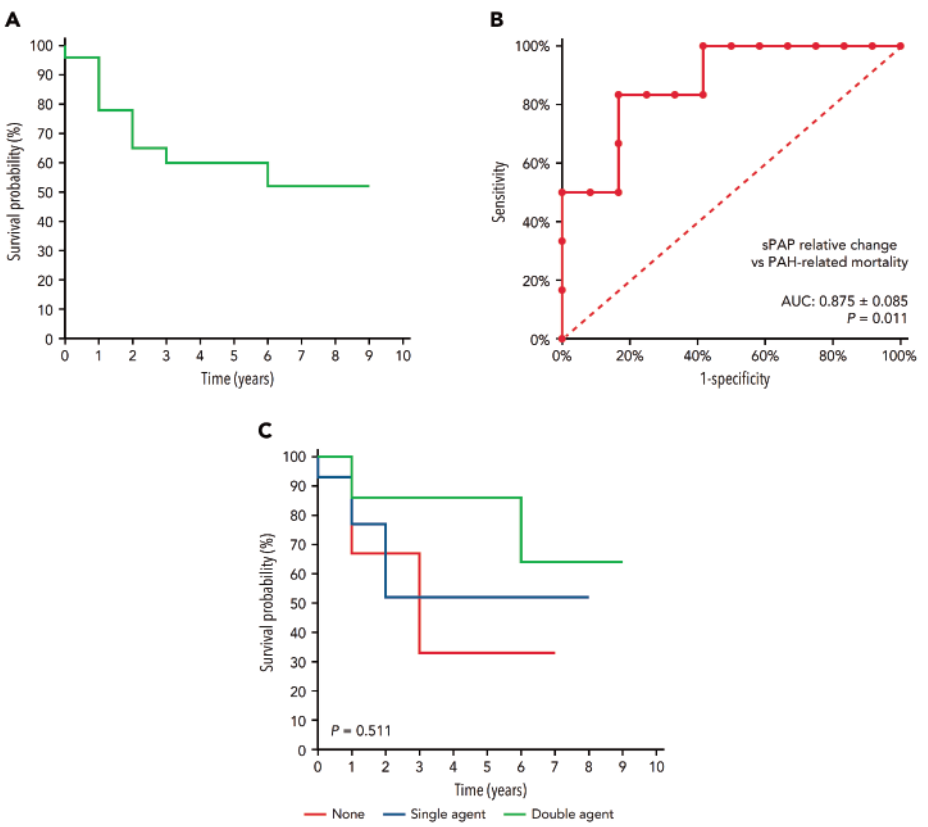
Figure 6. *Major heart and lung pathogenic features in patients with β-thalassaemia and pulmonary hypertension. Reproduced with permission from [51].*



Pulmonary hypertension appearing in response to left ventricular dysfunction and raised left atrial pressures generated by HFpEF or by HFrEF requires treatment geared to improve systolic and diastolic left ventricular function, or better, to prevent the development of dysfunction. In this instance, by iron chelation and maintenance of pretransfusion haemoglobin as high as practicable. Unloading the heart, using conventional heart failure medication where indicated, and a low threshold to consider SGLT2i for those at risk of or suffering from HFpEF, would appear to be the rational approach.

Intravascular haemolysis, with the liberation of free haemoglobin in the circulation, is believed to be an important factor in generating endothelial dysfunction in haemoglobinopathies. Thus, pulmonary arterial hypertension, where abnormalities of left ventricular function are excluded (pulmonary capillary wedge pressure <15 mmHg) and the abnormality rests within the pulmonary vasculature, is encountered where haemolysis is high, specifically in previously under-transfused patients, e.g. non-transfusion-dependent β-thalassaemia (β-thalassaemia intermedia) [52]. A recent longitudinal follow up of β-thalassaemia patients (major and intermedia) with pulmonary arterial hypertension revealed a survival of 60% at 5 years (Figure 7) [53], and improvement in pulmonary arterial pressure has been observed in patients receiving specific pulmonary arterial hypertension-related medications [53, 54], although data from randomised clinical trials in β-thalassaemia are still lacking.

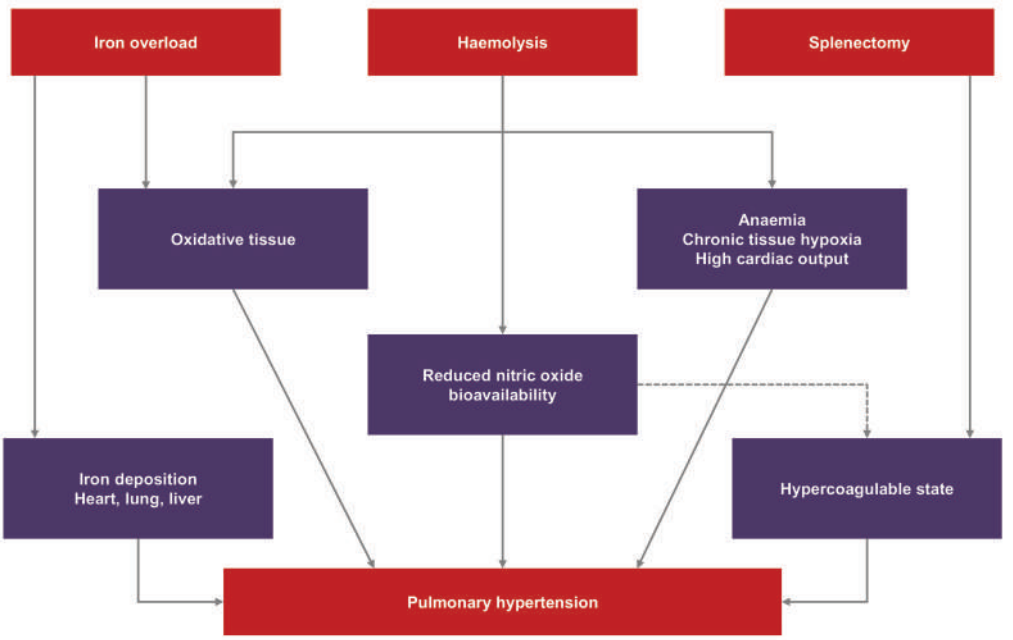
Figure 7. *Taken from a prospective study of patients with catheter proven PAH. (A) Kaplan-Meier survival curve for PAH-related mortality. (B) Receiver operating characteristic curve for relative change in sPAP as a predictor of PAH-related mortality. (C) Kaplan-Meier survival curve for P AH-related mortality by receipt of PAH-related therapy (one, two, or none). Abbreviations: PAH, pulmonary arterial hypertension; sPAP, systolic pulmonary artery pressure. AUC, area under the curve. Reproduced with permission from [53].*



Pulmonary thromboembolic disease is a risk in β-thalassaemia, particularly for those who have undergone splenectomy. It may account for up to 30% of pulmonary hypertension cases in β-thalassaemia according to one small study [55], but because of the possibility of treatment with anticoagulation, the potential contribution of pulmonary embolism to pulmonary hypertension should be sought in all β-thalassaemia patients.

Since pulmonary hypertension carries a risk for reduced survival, the clinical emphasis should be on preventing the development of pulmonary hypertension by addressing the underlying pathophysiology (Figure 8) [51], and focusing on maintaining good left ventricular function, minimising haemolysis rates, preventing thromboembolism, and ensuring healthy lungs.

Figure 8. *Pathophysiology and risk factors of pulmonary hypertension in β-thalassaemia. Redrawn with permission from [51].*



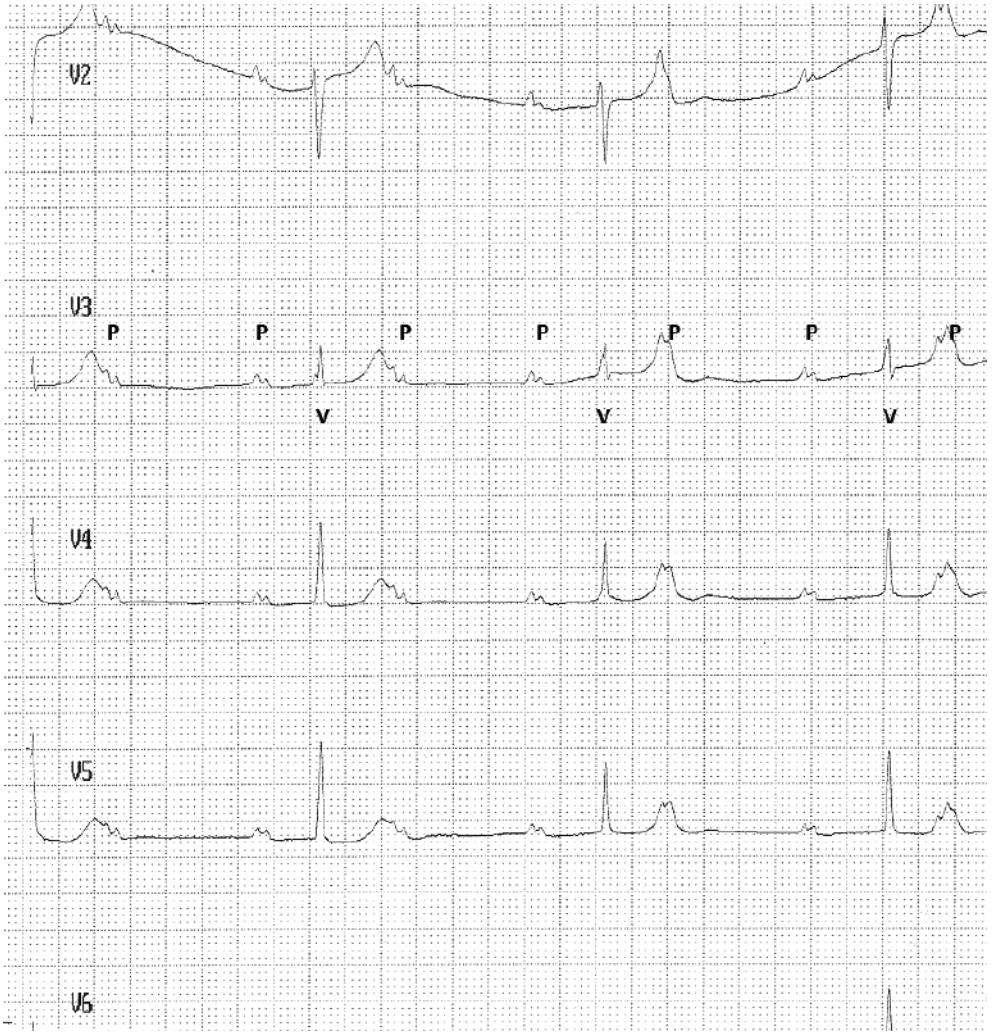
4.3. Arrhythmia

Arrhythmias are common in the iron-loaded population, with an increasing frequency as they age, where atrial fibrillation is the commonest clinical problem encountered [56]. It is crucial to consider the propensity to arrhythmia and management within the clinical context of the TDT patient, specifically their cardiac iron loading status and current underlying ventricular function.

When iron overload is severe, all forms of arrhythmia from heart block to tachycardias may be encountered and pose a medical emergency, especially if there is impaired ventricular function. From the earliest clinical descriptions of β-thalassaemia, complete heart block (CHB) was described, but is very infrequently encountered in current practice (Figure 9). Although CHB may reverse with immediate intense chelation with deferoxamine, a pacemaker is now usually implanted to stabilise

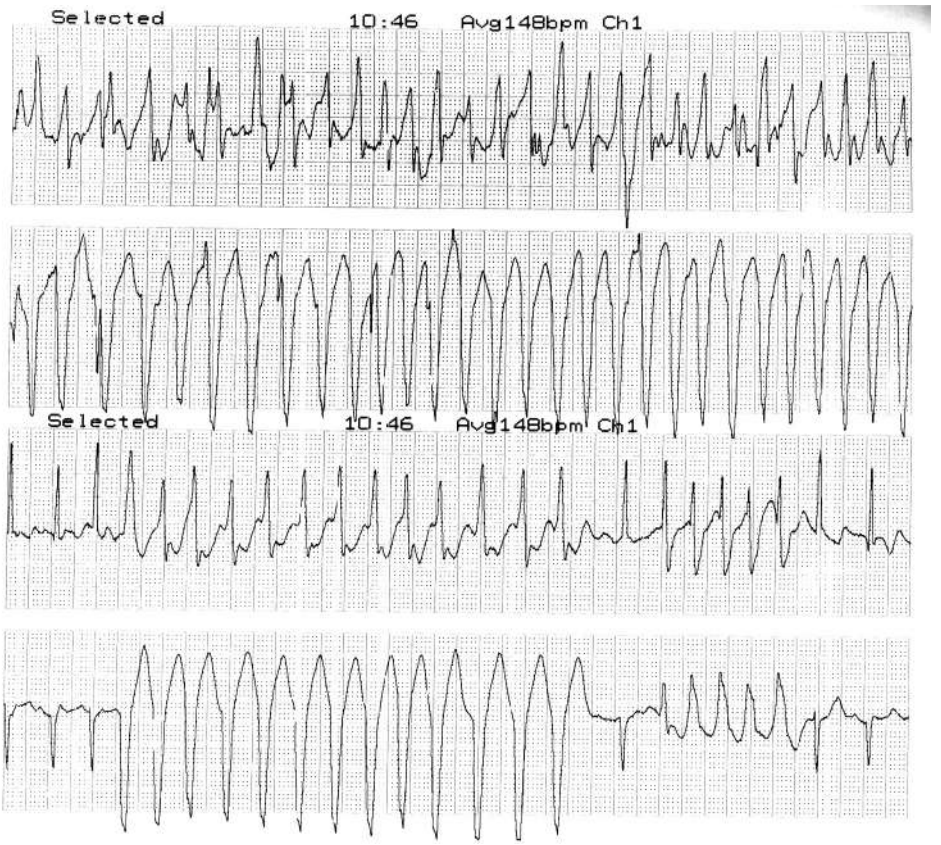
the situation and allow cardiac iron removal to occur over days and weeks. In some patients, normal conduction is never regained, despite "de-ironing" the heart. Permanent implantable devices, pacemakers, implantable cardioverter-defibrillators (ICD) and their leads are now, by default, compatible with MRI scanning.

Figure 9. *An electrocardiogram strip demonstrating complete heart block (p-waves marked "p", ventricular QRS complexes marked "v") from a young β-thalassaemia patient presenting with severe right heart failure due to iron overload. Figure courtesy of JM Walker.*



Ventricular tachycardia (Figure 10) is usually a manifestation of severe ventricular impairment and iron overload. It carries the risk of sudden death and deserves immediate admission to hospital for expert assessment and likely ICD implantation, despite responding to intensive parenteral chelation with deferoxamine presumably due to the immediate mopping up of toxic non-transferrin-bound iron.

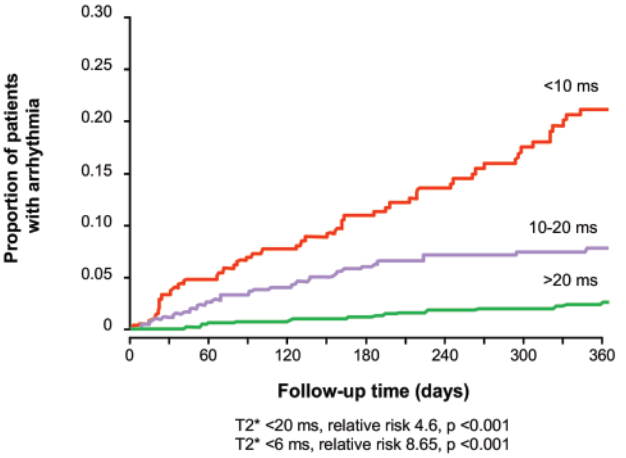
Figure 10. *A rhythm strip demonstrating a broad complex tachycardia in a 29-year-old patient with β-thalassaemia and heavy cardiac iron loading (T2* <6 ms), presenting to clinic with palpitations and near-syncope. Figure courtesy of JM Walker.*



Atrial fibrillation in a younger age group, is usually a manifestation of significant iron overload and in those patients can precipitate acute cardiac decompensation and needs to be reversed early, usually by urgent direct current cardioversion. Acute management follows the suggested lines for decompensated heart failure (Table 1) [37], with parenteral deferoxamine supported by oral deferiprone in the subacute stage.

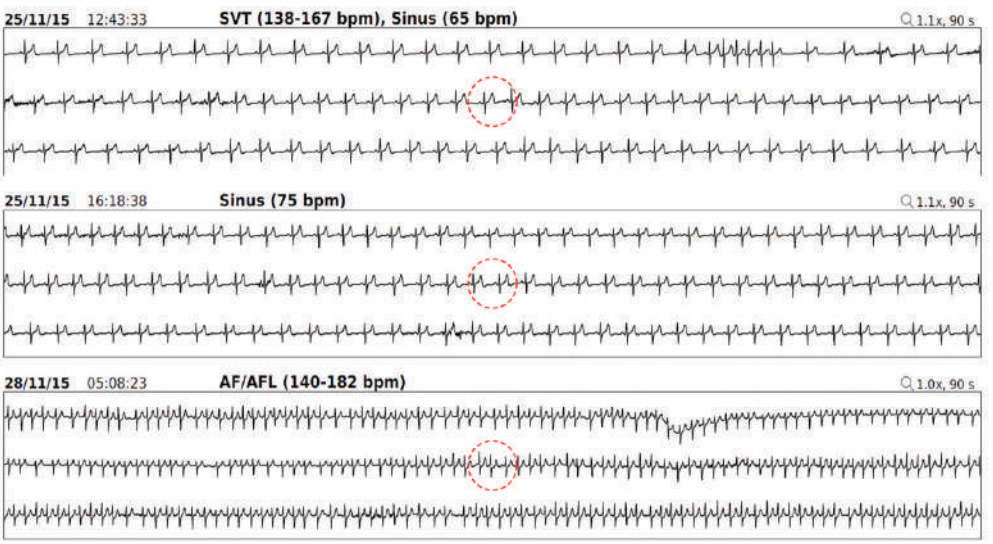
Thus, although there appears to be a relationship between atrial fibrillation and iron loading, as measured by the MRI T2*, it is not as strong or predictable a relationship as it is for T2* and the development of heart failure (Figure 11) [34].

Figure 11. *Risk of developing arrythmia within 12 months, according to the level of cardiac iron, measured by magnetic resonance imaging T2*. Modified with permission from [34].*



Paroxysmal atrial fibrillation is often encountered in older patients, with a prevalence of up to 40% in β-thalassaemia patients over 40 years of age [57, 58]. In this scenario, iron overload is frequently absent, but the risk factors for the development of atrial fibrillation include diabetes, a distant history of iron overload and non-specific electrocardiogram (ECG) abnormalities, such as right bundle branch block (RBBB) (Figure 12) [57].

Figure 12. *Wearable 7-day electrocardiogram recorder strips obtained from a 44-year-old transfusion-dependent β-thalassaemia patient with no cardiac iron (T2* 34 ms), but a history of congestive heart failure more than 20 years previously. The history was of occasional attacks of fast, uncomfortable palpitations without syncope. The top two samples during the day revealed normal sinus rhythm. The bottom strip shows a narrow QRS tachycardia (atrial fibrillation) with a fast heart rate in the early hours of the morning (this episode was asymptomatic). Figure courtesy of JM Walker.*



The risk of embolisation is increased in the haemoglobinopathy subpopulation, especially if they have had a splenectomy and anticoagulation should be considered. Increasingly, it is recognised that the burden of atrial fibrillation is important in making any decision about, what is likely to be life-long anticoagulation [59], particularly as it may affect younger age groups within the β-thalassaemia population. It is important to note that widely applied stroke risk calculators, such as the CHA_2DS_2-VA scores, applied to patients with atrial fibrillation to decide on the need for anticoagulation, should not be applied to patients with haemoglobinopathies [60].

A detailed description of the treatment of atrial fibrillation other than in broad principles is not appropriate in this Chapter but has been the subject of extensive review elsewhere [61, 62]. In all cases, patients will require an assessment of the frequency of attacks, the underlying cardiac status, including iron loading, cardiac function, chamber sizes (patients with large atria will be more difficult to treat than patients with normal or near-normal left atrium size), as well as assessment of diabetes and other endocrinopathies.

Infrequent atrial fibrillation of short duration in a patient with no cardiac iron overload may not require any intervention, other than reassurance. In other circumstances, the risk factors of iron, ventricular function and diabetes, will need to be optimised and anticoagulation discussed. Patients with difficult to control atrial fibrillation may require interventions such as ablation and/or pacemaker therapy.

4.4. Other forms of cardiovascular disease

4.4.1. Thromboembolism

The hypercoagulable state that exists for β-thalassaemia patients predisposes them to both arterial and venous thrombosis, a risk which appears to be 4-fold higher in non-transfusion-dependent β-thalassaemia than TDT patients who receive less or no transfusion [63]. It has been attributed to various underlying mechanisms including a thrombogenic potential of haemolysed red blood cells, platelet activation and thrombocytosis, iron-mediated endothelial damage, and coagulopathy, all which have been seen at higher rates in splenectomised adults [64, 65]. A thalassaemia-related thrombosis system (TRT-RSS) has been suggested and included patient age >35 years, haemoglobin level <9 g/dL, serum ferritin level ≥1000 ng/mL, splenectomy, and transfusion-independence as independent risk factors to be considered in the context of other conventional risk factors in local risk scoring systems and prophylaxis guidelines for medical and surgical patients [66]. Strokes, even amongst younger patients, are encountered and atrial fibrillation is clearly an important risk factor, as noted in a number of small case series [67-69].

4.4.2. Pericardial disease

Historically, pericardial involvement including pericarditis with severe iron overload was reported, but its prevalence in countries with access to good care and reduced tissue iron loading is now very low [52, 70].

4.4.3. Valvular disease

Co-existing valve disease may be encountered in TDT patients and should be managed conventionally, whilst bearing in mind the additional cardiac burden imposed on these patients by the chronic high output state imposed by a persisting, although mild, anaemia. An increased occurrence of degenerative aortic stenosis may be expected in ageing β-thalassaemia populations, although relative epidemiological data are missing [13].

4.4.4. Non-iron-related cardiomyopathy

Non-thalassaemia related inherited cardiomyopathies, such as hypertrophic cardiomyopathy, may very rarely occur coincidentally with β-thalassaemia. Their identification and characterisation may explain abnormalities of cardiac function or severe arrhythmia not due to iron overload or chronic anaemia; such diagnoses should be investigated and treated by expert specialist services.

4.4.5. Arteriosclerosis

Arteriosclerosis would be expected to be accelerated in the thalassaemic population, by virtue of their multi-system condition with intravascular haemolysis, chronic inflammation and additional "conventional" risk factors for arteriosclerosis, including diabetes. It has been detected in animal models of β-thalassaemia [71] and increases in intima-media thickness reported in children [72, 73], with changes in vascular compliance also documented [26]. However, classical arteriosclerotic coronary disease does not appear to be prevalent in the ageing β-thalassaemia cohorts in Europe. Nevertheless, it is not reasonable to withhold conventional risk factor treatment, or advice on healthy lifestyles to TDT patients, in the hope that they are protected in some, as yet, unidentified fashion.

4.4.6. Cancer

With an ageing population, it is inevitable that some β-thalassaemia patients will develop cancer. Some, such as hepatocellular carcinoma, are specifically prevalent due to underlying iron overload or hepatitis C virus infection and liver disease [74]. Although cancers should be treated according to current best practice, the potential for TDT patients to have higher cardiac toxicity from anthracycline-containing chemotherapy should be considered [75] and suitable intensive surveillance and cardio-protective measures need to be discussed with the oncology team and, if needed, a cardio-oncology expert [76].

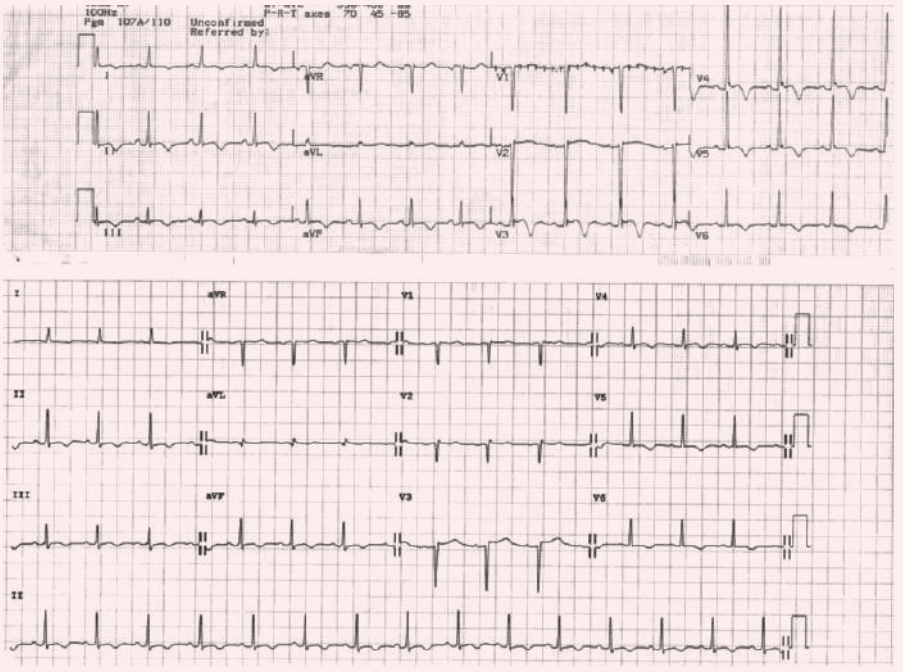
5. CARDIAC DIAGNOSTICS

5.1. Electrocardiogram

The ECG remains an essential part of a cardiovascular assessment, and non-specific abnormalities are frequently encountered in practice, but the essential clinical message is that a normal ECG does not exclude significant myocardial iron loading or imply a low risk of events (Figure 13).

Many arrhythmias are intermittent and require prolonged ECG monitoring to capture. The technology to achieve this has improved radically in recent years and includes conventional 24-hour Holter monitors, wearable patch ECGs lasting from 7 to 14 days or more, implantable loop recorders with a life of years, to patient worn smart devices. Choosing the appropriate tool is important to maximise the likelihood of detecting an intermittent, but potentially serious problem and has been reviewed elsewhere [77].

Figure 13. *Two electrocardiograms from the same adult with transfusion-dependent β-thalassaemia. The top trace was recorded when he was seriously iron overloaded with no abnormality of systolic cardiac function (by echo). The electrocardiogram shows sinus rhythm (with some electrical artifact in lead V1) with widespread, deep T-wave inversion (most marked in V3 and V4). The bottom trace was recorded some years later, when cardiac iron had normalised. T-wave inversion persists but is less marked. Figure courtesy of JM Walker.*



Many arrhythmias are intermittent and require prolonged ECG monitoring to capture. The technology to achieve this has improved radically in recent years and includes conventional 24-hour Holter monitors, wearable patch ECGs lasting from 7 to 14 days or more, implantable loop recorders with a life of years, to patient worn smart devices. Choosing the appropriate tool is important to maximise the likelihood of detecting an intermittent, but potentially serious problem and has been reviewed elsewhere [77].

5.2. Echocardiography

Echocardiography is a widely available, safe and painless technique that provides instant, bed-side cardiovascular assessment. It forms the basis for most cardiovascular surveillance and has developed significantly in recent years. Like all ultrasound imaging, there is an important problem with variability, which needs to be minimised by adherence to image acquisition techniques and appropriate rigorous training of sonographers to international standards [78].

Detailed analysis of chamber size and detection of acquired problems (e.g., intra-cardiac thrombus [79]) or developmental defects (e.g., atrial septal defects) allows for precision in cardiovascular assessment for TDT patients.

The LVEF is the ubiquitously used parameter of systolic function. It has an approximate 10% coefficient of variability that can be improved with modern techniques, using volumetric analysis (3D and 4D echo). The LVEF changes late with iron overload, although progressive reduction in LVEF or even right ventricular ejection fraction over time, whilst remaining within normal ranges, may alert clinicians to a developing problem, in this context, likely to be increasingly severe cardiac iron content.

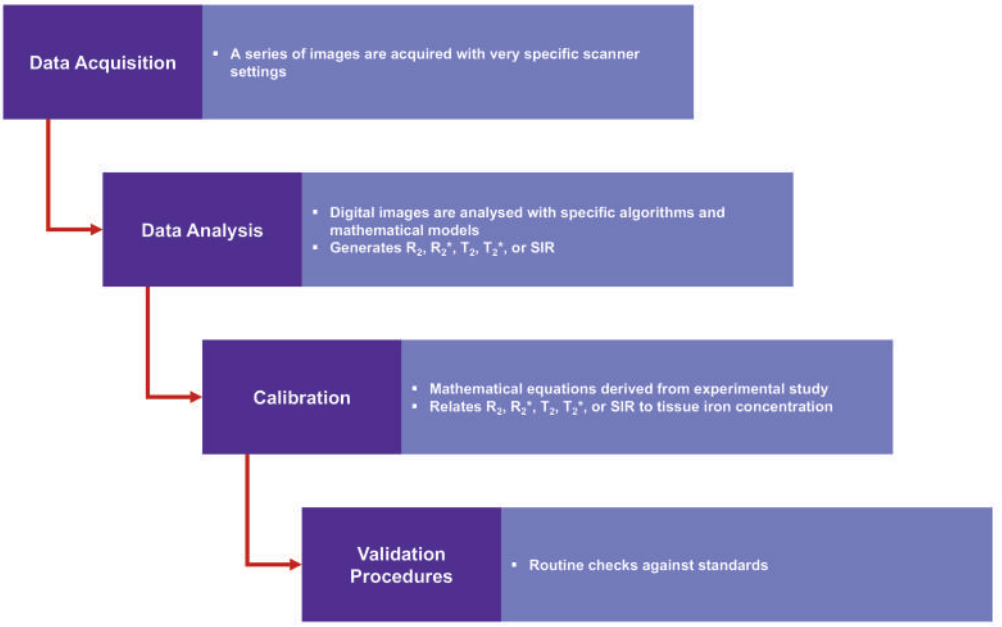
Newer modalities, such as tissue Doppler imaging (TDI) and strain measurements (global longitudinal strain [GLS]) have improved the sensitivity of echo assessment [80]. Including echo scanning in surveillance programmes allows changes in parameters to be used to triage individuals for more complex, expensive and less accessible tests, such as MRI scans [81]. In the absence of, or if there is likely to be a significant delay in obtaining an MRI, the observation of progressive deterioration in echo parameters should stimulate a change to more intensive therapy and/or encouragement to improve adherence of prescribed iron chelation.

Screening for the development of pulmonary hypertension can only practically be undertaken with echocardiography, where a tricuspid valve regurgitant jet velocity (TRVmax) of >3.2 m/s provides an upper limit above which pulmonary hypertension becomes likely (positive predictive value of 93.9% in a cross-sectional study of β-thalassaemia) [48], and has been associated with adverse outcomes [82]. Prospective studies to help determine the best criteria by which patients need to be selected for referral for diagnostic right heart catheterisation are not available, but clinicians should seek advice early to reduce the chance that vascular changes become irreversible. Importantly, potentially remediable causes of pulmonary hypertension, such as pulmonary embolism, need to be actively sought and excluded.

5.3. Cardiac magnetic resonance imaging

The development of MRI to non-invasively quantify tissue iron [83] has proven to be a highly successful clinical advance in the management of TDT, accredited with aiding a 70% fall in mortality [3]. The T2* has become the international standard for risk assessment related to tissue iron loading, but its reliability depends critically on strict adherence to scanning and calculation protocols (Figure 14) [84].

Figure 14. *Four key components for an MRI-based iron measurement method. It is essential that data acquisition, data analysis, and validation procedures match exactly to those used in the calibration study. Even small deviations from protocols will cause calibration shifts and hence inaccurate measurements. Abbreviations: MRI, magnetic resonance imaging; SIR, signal intensity ratio. Redrawn with permission from [84].*



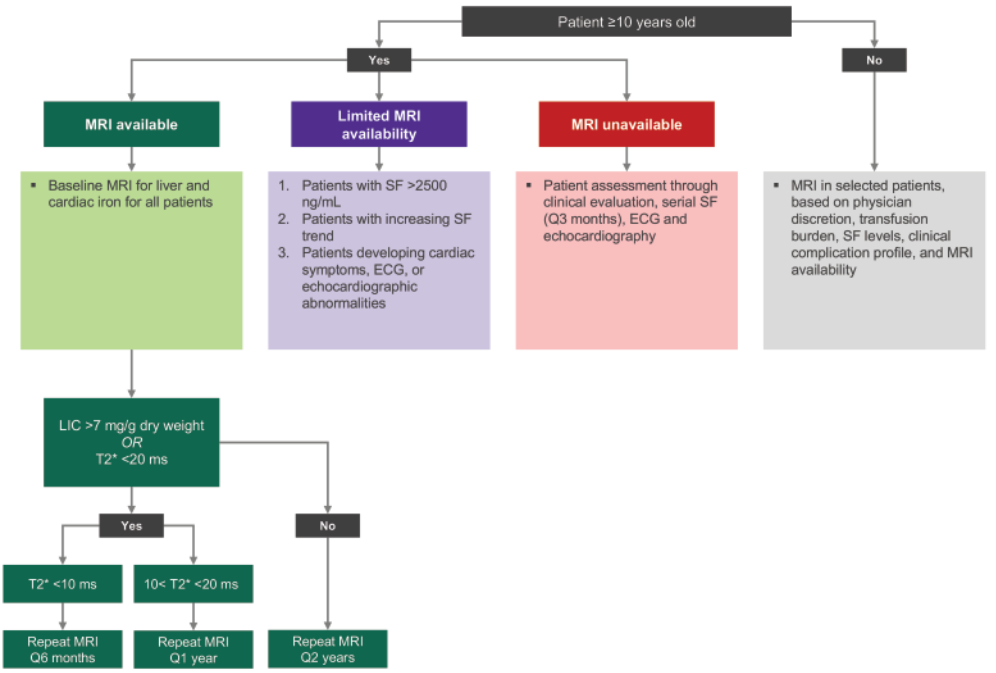
Iron content is assessed by estimation of relaxation parameters, such as R2*. But the T2* (reciprocal of R2*) obtained on a 1.5 Tesla (T) MRI is the only measure to have been validated against physical cardiac tissue measurement of iron content [85]. Many centres are replacing 1.5T MRIs with 3T machines and although direct tissue validation of T2* measurements on 3T MRI scanners is lacking, there is evidence that a good correlation for T2* obtained between scanners is possible, although with reduced reproducibility at low T2* values (high iron content) [86].

In general terms, the risk of heart failure rises with increasing cardiac iron content, shown by a falling T2* (Figure 5) [31, 34]. More recent analyses from the Italian MIOT group have demonstrated improved prediction of cardiovascular complications by incorporating assessment of left ventricular function, chamber size, and fibrosis into the risk profile [87].

The perceived complexity and expense of MRI has hampered adoption in some LMICs, but newer, faster, and cheaper scan sequences are addressing these issues [9, 88, 89]. To aid the speed and accessibility of MRI, the use of mapping sequences for T1 and T2*, which are commercially available and often provided with the scanner, has been explored [8]. These maps can provide an instant assessment of cardiac iron content, colour-coded into categories of severe, moderate, and no iron, obviating the need for third-party software tools to calculate T2*.

Although MRI has become an indispensable tool in the management of iron overload in TDT, hurdles exist in providing access in LMICs and ensuring reliable scanning techniques with validated sequences and appropriate quality control measures are in place to provide reliable results. An algorithm to aid patient selection and prioritization for MRI scanning when resources are limited has been proposed (Figure 15) [90].

Figure 15. *A proposed algorithm to guide MRI T2* use, according to local availability. Abbreviations: LIC, liver iron concentration; MRI, magnetic resonance imaging; SF, serum ferritin; ECG, electrocardiogram, Q, every. Modified with permission from [90].*



5.4. Blood biomarkers

Cardiac biomarkers such as N-terminal pro-B-type natriuretic peptide (NT-proBNP) and troponin have a very limited role in managing most patients with iron overload but are useful in acute decompensation and to manage fluid overload in chronic heart failure [36]. There is also some scope to include NT-proBNP assessment when screening for pulmonary hypertension, with the aim to improve diagnostic accuracy and risk assessment.

5.5. Cardiac catheterisation

Invasive right cardiac catheterisation is required to definitively diagnose pulmonary hypertension, with a mean pulmonary arterial pressure >25 mmHg at rest, in patients with suspected pulmonary hypertension by virtue of echocardiography screening. Catheterisation is generally reserved for those patients where there is doubt about the diagnosis of pulmonary hypertension or where pre-capillary pulmonary hypertension is a possibility and specific pulmonary arterial hypertension treatments are being contemplated.

6. PREVENTION AND SURVEILLANCE

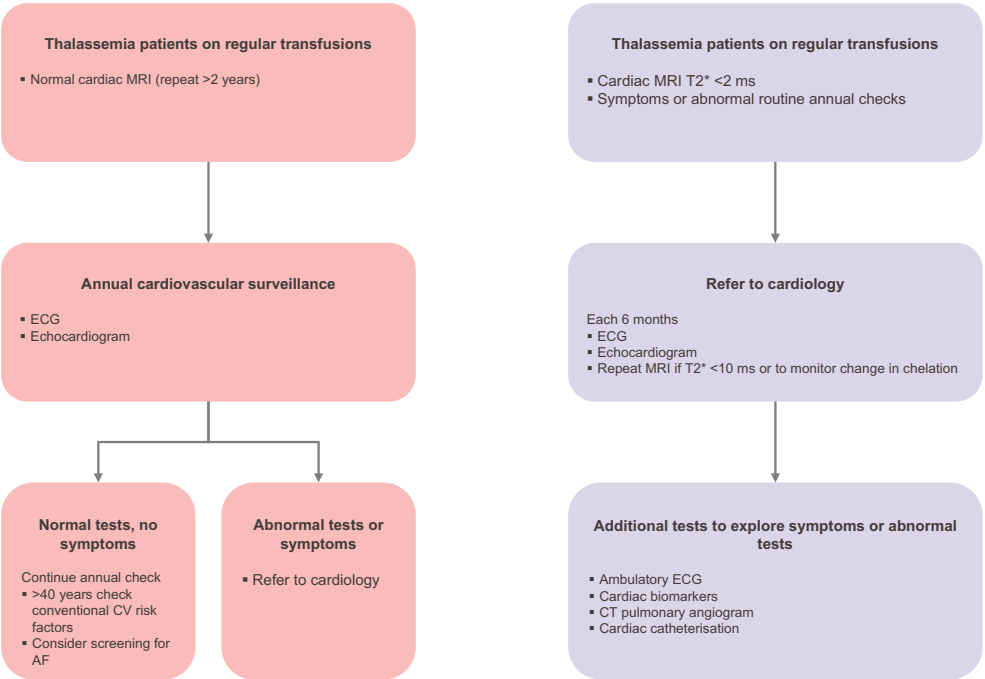
The approach to prevention of cardiovascular complications in TDT is now well-established [91], although incompletely successful [1], in that, despite a reduced mortality rate, cardiovascular disease still accounts for a high proportion of deaths (~ 50% of deaths in recent series from Europe) [92]. Regular blood transfusion to optimise pretransfusion haemoglobin and adequate chelation from a young age are the basic, unchanging principles in patients without access to curative therapy.

The use of calcium channel blockers, such as amlodipine, have shown some promise in slowing the accumulation of cardiac iron [93, 94], but have yet to be adopted generally in clinics. This may be partly due to the blood pressure lowering effect of calcium antagonists, making their introduction difficult in patients who already tend to have low blood pressure.

As the TDT population ages, the detection and management of other risk factors for the development of age-related cardiovascular conditions, need to be considered. Hypertension is very rare in this population, unless there are specific renal factors affecting the patient. Hyperlipidaemia is also rare. However, diabetes is common among other endocrinopathies, and these conditions need to be well controlled. CKD may also occur and predispose to cardiovascular disease.

Lifestyle modification, including regular exercise, healthy diet, weight control and smoking absence are crucial for cardiovascular disease prevention and should be strongly recommended. Meanwhile, surveillance for the early detection of cardiovascular complications is supported by international experience and each centre will need to develop their own specific patient-oriented programmes that take into account local factors. A simple proposal is illustrated in Figure 16.

Figure 16. *A basic algorithm for the cardiac evaluation of patients with transfusion-dependent β-thalassaemia. Abbreviations: MRI, magnetic resonance imaging; ECG, electrocardiogram; CV, cardiovascular; AF, atrial fibrillation; CT, computed tomography.*



7. GENERAL APPROACH TO CARE

Cardiovascular disease has been the single leading cause of mortality in TDT but is now significantly declining in patient populations with access to modern multi-disciplinary care and monitoring. The pathophysiology and phenotypes of cardiovascular disease depend on the interaction between the main disease and the applied disease-specific therapy. The cardiovascular spectrum consists of a wide range of disorders. Among them, iron overload cardiomyopathy has long been the main form of heart disease in TDT patients but is now being effectively prevented and managed with MRI-guided contemporary iron chelation therapy. Regular cardiovascular assessment should be part of a multidisciplinary monitoring programme and should ideally be performed by or in consultation with clinics or physicians with experience in cardiovascular disease in haemoglobinopathies and in close collaboration with the attending β-thalassaemia physician. The prevention and treatment of cardiovascular disease, besides cardioactive therapies and interventions, relies crucially on the optimisation of disease-specific therapy and the successful management of comorbid conditions. A lifestyle that promotes cardiovascular health is an important part of cardiovascular prevention, while the particularities of cardiovascular disease in TDT should be taken under consideration in the management of patients. Most importantly, cardiac dysfunction and heart failure may be reversible by timely therapy.

KEY POINTS AND RECOMMENDATIONS

1. Regular cardiovascular assessment should be part of a transfusion-dependent β-thalassaemia (TDT) patient's multidisciplinary monitoring programme (**Grade C**).
2. Cardiovascular assessment and management should ideally be performed by or in consultation with clinics or physicians with experience in cardiovascular disease in haemoglobinopathies and in close collaboration with the attending β-thalassaemia physician (**Grade C**).
3. Magnetic resonance imaging (MRI) T2*-guided iron chelation therapy represents the best available approach to prevent cardiac dysfunction related to iron overload (**Grade B**).
4. In places lacking cardiac MR T2* assessments, worsening of left ventricular function in serial echocardiograms in TDT patients, particularly those with poor compliance with iron chelation regimens, may be used as a red flag for iron toxicity and should prompt aggressive and sustained escalation of chelation therapy (**Grade B**).
5. Echocardiographic screening should be part of annual cardiovascular assessment starting the age of 10 years. In addition to routine parameters of function and chamber size, screening for pulmonary hypertension should be undertaken. A tricuspid valve regurgitant jet velocity (TRVmax) >3.2 m/s should be referred for specialist input, including the consideration of cardiac catheterisation to confirm the diagnosis of pulmonary hypertension if a proximate cause, such as pulmonary embolism cannot be identified and corrected (**Grade B**).
6. Combined therapy with deferoxamine and deferiprone represents the best available intensive chelation for TDT patients with cardiac iron overload, with or without overt cardiac dysfunction or heart failure (**Grade B**).
7. Diagnosis of cardiovascular disease should prompt optimisation of disease-specific therapy in addition to cardioactive treatment (**Grade C**).
8. Regular electrocardiogram (ECG) monitoring should be considered. Opportunistic screening for atrial fibrillation in older patients and, when indicated, ambulatory ECG monitoring should be considered for the detection of atrial fibrillation, followed by evidence-based anticoagulation therapy when significant atrial fibrillation burden is detected (**Grade C**).
9. Screening and treatment of endocrine and metabolic comorbidities is crucial for the prevention and management of cardiovascular disease (**Grade C**).
10. Management of cardioactive therapies must account for a patient's unique physiology compared with the general population (**Grade C**).
11. Cardiac abnormalities including ventricular dysfunction, heart failure, and arrhythmias are often reversible following intensification of disease-specific therapy, albeit after several weeks or months (**Grade C**).
12. Lifestyle choices that promote cardiovascular health (absence of smoking, physical exercise, weight control, healthy diet) should be vigorously promoted in TDT patients (**Grade C**).
13. Venous thromboembolic risk assessment and prophylaxis should be conducted for TDT patients in medical and surgical settings based on local guidelines, especially in older patients who are splenectomised, with low pretransfusion haemoglobin, or pregnant (**Grade C**).

REFERENCES

1. Pepe A, Pistoia L, Gamberini MR, Cuccia L, Lisi R, Cecinati V, Maggio A, Sorrentino F, Filosa A, Rosso R, Messina G, Missere M, Righi R, Renne S, Vallone A, Dalmiani S, Positano V, Midiri M, Meloni A. National networking in rare diseases and reduction of cardiac burden in thalassemia major. Eur Heart J 2022;43(26):2482-2492.
2. Voskaridou E, Ladis V, Kattamis A, Hassapopoulou E, Economou M, Kourakli A, Maragkos K, Kontogianni K, Lafioniatis S, Vrettou E, Koutsouka F, Papadakis A, Mihos A, Eftihiadis E, Farmaki K, Papageorgiou O, Tapaki G, Maili P, Theohari M, Drosou M, Kartasis Z, Aggelaki M, Basileiadi A, Adamopoulos I, Lafiatis I, Galanopoulos A, Xanthopoulidis G, Dimitriadou E, Mprimi A, Stamatopoulou M, Haile ED, Tsironi M, Anastasiadis A, Kalmanti M, Papadopoulou M, Panori E, Dimoxenou P, Tsirka A, Georgakopoulos D, Drandrakis P, Dionisopoulou D, Ntalamaga A, Davros I, Karagiorga M, Greek Haemoglobinopathies Study G. A national registry of haemoglobinopathies in Greece: deducted demographics, trends in mortality and affected births. Ann Hematol 2012;91(9):1451-1458.
3. Modell B, Khan M, Darlison M, Westwood MA, Ingram D, Pennell DJ. Improved survival of thalassaemia major in the UK and relation to T2* cardiovascular magnetic resonance. J Cardiovasc Magn Reson 2008;10(1):42.
4. Aydinok Y. Combination chelation therapy. Ann N Y Acad Sci 2023;1529(1):33-41.
5. Tantiworawit A, Kamolsripat T, Piriyakhuntorn P, Rattanathammethee T, Hantrakool S, Chai-Adisaksopha C, Rattarittamrong E, Norasetthada L, Fanhchaksai K, Charoenkwan P. Survival and causes of death in patients with alpha and beta-thalassemia in Northern Thailand. Ann Med 2024;56(1):2338246.
6. Daar S, Al-Naamani K, De Sanctis V, Al Rahbi S, Al Zadjali S, Khan H, Panjwani V, Al-Khabori M. Mortality and complications in Omani patients with beta-thalassemia major: a long-term follow-up study. Acta Biomed 2023;94(4):e2023191.
7. Koohi F, Kazemi T, Miri-Moghaddam E. Cardiac complications and iron overload in beta thalassemia major patients-a systematic review and meta-analysis. Ann Hematol 2019;98(6):1323-1331.
8. Abdel-Gadir A, Vorasettakarnkij Y, Ngamkasem H, Nordin S, Ako EA, Tumkosit M, Sucharitchan P, Uaprasert N, Kellman P, Piechnik SK, Fontana M, Fernandes JL, Manisty C, Westwood M, Porter JB, Walker JM, Moon JC. Ultrafast Magnetic Resonance Imaging for Iron Quantification in Thalassemia Participants in the Developing World: The TIC-TOC Study (Thailand and UK International Collaboration in Thalassaemia Optimising Ultrafast CMR). Circulation 2016;134(5):432-434.
9. Medina KM, Abdel-Gadir A, Ganga K, Ojha V, Pratap S, Boubertakh R, McGrath L, Augusto JB, Rikowski A, Mughal N, Khanna VK, Seth T, Sharma S, Mahajan A, Bansal RK, Srivastava P, Mahajan H, Mahajan V, Walker J, Seldon T, Ako E, Moon JC, Walker JM. Use of rapid cardiac magnetic resonance imaging to guide chelation therapy in patients with transfusion-dependent thalassaemia in India: UMIMI study. Eur Heart J Qual Care Clin Outcomes 2022;8(3):289-297.
10. Cappellini MD, Porter JB, Viprakasit V, Taher AT. A paradigm shift on beta-thalassaemia treatment: How will we manage this old disease with new therapies? Blood Rev 2018;32(4):300-311.
11. Hokland P, Daar S, Khair W, Sheth S, Taher AT, Torti L, Hantaweepant C, Rund D. Thalassaemia-A global view. Br J Haematol 2023;201(2):199-214.
12. Akhtar M, Arshad A, Junaid K, Tufail M, Afzal S. Complications of thalassemia and life expectancy among children and adults in South Asia a systematic review. J Soc of Prev Advocacy Res KEMU 2024;2(2):77-90.
13. Farmakis D, Giakoumis A, Angastiniotis M, Eleftheriou A. The changing epidemiology of the ageing thalassaemia populations: A position statement of the Thalassaemia International Federation. Eur J Haematol 2020;105(1):16-23.

14. Wood JC. Cardiac complications in thalassemia throughout the lifespan: Victories and challenges. Ann Ny Acad Sci 2023;1530(1):64-73.
15. Farmakis D, Triposkiadis F, Lekakis J, Parissis J. Heart failure in haemoglobinopathies: pathophysiology, clinical phenotypes, and management. Eur J Heart Fail 2017;19(4):479-489.
16. Coates TD. Higher hemoglobin is better in thalassemia. Blood 2024;143(10):842-844.
17. Musallam KM, Barella S, Origa R, Ferrero GB, Lisi R, Pasanisi A, Longo F, Gianesin B, Forni GL. Pretransfusion hemoglobin level and mortality in adults with transfusion-dependent beta-thalassemia. Blood 2024;143(10):930-932.
18. Modell B, Khan M, Darlison M. Survival in beta-thalassaemia major in the UK: data from the UK Thalassaemia Register. Lancet 2000;355(9220):2051-2052.
19. Musallam KM, Vitrano A, Meloni A, Pollina SA, Karimi M, El-Beshlawy A, Hajipour M, Di Marco V, Ansari SH, Filosa A, Ricchi P, Ceci A, Daar S, Vlachaki E, Singer ST, Naserullah ZA, Pepe A, Scondotto S, Dardanoni G, Bonifazi F, Sankaran VG, Vichinsky E, Taher AT, Maggio A, International Working Group on T. Risk of mortality from anemia and iron overload in nontransfusion-dependent beta-thalassemia. Am J Hematol 2022;97(2):E78-E80.
20. Musallam KM, Vitrano A, Meloni A, Pollina SA, Karimi M, El-Beshlawy A, Hajipour M, Di Marco V, Ansari SH, Filosa A, Ricchi P, Ceci A, Daar S, Vlachaki E, Singer ST, Naserullah ZA, Pepe A, Scondotto S, Dardanoni G, Bonifazi F, Sankaran VG, Vichinsky E, Taher AT, Maggio A. Survival and causes of death in 2,033 patients with non-transfusion-dependent beta-thalassemia. Haematologica 2021;106(9):2489-2492.
21. Musallam KM, Cappellini MD, Daar S, Taher AT. Morbidity-free survival and hemoglobin level in non-transfusion-dependent beta-thalassemia: a 10-year cohort study. Ann Hematol 2022;101(1):203-204.
22. Musallam KM, Cappellini MD, Taher AT. Variations in hemoglobin level and morbidity burden in non-transfusion-dependent beta-thalassemia. Ann Hematol 2021;100(7):1903-1905.
23. Fernandes JL, Silveira MA, Fertrin K, Lauar S, Fattori A, Coelho O, Junqueira FP, da Cunha GM, Coutinho AC, Jr., Pereira FB, Verissimo M, Saad ST. Left and right ventricular function and volume assessment in young thalassemia major patients with no related myocardial iron overload. Ann Hematol 2012;91(12):1839-1844.
24. Westwood MA, Anderson LJ, Maceira AM, Shah FT, Prescott E, Porter JB, Wonke B, Walker JM, Pennell DJ. Normalized left ventricular volumes and function in thalassemia major patients with normal myocardial iron. J Magn Reson Imaging 2007;25(6):1147-1151.
25. Veglio F, Melchio R, Rabbia F, Molino P, Genova GC, Martini G, Schiavone D, Piga A, Chiandussi L. Blood pressure and heart rate in young thalassemia major patients. Am J Hypertens 1998;11(5):539-547.
26. Stakos DA, Margaritis D, Tziakas DN, Kotsianidis I, Chalikias GK, Tsatalas K, Bourikas G, Boudoulas H. Cardiovascular involvement in patients with beta-thalassemia major without cardiac iron overload. Int J Cardiol 2009;134(2):207-211.
27. Cheung YF, Ha SY, Chan GC. Ventriculo-vascular interactions in patients with beta thalassaemia major. Heart 2005;91(6):769-773.
28. Chinprateep B, Ratanasit N, Kaolawanich Y, Karaketklang K, Saiviroonporn P, Viprakasit V, Krittayaphong R. Prevalence of left ventricular diastolic dysfunction by cardiac magnetic resonance imaging in thalassemia major patients with normal left ventricular systolic function. BMC Cardiovasc Disord 2019;19(1):245.
29. Gardenghi S, Grady RW, Rivella S. Anemia, ineffective erythropoiesis, and hepcidin: interacting factors in abnormal iron metabolism leading to iron overload in beta-thalassemia. Hematol Oncol Clin North Am 2010;24(6):1089-1107.
30. Sawicki KT, De Jesus A, Ardehali H. Iron Metabolism in Cardiovascular Disease: Physiology, Mechanisms, and Therapeutic Targets. Circ Res 2023;132(3):379-396.
31. Kremastinos DT, Farmakis D. Iron overload cardiomyopathy in clinical practice. Circulation 2011;124(20):2253-2263.

32. Oudit GY, Sun H, Trivieri MG, Koch SE, Dawood F, Ackerley C, Yazdanpanah M, Wilson GJ, Schwartz A, Liu PP, Backx PH. L-type Ca2+ channels provide a major pathway for iron entry into cardiomyocytes in iron-overload cardiomyopathy. Nat Med 2003;9(9):1187-1194.
33. Link G, Pinson A, Hershko C. Iron loading of cultured cardiac myocytes modifies sarcolemmal structure and increases lysosomal fragility. J Lab Clin Med 1993;121(1):127-134.
34. Kirk P, Roughton M, Porter JB, Walker JM, Tanner MA, Patel J, Wu D, Taylor J, Westwood MA, Anderson LJ, Pennell DJ. Cardiac T2* magnetic resonance for prediction of cardiac complications in thalassemia major. Circulation 2009;120(20):1961-1968.
35. Anderson LJ, Westwood MA, Holden S, Davis B, Prescott E, Wonke B, Porter JB, Walker JM, Pennell DJ. Myocardial iron clearance during reversal of siderotic cardiomyopathy with intravenous desferrioxamine: a prospective study using T2* cardiovascular magnetic resonance. Br J Haematol 2004;127(3):348-355.
36. Pennell DJ, Udelson JE, Arai AE, Bozkurt B, Cohen AR, Galanello R, Hoffman TM, Kiernan MS, Lerakis S, Piga A, Porter JB, Walker JM, Wood J. Cardiovascular function and treatment in beta-thalassemia major: a consensus statement from the American Heart Association. Circulation 2013;128(3):281-308.
37. Pennell DJ, Udelson JE, Arai AE, Bozkurt B, Cohen AR, Galanello R, Hoffman TM, Kiernan MS, Lerakis S, Piga A, Porter JB, Walker JM, Wood J, Fa AHACH. Cardiovascular Function and Treatment in -Thalassemia Major: A Consensus Statement From the American Heart Association. Circulation 2013;128(3):281-308.
38. McDonagh TA, Metra M, Adamo M, Gardner RS, Baumbach A, Bohm M, Burri H, Butler J, Celutkiene J, Chioncel O, Cleland JGF, Coats AJS, Crespo-Leiro MG, Farmakis D, Gilard M, Heymans S, Hoes AW, Jaarsma T, Jankowska EA, Lainscak M, Lam CSP, Lyon AR, McMurray JJV, Mebazaa A, Mindham R, Muneretto C, Francesco Piepoli M, Price S, Rosano GMC, Ruschitzka F, Kathrine Skibelund A, Group ESCSD. 2021 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure. Eur Heart J 2021;42(36):3599-3726.
39. Wilcox JE, Fang JC, Margulies KB, Mann DL. Heart Failure With Recovered Left Ventricular Ejection Fraction: JACC Scientific Expert Panel. J Am Coll Cardiol 2020;76(6):719-734.
40. Tang H, Zhang N, Deng J, Zhou K. Changing trends in the prevalence of heart failure impairment with Thalassemias over three decades. Eur J Clin Invest 2024;54(1):e14098.
41. Galanello R, Agus A, Campus S, Danjou F, Giardina P, Grady RW. Combined iron chelation therapy. Ann Ny Acad Sci 2010;1202(1):79-86.
42. Tanner MA, Galanello R, Dessi C, Smith GC, Westwood MA, Agus A, Pibiri M, Nair SV, Walker JM, Pennell DJ. Combined chelation therapy in thalassemia major for the treatment of severe myocardial siderosis with left ventricular dysfunction. J Cardiovasc Magn Reson 2008;10(1):12.
43. Tanner MA, Galanello R, Dessi C, Smith GC, Westwood MA, Agus A, Roughton M, Assomull R, Nair SV, Walker JM, Pennell DJ. A randomized, placebo-controlled, double-blind trial of the effect of combined therapy with deferoxamine and deferiprone on myocardial iron in thalassemia major using cardiovascular magnetic resonance. Circulation 2007;115(14):1876-1884.
44. Arandi N, Haghpanah S, Safaei S, Zahedi Z, Ashrafi A, Eatemadfar P, Zarei T, Radwan AH, Taher AT, Karimi M. Combination therapy - deferasirox and deferoxamine - in thalassemia major patients in emerging countries with limited resources. Transfus Med 2015;25(1):8-12.
45. Vogel M, Anderson LJ, Holden S, Deanfield JE, Pennell DJ, Walker JM. Tissue Doppler echocardiography in patients with thalassaemia detects early myocardial dysfunction related to myocardial iron overload. Eur Heart J 2003;24(1):113-119.
46. Westwood MA, Wonke B, Maceira AM, Prescott E, Walker JM, Porter JB, Pennell DJ. Left ventricular diastolic function compared with T2* cardiovascular magnetic resonance for early detection of myocardial iron overload in thalassemia major. J Magn Reson Imaging 2005;22(2):229-233.

47. McDonagh TA, Metra M, Adamo M, Gardner RS, Baumbach A, Bohm M, Burri H, Butler J, Celutkiene J, Chioncel O, Cleland JGF, Crespo-Leiro MG, Farmakis D, Gilard M, Heymans S, Hoes AW, Jaarsma T, Jankowska EA, Lainscak M, Lam CSP, Lyon AR, McMurray JJV, Mebazaa A, Mindham R, Muneretto C, Francesco Piepoli M, Price S, Rosano GMC, Ruschitzka F, Skibelund AK, Group ESCSD. 2023 Focused Update of the 2021 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure. Eur Heart J 2023;44(37):3627-3639.
48. Derchi G, Galanello R, Bina P, Cappellini MD, Piga A, Lai ME, Quarta A, Casu G, Perrotta S, Pinto V, Musallam KM, Forni GL, Webthal Pulmonary Arterial Hypertension G. Prevalence and risk factors for pulmonary arterial hypertension in a large group of beta-thalassemia patients using right heart catheterization: a Webthal study. Circulation 2014;129(3):338-345.
49. Mohammad AM, Dawad MM, Kashmoola MA, Al-Allawi N. Doppler-defined pulmonary hypertension in beta-thalassemia major in Kurdistan, Iraq. PLoS One 2020;15(12):e0243648.
50. Gupta V, Vijayakumar V, Aggarwal P, Kumar I, Agrawal V. Pulmonary Artery Hypertension in Transfusion-Dependent Thalassemia. Indian Pediatr 2024;61:49-52.
51. Anthi A, Orfanos SE, Armaganidis A. Pulmonary hypertension in beta thalassaemia. Lancet Respir Med 2013;1(6):488-496.
52. Aessopos A, Farmakis D, Karagiorga M, Voskaridou E, Loutradi A, Hatziliami A, Joussef J, Rombos J, Loukopoulos D. Cardiac involvement in thalassemia intermedia: a multicenter study. Blood 2001;97(11):3411-3416.
53. Pinto VM, Musallam KM, Derchi G, Graziadei G, Giuditta M, Origa R, Barella S, Casu G, Pasanisi A, Longo F, Casale M, Miceli R, Merella P, Tartaglione I, Piga A, Cappellini MD, Gianesin B, Forni GL, Webthal p. Mortality in beta-thalassemia patients with confirmed pulmonary arterial hypertension on right heart catheterization. Blood 2022;139(13):2080-2083.
54. Anthi A, Tsiapras D, Karyofyllis P, Voudris V, Armaganidis A, Orfanos SE. The wide spectrum of beta-thalassaemia intermedia-induced pulmonary hypertension: two case reports on the possible role of specific pulmonary arterial hypertension therapy. Pulm Circ 2021;11(3):20458940211030490.
55. Karyofyllis P, Demerouti E, Tsetika EG, Apostolopoulou S, Tsiapras P, Iakovou I, Tsiapras D. Haemolytic Anaemia-Related Pulmonary Hypertension. Life (Basel) 2024;14(7).
56. Taher AT, Cappellini MD. How I manage medical complications of beta-thalassemia in adults. Blood 2018;132(17):1781-1791.
57. Bell RM, Mohamed S, Ako EA, Chatterjee R, Bajoria R, Porter J, Walker JM. The prevalence and risk factors for atrial fibrillation in beta-thalassemia major: a cross-sectional study in a UK specialist cardio-haematology clinic. Eur Heart J 2015;36:916-916.
58. Abdelmottaleb W, Maraey A, Ozbay M, Elsharnoby H, Khalil M, Natale A, Al Rawahi M. Association Between beta-thalassemia and Atrial Fibrillation: Insights from the United States National Inpatient Sample. J Innov Card Rhythm Manag 2023;14(9):5566-5569.
59. Chen LY, Chung MK, Allen LA, Ezekowitz M, Furie KL, McCabe P, Noseworthy PA, Perez MV, Turakhia MP, American Heart Association Council on Clinical C, Council on C, Stroke N, Council on Quality of C, Outcomes R, Stroke C. Atrial Fibrillation Burden: Moving Beyond Atrial Fibrillation as a Binary Entity: A Scientific Statement From the American Heart Association. Circulation 2018;137(20):e623-e644.
60. Van Gelder IC, Rienstra M, Bunting KV, Casado-Arroyo R, Caso V, Crijns H, De Potter TJR, Dwight J, Guasti L, Hanke T, Jaarsma T, Lettino M, Lochen ML, Lumbers RT, Maesen B, Molgaard I, Rosano GMC, Sanders P, Schnabel RB, Suwalski P, Svennberg E, Tamargo J, Tica O, Traykov V, Tzeis S, Kotecha D, Group ESCSD. 2024 ESC Guidelines for the management of atrial fibrillation developed in collaboration with the European Association for Cardio-Thoracic Surgery (EACTS). Eur Heart J 2024;45(36):3314-3414.

61. Camm AJ, Kirchhof P, Lip GY, Schotten U, Savelieva I, Ernst S, Van Gelder IC, Al-Attar N, Hindricks G, Prendergast B, Heidbuchel H, Alfieri O, Angelini A, Atar D, Colonna P, De Caterina R, De Sutter J, Goette A, Gorenek B, Heldal M, Hohloser SH, Kolh P, Le Heuzey JY, Ponikowski P, Rutten FH, Guidelines ESCCfP. Guidelines for the management of atrial fibrillation: the Task Force for the Management of Atrial Fibrillation of the European Society of Cardiology (ESC). Europace 2010;12(10):1360-1420.
62. NICE, 196 G. Atrial fibrillation: diagnosis and management. 2021.
63. Taher A, Isma'eel H, Mehio G, Bignamini D, Kattamis A, Rachmilewitz EA, Cappellini MD. Prevalence of thromboembolic events among 8,860 patients with thalassaemia major and intermedia in the Mediterranean area and Iran. Thromb Haemost 2006;96(4):488-491.
64. Eldor A, Rachmilewitz EA. The hypercoagulable state in thalassemia. Blood 2002;99(1):36-43.
65. Taher AT, Cappellini MD, Bou-Fakhredin R, Coriu D, Musallam KM. Hypercoagulability and Vascular Disease. Hematol Oncol Clin North Am 2018;32(2):237-245.
66. Taher AT, Cappellini MD, Musallam KM. Development of a thalassemia-related thrombosis risk scoring system. Am J Hematol 2019;94(8):E207-E209.
67. Karimi M, Khanlari M, Rachmilewitz EA. Cerebrovascular accident in beta-thalassemia major (beta-TM) and beta-thalassemia intermedia (beta-TI). Am J Hematol 2008;83(1):77-79.
68. Vassilopoulou S, Anagnostou E, Paraskevas G, Spengos K. Etiology and treatment of ischaemic stroke in patients with beta-thalassemia major. Eur J Neurol 2011;18(12):1426-1428.
69. Aessopos A, Farmakis D, Karagiorga M, Rombos I, Loucopoulos D. Pseudoxanthoma elasticum lesions and cardiac complications as contributing factors for strokes in beta-thalassemia patients. Stroke 1997;28(12):2421-2424.
70. Aessopos A, Farmakis D, Hatziliami A, Fragodimitri C, Karabatsos F, Joussef J, Mitilineou E, Diamanti-Kandaraki E, Meletis J, Karagiorga M. Cardiac status in well-treated patients with thalassemia major. Eur J Haematol 2004;73(5):359-366.
71. Hurtado J, Sellak H, Joseph G, Lewis CV, Naudin CR, Garcia S, Wodicka JR, Archer DR, Taylor WR. Accelerated atherosclerosis in beta-thalassemia. Am J Physiol-Heart C 2023;325(5):H1133-H1143.
72. Sherief LM, Dawood O, Ali A, Sherbiny HS, Kamal NM, Elshanshory M, Alazez OA, Alhady MA, Nour M, Mokhtar WA. Premature atherosclerosis in children with beta-thalassemia major: New diagnostic marker. BMC Pediatr 2017;17(1):69.
73. Kumaravel KS, Sampathkumar D, Punitha P, Anurekha V, Hema M, Rameshbabu B. Premature Atherosclerosis in Children With Transfusion-Dependent Thalassemia: A Twin-Center Cross-Sectional Study. Indian Pediatr 2022;59(12):925-928.
74. De Sanctis V, Soliman AT, Daar S, Alansary N, Kattamis A, Skafida M, Galati MC, Christou S, Campisi S, Messina G, Yassin MA, Canatan D, Di Maio S, Al Jaouni S, Raiola G, Karimi M, Kaleva V, Kakkar S, Mariannis D, Kattamis C. A Concise Review on the Frequency, Major Risk Factors and Surveillance of Hepatocellular Carcinoma (HCC) in beta-Thalassemias: Past, Present and Future Perspectives and the ICET-A Experience. Mediterr J Hematol Infect Dis 2020;12(1):e2020006.
75. Nazir MS, Lyon AR, Southworth R. Iron replacement in anthracycline-related cardiac dysfunction: fuel on the fire? Eur Heart J 2024;45(34):3132-3134.
76. Lyon AR, Lopez-Fernandez T, Couch LS, Asteggiano R, Aznar MC, Bergler-Klein J, Boriani G, Cardinale D, Cordoba R, Cosyns B, Cutter DJ, de Azambuja E, de Boer RA, Dent SF, Farmakis D, Gevaert SA, Gorog DA, Herrmann J, Lenihan D, Moslehi J, Moura B, Salinger SS, Stephens R, Suter TM, Szmit S, Tamargo J, Thavendiranathan P, Tocchetti CG, van der Meer P, van der Pal HJH, Group ESCSD. 2022 ESC Guidelines on cardio-oncology developed in collaboration with the European Hematology Association (EHA), the European Society for Therapeutic Radiology and Oncology (ESTRO) and the International Cardio-Oncology Society (IC-OS). Eur Heart J 2022;43(41):4229-4361.

77. Steinberg JS, Varma N, Cygankiewicz I, Aziz P, Balsam P, Baranchuk A, Cantillon DJ, Dilaveris P, Dubner SJ, El-Sherif N, Krol J, Kurpesa M, La Rovere MT, Lobodzinski SS, Locati ET, Mittal S, Olshansky B, Piotrowicz E, Saxon L, Stone PH, Tereshchenko L, Turitto G, Wimmer NJ, Verrier RL, Zareba W, Piotrowicz R. 2017 ISHNE-HRS expert consensus statement on ambulatory ECG and external cardiac monitoring/telemetry. Heart Rhythm 2017;14(7):e55-e96.
78. Mitchell C, Rahko PS, Blauwet LA, Canaday B, Finstuen JA, Foster MC, Horton K, Ogunyankin KO, Palma RA, Velazquez EJ. Guidelines for Performing a Comprehensive Transthoracic Echocardiographic Examination in Adults: Recommendations from the American Society of Echocardiography. J Am Soc Echocardiogr 2019;32(1):1-64.
79. Welhenge C, Ranasinghe R, Rajapakse S, Premawardhena A. Extensive systemic thrombo-embolism including intra-cardiac thrombosis mimicking an atrial myxoma in a patient with beta thalassaemia major - a case report. BMC Cardiovasc Disord 2023;23(1):532.
80. Patsourakos D, Aggeli C, Dimitroglou Y, Delicou S, Xydaki K, Koukos M, Tsartsalis D, Gialeli F, Gatzoulis KA, Tousoulis D, Tsioufis K. Speckle tracking echocardiography and β-thalassemia major. A systematic review. Ann Hematol 2023.
81. Fianza PI, Pramono AA, Ghozali M, Sihite TA, Setiabudi D, Syamsunarno M, Fucharoen S, Panigoro R. Diagnostic Modalities in Detecting Cardiovascular Complications of Thalassemia. Rev Cardiovasc Med 2022;23(8):267.
82. Derchi G, Musallam KM, Pinto VM, Graziadei G, Giuditta M, Barella S, Origa R, Casu G, Pasanisi A, Longo F, Casale M, Miceli R, Merella P, Gianesin B, Ameri P, Tartaglione I, Perrotta S, Piga A, Cappellini MD, Forni GL. Tricuspid-valve regurgitant jet velocity as a risk factor for death in beta-thalassemia. Haematologica 2022;107(7):1714-1718.
83. Anderson LJ, Holden S, Davis B, Prescott E, Charrier CC, Bunce NH, Firmin DN, Wonke B, Porter J, Walker JM, Pennell DJ. Cardiovascular T2-star (T2*) magnetic resonance for the early diagnosis of myocardial iron overload. Eur Heart J 2001;22(23):2171-2179.
84. Quinn CT, St Pierre TG. MRI Measurements of Iron Load in Transfusion-Dependent Patients: Implementation, Challenges, and Pitfalls. Pediatr Blood Cancer 2016;63(5):773-780.
85. Carpenter JP, He T, Kirk P, Roughton M, Anderson LJ, de Noronha SV, Sheppard MN, Porter JB, Walker JM, Wood JC, Galanello R, Forni G, Catani G, Matta G, Fucharoen S, Fleming A, House MJ, Black G, Firmin DN, St Pierre TG, Pennell DJ. On T2* magnetic resonance and cardiac iron. Circulation 2011;123(14):1519-1528.
86. Alam MH, Auger D, McGill LA, Smith GC, He T, Izgi C, Baksi AJ, Wage R, Drivas P, Firmin DN, Pennell DJ. Comparison of 3 T and 1.5 T for T2* magnetic resonance of tissue iron. J Cardiovasc Magn Reson 2016;18(1):40.
87. Meloni A, Pistoia L, Gamberini MR, Cuccia L, Lisi R, Cecinati V, Ricchi P, Gerardi C, Restaino G, Righi R, Positano V, Cademartiri F. Multi-Parametric Cardiac Magnetic Resonance for Prediction of Heart Failure Death in Thalassemia Major. Diagnostics (Basel) 2023;13(5).
88. Medina KM, Abdel-Gadir A, Ganga K, Ojha V, Pratap S, Boubertakh R, McGrath L, Augusto JB, Rikowski A, Mughal N, Khanna VK, Seth T, Sharma S, Mahajan A, Bansal RK, Srivastava P, Mahajan H, Mahajan V, Walker J, Seldon MT, Ako E, Moon JC, Walker JM. Use of rapid cardiac magnetic resonance imaging to guide chelation therapy in patients with transfusion-dependent thalassaemia in India: UMIMI study. Eur Heart J-Qual Car 2022;8(3):289-297.
89. Menacho KD, Ramirez S, Perez A, Dragonetti L, Perez de Arenaza D, Katekaru D, Illatopa V, Munive S, Rodriguez B, Shimabukuro A, Cupe K, Bansal R, Bhargava V, Rodriguez I, Seraphim A, Knott K, Abdel-Gadir A, Guerrero S, Lazo M, Uscamaita D, Rivero M, Amaya N, Sharma S, Peix A, Treibel T, Manisty C, Mohiddin S, Litt H, Han Y, Fernandes J, Jacob R, Westwood M, Ntusi N, Herrey A, Walker JM, Moon J. Improving cardiovascular magnetic resonance access in low- and middle-income countries for cardiomyopathy assessment: rapid cardiovascular magnetic resonance. Eur Heart J 2022;43(26):2496-2507.

90. Viprakasit V, Ajlan A, Aydinok Y, Al Ebadi BAA, Dewedar H, Ibrahim AS, Ragab L, Trad O, Wataify AS, Wong LLL, Taher AT. MRI for the diagnosis of cardiac and liver iron overload in patients with transfusion-dependent thalassemia: An algorithm to guide clinical use when availability is limited. Am J Hematol 2018;93(6):E135-E137.
91. Shah FT, Porter JB, Sadasivam N, Kaya B, Moon JC, Velangi M, Ako E, Pancham S. Guidelines for the monitoring and management of iron overload in patients with haemoglobinopathies and rare anaemias. Br J Haematol 2022;196(2):336-350.
92. Forni GL, Gianesin B, Musallam KM, Longo F, Rosso R, Lisi R, Gamberini MR, Pinto VM, Graziadei G, Vitucci A, Bonetti F, Musto P, Piga A, Cappellini MD, Borgna-Pignatti C, Webthal p. Overall and complication-free survival in a large cohort of patients with beta-thalassemia major followed over 50 years. Am J Hematol 2023;98(3):381-387.
93. Fernandes JL, Loggetto SR, Verissimo MP, Fertrin KY, Baldanzi GR, Fioravante LA, Tan DM, Higa T, Mashima DA, Piga A, Coelho OR, Costa FF, Saad ST. A randomized trial of amlodipine in addition to standard chelation therapy in patients with thalassemia major. Blood 2016;128(12):1555-1561.
94. Aliasgharian A, Karami H, Zahedi M, Jahanshahi R, Bakhtiari-Dovvombaygi H, Nasirzadeh A, Naderisorki M, Kosaryan M, Salehifar E, Ghazaiean M, Bitaraf S, Darvishi-Khezri H. Amlodipine Therapy in β-Thalassemia Patients: A Systematic Review and Meta-Analysis on Ferritin Levels and Liver MRI T2. Thalass Rep 2023;13(4):241-252.

05 LIVER DISEASE

Authors: **Alessandra Mangia[1], Nicola Pugliese[2,3], Carlotta La Floresta[1], Alessio Aghemo[2,3]**

[1.] Liver Unit, Department of Medical Sciences, Fondazione "Casa Sollievo della Sofferenza" IRCCS, San Giovanni Rotondo, Italy

[2.] Department of Biomedical Sciences, Humanitas University, Pieve Emanuele, Milan, Italy

[3.] Division of Internal Medicine and Hepatology, Department of Gastroenterology, IRCCS Humanitas Research Hospital, Rozzano, Milan, Italy

Competing interest: AM has served as speaker for Bristol Myers Squibb, Gilead Sciences, Janssen, and MSD. NP has served as speaker for Gilead Sciences, AlfaSigma, Advanz Pharma, Novo Nordisk, and Gore. AA reports grant and research support from AbbVie and Gilead Sciences; being on advisory boards for AbbVie, Gilead Sciences, MSD, Mylan, Intercept, Sobi, and Takeda. The remaining authors have no competing interests to disclose. All competing interests are outside the present work.

Acknowledgements: The authors would like to thank Prof. Geoffrey M Dusheiko (University College London and Kings College Hospital, London, United Kingdom) for his review and input on this Chapter.

1. INTRODUCTION

Liver diseases are among the top causes of mortality with the latest data coming from the Global Burden of Diseases Study identifying cirrhosis as the 18th leading cause of death worldwide [1]. Patients with transfusion-dependent β-thalassaemia (TDT) often have concomitant liver diseases as a consequence of post-transfusion viral hepatitis B (HBV) and C (HCV) or iron overload. Recent epidemiological data identify liver-related mortality as the 2nd (Global) cause of death in non-transfusion-dependent β-thalassaemia (NTDT) patients [2] and the 2nd (Greece) [3] or 4th (Italy) cause of death among patients with TDT [4]. Mortality from liver disease is the consequence of the development of cirrhosis and its complications, namely hepatocellular carcinoma (HCC), end-stage liver disease, and oesophageal/gastric variceal bleeding. In patients with chronic liver disease, cirrhosis takes decades to develop, but its appearance can be accelerated by environmental factors such as obesity and alcohol consumption [5]. Aetiological treatment in patients with chronic hepatitis without cirrhosis eliminates the risk of progression to cirrhosis thus affecting liver-related mortality [6, 7]. In patients with cirrhosis, effective treatment of the underlying cause is associated with cirrhosis/fibrosis regression, while six monthly ultrasound surveillance aimed at early identification of HCC as well as prophylactic treatment of portal hypertension in patients with clinically significant portal hypertension (CSPH) to prevent variceal bleeding is demonstrated to increase survival [6-8].

The management of liver disease in patients with TDT should thus focus on identifying the cause of liver damage and ideally mitigating the damage, staging the severity of liver disease to identify who should enter lifelong prevention for the complications of cirrhosis and counselling patients on co-factors associated with liver damage (obesity, diabetes, alcohol).

2. DIAGNOSIS AND QUANTIFICATION OF LIVER DAMAGE IN PATIENTS WITH THALASSAEMIA

The approach to liver disease includes clinical parameters, biochemical/serological/molecular tests and imaging techniques in order to identify the aetiological factor(s) and assess the severity of the injury.

- **Clinical examination:** may reveal signs of systemic iron excess such as skin pigmentation and hepatomegaly while in later stages stigmata of decompensated chronic liver disease (i.e., palmar erythema, spider naevi), ascites, and encephalopathy may follow.
- **Laboratory tests:** may show moderate elevations (2-3 times higher than the upper limit of normal) of the aminotransferases, aspartate transaminase (AST) and alanine transaminase (ALT) and a mild increase of alkaline phosphatase and gamma glutamyl transferase (γGT). In the presence of severe hepatic impairment, prolonged prothrombin time, low albumin, and high serum bilirubin are found.
- **Imaging techniques:** ultrasound remains the most accessible method to evaluate hepatic morphology and to recognise signs of fibrosis and cirrhosis.
- **Other non-invasive tests:** non-invasive tests have been developed to identify patients with cirrhosis or compensated advanced chronic liver disease (cACLD). Non-invasive tests include serum tests such as the fibrosis-4 score (FIB-4) and vibration controlled hepatic transient elastography (TE), a pulse-echo ultrasound technique for liver stiffness measurement (LSM), a surrogate that can reduce the need for liver biopsy as a method to stage liver fibrosis. FIB-4 is a simple model based on biochemical parameters of ALT, AST, platelet count, and the patient's age. Its accuracy in patients with β-thalassaemia can be affected by splenectomy and has been generally shown to be suboptimal when compared to TE. LSM, measured by TE, correlates well with stages of fibrosis in patients with β-thalassaemia [9-11]. TE >25 kPa also identifies patients with CSPH that should receive prophylactic pharmacological treatment. TE is an easy-to-perform tool which has now supplanted liver biopsy for routine examination for the evaluation of liver fibrosis and the identification of CSPH. In patients with thalassaemia, it has moderate to high accuracy, especially in thalassaemic patients with chronic HCV infection [12].

3. IRON-MEDIATED LIVER DISEASE

Iron overload and chelation therapy are extensively covered in Chapter 3. Elevated serum ferritin and liver iron concentration (LIC) have been consistently linked to an increased risk of liver disease in patients with β-thalassaemia [13-16]. Effective iron monitoring and chelation therapy have also been associated with reversal or stabilisation of liver fibrosis and reduced liver disease-related mortality in β-thalassaemia [2, 17]. Chelator-associated liver toxicity may also require close monitoring and dose modifications.

4. THALASSAEMIA AND HEPATITIS B VIRUS

HBV is a widespread global health concern, with an estimated 257 million people (3.2%) affected by chronic HBV infection by 2022 [18-20]. Approximately, 1.5 million new infections occur each year, and HBV-related complications such as cirrhosis and HCC were responsible for 820,000 deaths

in 2019 [18, 19]. In the European Union and European Economic Area (EU/EEA) alone, approximately 3.6 million individuals are living with chronic hepatitis B, reflecting the ongoing burden of this disease in both developed and developing regions [21-23].

TDT patients, in particular, are highly susceptible to HBV infection due to their dependence on regular blood transfusions. Prior to the introduction of comprehensive blood screening protocols, infected transfusions were a major route of HBV transmission, contributing significantly to the infection rates in this population. Globally, the prevalence of HBV infection in β-thalassaemia patients varies, with hepatitis B surface antigen (HBsAg) positivity rates ranging from 0.3% to 5.7%. The highest rates are often observed in Asian and Southeast Asian countries, where both HBV and thalassaemia are endemic [24-32]. However, the introduction of mandatory blood donor screening, including nucleic acid testing (NAT) for HBV DNA, has significantly reduced the risk of transfusion-related HBV transmission. In countries with advanced healthcare systems, such as the United States, Canada, and parts of Europe, the risk of transfusion-transmitted HBV is now estimated to be very low [33-35].

Vaccination against HBV has also played a crucial role in preventing new infections, particularly in high-risk populations such as TDT patients. Universal vaccination programmes, which confer long-term immunity against HBV, have been shown to dramatically reduce the prevalence and incidence of HBV-related liver disease, such as cirrhosis and HCC, in countries that have adopted these measures. [36].

Recent epidemiological data reflect the success of preventive measures in reducing HBV transmission in β-thalassaemia patients, particularly in countries with robust vaccination and blood safety programmes. For example, the Italian Thalassaemia Registry reports that 5% of thalassaemia patients with HCC are currently HBsAg-positive, with 58% showing evidence of past HBV infection based on hepatitis B core antibody (HBcAb) positivity [37]. These figures represent a significant reduction from historical levels, largely due to widespread vaccination and improved blood donor screening. However, in regions with less stringent health protocols, the burden of chronic HBV infection remains substantial, and thalassaemia patients in these areas continue to be at increased risk of liver-related complications.

Chronic HBV infection is a major cause of liver disease in β-thalassaemia patients, as long-term persistence of the virus can lead to progressive liver damage, with studies showing that the 5-year cumulative incidence of cirrhosis in patients with chronic HBV ranges from 8% to 20%. In patients who develop cirrhosis, the annual risk of developing HCC is between 2% and 5%, and the 5-year cumulative incidence of liver failure is approximately 20% [38].

Treatment of chronic HBV infection in TDT patients includes long-term antiviral therapy with nucleos(t)ide analogues aimed at suppressing viral replication and preventing disease progression and HCC. Current European Association for the Study of the Liver (EASL) guidelines recommend treatment initiation based on HBV DNA levels, liver enzyme (ALT) levels, the presence of liver fibrosis, and family history of HCC [39]. First-line treatments, such as tenofovir and entecavir, are highly effective in suppressing HBV replication and reducing the risk of cirrhosis and HCC [39]. Lifelong antiviral therapy is generally required. HCC in β-thalassaemia patients with HBV often develops in the presence of co-infection with HCV or severe concomitant iron overload. The exact mechanisms underlying HBV-associated HCC in β-thalassaemia patients are not fully understood, but chronic viral infection and iron-induced oxidative stress are thought

to contribute to liver carcinogenesis [37, 40, 41]. Biannual screening for HCC using ultrasound is recommended for all at-risk TDT patients to ensure early detection and treatment.

5. THALASSAEMIA AND HEPATITIS C VIRUS

HCV infection has represented for decades the most important cause of liver disease in patients with TDT. The estimated rate of HCV seroprevalence in TDT in the early 1990s, at the time of HCV discovery, was reported to range from 4.4% in Turkey to 85.4% in Italy [42].

TDT patients have a well-defined source and timing of HCV infection. In fact, before discovery of HCV and HCV screening implementation after 1990, all β-thalassaemia patients who received transfusions were potentially infected. However, the impact of HCV on patients with TDT has fortunately been significantly reduced, particularly in high-income countries, where donor screening has eliminated the risk of post-transfusion infection. Unfortunately, in resource-limited countries, up to 25% of TDT patients continue to receive unscreened transfusions [43].

Currently, all TDT patients transfused before 1990 should undergo HCV antibody (HCV-Ab) screening by enzyme linked immunoassay, and in case of a positive result, viremia should be ruled out by either reflex HCV RNA tested by quantitative polymerase chain reaction (PCR) with a low detection limit ≤15 IU/mL, or by a HCV core antigen (HCcAg) test [44]. Genotyping identification is not obligatory, given the availability of pan-genotypic treatment regimens.

A chronic test indicating chronic HCV should be followed assessment of the severity of liver disease. It should be highlighted that patients with TDT are at risk of accelerated fibrosis due to concomitant iron overload [14], therefore performing an evaluation of the stage of fibrosis at baseline and monitoring is recommended in TDT patients. Non-invasive assessment of liver fibrosis by LSM that measures liver stiffness in kPa is currently adopted.

12.5 kPa is considered the threshold to rule in fibrosis, as results higher than this threshold are commonly associated with advanced liver disease. When LSM is not available, serum scores such as the AST to platelet ratio index (APRI) or FIB-4 can be used but only in patients who have not undergone splenectomy. Platelet counts are constituents of these scores, thus in patients with splenectomy, the high reactive thrombocytosis count will not reliably rule in portal hypertension.

From 1999 to 2014 the use of (pegylated) interferon (Peg-IFN) in combination with ribavirin was the mainstay of treatment. However, the unsatisfactory sustained virological response (SVR) rates, and undesirable safety profile, of the combination (ribavirin causes red blood cell consumption) was unacceptable [45, 46]. As in non-thalassaemic patients, higher response rates were observed in patients with HCV genotype 2 and 3, in the absence of cirrhosis or advanced fibrosis, in subjects with a favourable genetic profile, and in patients at their first course of treatment. In addition to these reported factors, for patients with TDT, an additional favourable predictor of response was low liver iron content [47].

The impact of HCV on the burden of liver disease has been markedly reduced due to the advent of new, safe, and highly effective oral direct antiviral drugs (DAA) [48, 49]. DAA have been introduced since 2014. These antiviral drugs selectively target different HCV non-structural (NS) proteins such as NS3/protease, the NS5A replication associated protein, and NS5B, the viral RNA polymerase. The most recent and significant 'game changers' have been the pan-genotypic regimens [44]. Several

regimens based on the nucleoside polymerase inhibitor sofosbuvir plus NS3 or NS5 inhibitors, or a non-nucleoside polymerase inhibitor plus NS3 or NS5 inhibitors, or on a combination of NS3 and NS5 inhibitors have been largely used (Table 1) [50]. These regimens are associated with SVR of 95% or higher regardless of genotype and have an excellent safety profile with negligible side effects as compared to those observed with interferon-alpha containing regimens.

Table 1. *Direct antiviral drugs regimens for hepatitis C virus treatment.*

Regimen	Sofosbuvir / Velpatasvir	Grazoprevir / Elbasvir	Glecaprevir / Pibrentasvir
Pan-genotypic	Yes	No	Yes
HCV targeted region	NS5A/NS5B	NS5A/NS3	NS5A/NS3
Posology	One tablet once daily	One tablet once daily	Three tablets once daily with food
Treatment duration	12 weeks	12-16 weeks	8-12 weeks
Cirrhosis treatment allowed for	Child-Pugh A, B, C	Child-Pugh A only	Child-Pugh A only

Abbreviations: HCV, hepatitis C virus; NS, non-structural.

Two different pan-genotypic regimens are currently available: the single pill fixed combination of sofosbuvir and velpatasvir and the combination of the NS3 inhibitor glecaprevir with the NS5A protease inhibitor pibrentasvir, to be administered as three pills together once daily with food [44]. Rates of SVR attained with either regimen in real life were higher than 95% without significant differences across genotypes [44]. The combination of sofosbuvir and velpatasvir is administered at a fixed duration for 12 weeks regardless of the fibrosis status; the combination of glecaprevir and pibrentasvir is administered for 8 weeks in patients with or without cirrhosis if previously untreated, for 12 weeks in cirrhotic patients who failed a previous course of treatment and for 16 weeks in cirrhotic patients with genotype 3 infection who failed a previous treatment course [44, 51]. Treatment protocols for TDT patients do not differ from those for the general population [52, 53].

It is critical to evaluate potential drug-to-drug interactions before the start of treatment. The Liverpool University site is the predominantly used web-based tool to exclude potential drug-to-drug interactions [44]. Given the polypharmacy burden experienced by patients with TDT, a regimen not including protease inhibitors, thus associated with a lower risk of a drug-to-drug interaction, might be preferred. Real-life experiences with DAA in β-thalassaemia patients have been reported worldwide demonstrating excellent efficacy and safety [50-55].

6. CIRRHOSIS AND RELATED COMPLICATIONS

Cirrhosis is a common consequence of the long clinical course of all chronic liver diseases. It is characterised by tissue fibrosis and disturbance of the liver architecture into structurally abnormal nodules [56]. The prevalence of cirrhosis in β-thalassaemia patients ranges from 10 to 20%, as reported in series from the US, Italy, Greece, and Iran [57-60].

The histological definition commonly uses the METAVIR histological scoring system (stage F4). However, a more refined classification of cirrhosis suggests the existence of several sub-stages of cirrhosis [61]. Five clinical stages based on oesophageal varices, bleeding, and ascites fit the clinical course of cirrhosis and may be predicted by different factors. This refined classification supports the evidence that fibrosis and even cirrhosis are to an extent reversible, a concept that has become apparent after the development of specific therapies for liver disease, including DAA for HCV treatment. Regression has been observed in patients with viral hepatitis, as well as in autoimmune hepatitis, alcohol-induced liver injury and copper overload [62]. Muretto and colleagues showed in 2002 that after stem cell transplantation and cured β-thalassaemia, cirrhosis is reversible after iron removal treatment [63]. Treatment with the oral iron chelator deferasirox for three or more years also reversed or stabilised liver fibrosis in 83% of patients with hepatic iron-overloaded β-thalassaemia. This therapeutic effect was independent of reduced concentration of liver iron or previous exposure to hepatitis C virus [17].

The natural history of cirrhosis is characterised by a silent course until the development of portal hypertension and decreasing liver function result in clinical signs of decompensation including ascites, portal hypertensive gastrointestinal bleeding, encephalopathy, and jaundice. In the silent phase, cirrhosis is defined as compensated and diagnosed histologically or non-invasively. Following the first appearance of clinical signs, it is defined as decompensated and diagnosed clinically. Cumulative survival in compensated cirrhosis is 78% at 20 years [64]. In contrast, the median survival of decompensated patients is 3-4 years [64]. Decompensation of cirrhosis requires that the hepatic venous pressure gradient (HVPG) increases above the critical threshold of 10 mmHg, with the normal value ranging from 1 to 5 mmHg [65]. Progression of cirrhosis may be accelerated by the development of any other complications such as renal impairment, hepato-pulmonary syndrome, and sepsis. A multicentre study in patients with β-thalassaemia has shown that at the time of diagnosis of HCC, the majority of patients had cirrhosis and a median age of 45 years [37]. In a prospective study, the prevalence of HCC among cirrhotic patients was 78% increasing to 86% among those who died within 6 months [66]. HCC develops along the whole course of cirrhosis and survival depends on the severity of underlying cirrhosis, the degree of portal hypertension, and the extent of the tumour and vascular invasion. The five-year incidence of HCC in patients with cirrhosis is about 15-17% [67].

7. HEPATOCELLULAR CARCINOMA

7.1. Incidence, aetiology, and pathophysiology

HCC has been reported as the second most common cause of death in recent registries of TDT patients from Europe [3]. Since the early 2000s, the reduction in cardiac iron overload-related mortality together with the increased survival, has resulted in hepatic disease-related deaths, especially from HCC, becoming relatively more prevalent and surpassing deaths from cardiac disease in recent years [68].

HCC is the most prevalent and numerically increasing malignancy in both TDT and NTDT patients [69]. The survey of Borgna-Pignatti and colleagues which collected data from most Italian thalassaemia treatment centres in 2004 and in 2014, described 22 and later 60 new cases of HCC among 5,855 thalassaemia patients followed for 10 years, and demonstrated in the latter publication an incidence of 0.75% and 1.74%, respectively in TDT and NTDT [37, 70].

More recently, data on 4631 Italian patients with haemoglobinopathies followed from 1970 to 2021 (55.6%, TDT), showed that of 188 patients who developed cancer, almost half (41.5%) had HCC, with a crude incidence of 49.5/100,000 people [69]. The age-adjusted incidence rate of HCC was almost 9 times higher in patients with haemoglobinopathy than in the general population [69]. The age at diagnosis also appears significantly younger [71]. Chronic infection with HCV or HBV, metabolic dysfunction-associated steatohepatitis, chronic alcohol abuse, and any liver damage responsible of necro-inflammation results in HCC. In addition to HCV, which is the main aetiological factor associated with HCC in TDT patients, iron overload is also a key risk factor. Indeed, inadequate chelation seems to increase HCC risk in TDT, while liver iron overload plays a pivotal role in favouring fibrosis progression and increasing cancer risk in NTDT, even in the absence of HCV [72]. It has been shown that the combination of HCV infection and iron overload in thalassaemia synergistically accelerates fibrosis progression more than each of them alone [14]. Through the activation of stellate cells, iron overload is responsible for oxidative stress, inflammation, and cell damage [72, 73]. Finally, iron can induce immunological dysregulation, reducing cancer immuno-surveillance [74].

The increasing prevalence of liver steatosis, obesity, diabetes, and metabolic syndrome is an important antecedent of chronic liver disease. The rate of HCC related to metabolic dysfunction-associated steatohepatitis (MASH) in the general population is 2-13% [75, 76]. Moreover, TDT patients may have impaired pancreatic function due to pancreatic siderosis. Diabetes mellitus has been reported in 6.5% and impaired fasting sugar in 16.2% of cases [77]. In non-thalassaemic subjects, those with a history of type 2 diabetes mellitus at baseline show a hazard ratio of 2.14 (95% confidence interval, 1.69-2.71) of developing HCC compared to those without [40]. Corresponding data in TDT patients are lacking. Given the direct relationship between diabetes and cancer risk, irrespective of chronic viral hepatitis and haemochromatosis, an increased risk for MASH-associated HCC in TDT could be expected.

Only a minority of patients with β-thalassaemia and HCC are HBsAg positive, probably because of improved risk control due to HBV vaccination. In the Italian series of thalassaemia patients with HCC published in 2014, 5% were HBsAg positive and 58% had evidence of past HBV infection suggesting rates of annual occurrence comparable to the 2.3% registered in the general population [70].

7.2. Screening for hepatocellular carcinoma

A key point in HCC management and treatment is early diagnosis. Early diagnosis can be achieved through surveillance. Surveillance identifies small nodules eligible for curative treatments, and thus longer survival [78, 79]. According to the EASL clinical guidelines update presented at International Liver Congress in Milan (5-8 June 2024), an ultrasound examination of the liver every 6 months plus alpha fetoprotein (AFP) evaluation are required [80]. In a previous meta-analysis, ultrasonography combined with AFP provided 63% sensitivity, higher than the 45% sensitivity with ultrasonography alone, and therefore AFP should be

recommended in conjunction with ultrasound surveillance in TDT patients [81, 82]. Considering cost-effectiveness, safety, and waiting time multi-phasic computed tomography and multi-phasic magnetic resonance imaging (MRI) are reserved for further diagnostic evaluation [83]. EASL guidelines suggest HCC surveillance in patients with cirrhosis with a Child-Pugh class A or B, not in Child-Pugh class where HCC treatment is not suitable [78].

Surveillance is required in patients with TDT and chronic viral hepatitis. Although HCV remains the viral infection with the highest risk, the carcinogenic role of HBV is direct and related to viral DNA integration [84]. In HBV positive patients with cirrhosis and in patients without cirrhosis but with first-degree family member with a history of HCC, in Asian or Black men, in Asian women, and in those with hepatitis D virus (HDV) co-infection the World Health Organization (WHO) recommends HCC surveillance [85]. On the contrary, the mechanisms by which HCV results in carcinogenesis are debated. Currently, it is generally accepted that HCV is not directly carcinogenic, but inducing inflammation and a pro-fibrotic response which leads to cirrhosis and consequently to HCC.

Successful treatment-induced HCV-RNA clearance is associated with a substantial decrease in the rate of HCC development. Subjects with advanced fibrosis, who achieve SVR after DAA therapy, are still at a long-term risk for progression to liver cirrhosis and HCC and should receive HCC surveillance. HCC cab occur in patients who become HCV-RNA negative after treatment with different regimens (Peg-IFN alone or Peg-IFN plus ribavirin or DAAs) [43, 86]. Likewise, HCC may occur in patients on treatment with nucleoside analogues. After SVR, HCC surveillance is equally required according to EASL guidelines both in patients with METAVIR score F3 and F4 cirrhotic patients [80]. Considering the residual risk linked to iron overload, this needs absolute applicability to TDT patients.

HCC risk stratification and thus surveillance protocols for patients with chronic HCV can be assessed though risk prediction models such as GALAD (Gender, Age, AFP-L3, AFP and Des-carboxy-prothrombin) [87]. For chronic HBV infection, risk stratification and surveillance can also be based on predictive models such as PAGE-B (Platelet-Age-Gender-Hepatitis B) or REACH-B (Risk Estimation for Hepatocellular Carcinoma in Chronic Hepatitis B) [88, 89]. Those models can be applied to non-splenectomised patients with TDT.

Surveillance in TDT should be prioritised, but not restricted to cirrhotic patients [90]. Thalassaemia experts suggest that surveillance in TDT with iron overload in the absence of cirrhosis should be based on age, with a threshold of 45 years [69]. This suggestion stems from the consideration that independent of the severity of liver damage, not only the degree of accumulation but also the length of exposure increases iron-related HCC risk.

Assessment of severity of iron overload is also part of the HCC screening. See Chapter 3 for recommendations on iron overload assessment by serum ferritin and liver MRI.

7.3. Hepatocellular carcinoma treatment

Treatment of HCC in thalassaemia patients should not differ from the general population. In the Italian survey by Borgna-Pignatti and colleagues, 22 patients were treated with transarterial chemoembolisation (TACE) alone, 13 with thermoablation, 16 underwent surgical resection preceded or followed by TACE or thermoablation, 3 patients underwent liver transplant, 18 received palliative therapy and 3 received sorafenib. Two of the transplanted patients died of causes independent of thalassaemia [37, 70]. In the general population, the 5-year recurrence rate after a curative resection is to be 50-70% [91].

In addition to the potential curative surgical or ablative treatments, and to loco-regional treatments, systemic therapy has improved therapeutic options for patients with advanced HCC (stage C according to the Barcelona Clinic Liver Cancer [BCLC] staging), including TDT patients. Patients in BCLC stage C are unsuitable for or unresponsive to TACE or transarterial radioembolisation (TARE) [92]. Since the introduction of sorafenib [93], the small tyrosine kinase inhibitor (TKI) able to block tumoral proliferation, a number of active compounds were made available for systemic treatment as monotherapy or in combination.

In addition to TKIs [93, 94], a different class of drugs including immune checkpoint inhibitors has become available since 2020 [95]. Atezolizumab is a monoclonal antibody targeting cell death programming receptor PD1; it is combined with bevacizumab, another monoclonal antibody able to block vessel overgrowth associated with neoplasia (vascular endothelial growth factor [VEGF]-targeted) [96, 97]. Another immune checkpoint inhibitor combination based on durvalumab, an anti-PD1, plus tremelimumab, an antiCTLA4, is now also available [98, 99] (Table 2). Second-line options for systemic treatment of HCC have been reviewed extensively [100].

Table 2. *First-line systemic treatment for hepatocellular carcinoma.*

Study	Treatment	Adverse events	Serious adverse events (Grade ≥3)
Llovet et al 2008 [93]	Sorafenib	Diarrhoea, weight loss, hand-foot skin reaction, alopecia, anorexia, voice changes, hypophosphatemia, thrombocytopenia	52%
Kudo et al 2018 [94]	Lenvatinib	Hypertension, diarrhoea, decreased appetite, weight loss	75%
Finn et al 2020 [96]	Atezolizumab + Bevacizumab	Hypertension, immune-mediated events	56.6%
Yau et al 2022 [95]	Nivolumab	Skin, liver enzyme increase, hypertension	12%
Abou-Alfa 2022 [99]	Durvalumab + Tremelimumab	Immuno-mediated events	50.5%

It is essential that since initial diagnosis, TDT patients with HCC are managed by a multidisciplinary group. A multidisciplinary approach involving hepatologists, hepatobiliary surgeons, interventional radiologists, and oncologists is required for these patients. When planning surgery, the burden of relevant co-morbidities including hypogonadism, myocardiopathy, hypothyroidism, osteoporosis, diabetes, and chronic renal failure that characterise TDT should be taken into account [72].

7.4. Liver transplant for hepatocellular carcinoma in β-thalassaemia patients

Liver transplantation has long been denied to TDT patients mainly due to cardiac co-morbidities [101]. Based on improvements reported in case series, a diagnosis of β-thalassaemia per se is no longer considered a contraindication to liver transplant. In an Italian study of thalassaemia with HCC, survival of the only transplanted patient was 69 months compared with 25.25 ± 23.65 months (range, 3-64 months) of the 8 un-transplanted patients [102]. Mancuso and colleagues described two additional patients undergoing successful liver transplant with satisfactory post-transplant follow-up up to 2 years [71]. Liver transplant is now considered the treatment of choice for HCC, in patients without severe pulmonary hypertension and subclinical heart failure. The relevant survival benefit of liver transplant compared to the standard alternative treatments (transplant benefit) should be considered. Given the high percentage of panel reactive human leukocyte antigens (HLA) antibodies in thalassaemia, a regular evaluation of circulating donor-specific anti-HLA antibodies (DSA) has to be adopted in thalassaemia patients [72].

KEY POINTS AND RECOMMENDATIONS

1. Liver-related morbidity and mortality in transfusion-dependent β-thalassaemia (TDT) should be closely monitored due to the significant risk posed by viral hepatitis and iron overload, which can lead to cirrhosis and hepatocellular carcinoma (HCC) (**Grade B**).
2. Patients with TDT should be closely monitored and adequately managed for iron overload, using serial serum ferritin and hepatic magnetic resonance imaging (MRI) assessments per standards of care. They should also be monitored for iron chelator-related hepatic toxicity per local prescribing information (see Chapter 3) (**Grade A**).
3. Regular clinical examination and liver function tests (every three months or more frequently if abnormal), with aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase, gamma glutamyl transferase (γGT), and bilirubin are recommended in all TDT patients to monitor for hepatic impairment. In the presence of severe hepatic impairment, prothrombin time and albumin should be added. Hepatic ultrasound should also be considered in all TDT patients ≥18 years to be done annually or every 6 months in case of evidence of advances liver damage (**Grade B**).
4. Non-invasive tests, such as transient elastography (TE) and fibrosis-4 score (FIB-4, in non-splenectomised patients), are preferred for assessing liver fibrosis and cirrhosis in patients with TDT. These methods provide an effective and less invasive alternative to liver biopsy (**Grade B**).
5. In patients with liver disease, vaccination against hepatitis A virus is recommended in TDT (**Grade C**).
6. TDT patients are susceptible to hepatitis B virus (HBV) infection due to frequent blood transfusions. However, the implementation of vaccination programmes and improved screening methods have significantly reduced the rate of HBV infections and related liver disease. Vaccination against HBV prior to the initiation of any planned blood transfusion therapy, with regular monitoring of antibody titres is recommended in all TDT patients who are seronegative for HBV markers. Unvaccinated patients should be monitored with annual hepatitis B surface antigen (HBsAg) (**Grade A**).
7. Administer antiviral therapy in consultation with the hepatologist to effectively manage chronic HBV infection in TDT patients and lower the risk of developing cirrhosis and HCC (**Grade A**).
8. The historical risk of hepatitis C virus (HCV) infection in TDT patients who received transfusions before the implementation of HCV screening in 1990 is important. Today, HCV remains a major transfusion-related risk mainly in low-income countries where screening is limited (Grade B). In case of a high-risk context, screen TDT patients annually for HCV antibodies using enzyme-linked immunoassay (ELISA). If the HCV antibody test is positive, perform HCV RNA molecular evaluation using quantitative polymerase chain reaction (PCR) with a detection limit of ≤15 IU/mL (**Grade A**).
9. Treat HCV infection in TDT patients in consultation with the hepatologist using pan-genotypic regimens. The recommended options are the fixed-dose combination of sofosbuvir and velpatasvir in a single pill or the combination of the non-structural (NS)3 inhibitor glecaprevir with the NS5 inhibitor pibrentasvir, taken as three pills once daily with food (**Grade A**).

10. Early diagnosis of HCC can be achieved through surveillance based on ultrasound examination of the liver every 6 months plus alpha fetoprotein (AFP) evaluation. This should be conducted in all cirrhotic TDT patients, and in non-cirrhotic patients with severe hepatic iron overload, age >45 years, or chronic viral hepatitis. HCC risk stratification models for chronic HBV and HCV used in the general population can be used in TDT (**Grade B**).

11. Management of HCC in TDT should follow that in the general population. It is essential that TDT patients with HCC are managed through a multidisciplinary approach involving hepatologists, hepatobiliary surgeons, interventional radiologists, and oncologists in combination with the treating haematologist **(Grade A).**

REFERENCES

1. GBD 2021 Diseases and Injuries Collaborators. Global incidence, prevalence, years lived with disability (YLDs), disability-adjusted life-years (DALYs), and healthy life expectancy (HALE) for 371 diseases and injuries in 204 countries and territories and 811 subnational locations, 1990-2021: a systematic analysis for the Global Burden of Disease Study 2021. Lancet 2024;403(10440):2133-2161.
2. Musallam KM, Vitrano A, Meloni A, Pollina SA, Karimi M, El-Beshlawy A, Hajipour M, Di Marco V, Ansari SH, Filosa A, Ricchi P, Ceci A, Daar S, Vlachaki E, Singer ST, Naserullah ZA, Pepe A, Scondotto S, Dardanoni G, Bonifazi F, Sankaran VG, Vichinsky E, Taher AT, Maggio A. Survival and causes of death in 2,033 patients with non-transfusion-dependent beta-thalassemia. Haematologica 2021;106(9):2489-2492.
3. Voskaridou E, Kattamis A, Fragodimitri C, Kourakli A, Chalkia P, Diamantidis M, Vlachaki E, Drosou M, Lafioniatis S, Maragkos K, Petropoulou F, Eftihiadis E, Economou M, Klironomos E, Koutsouka F, Nestora K, Tzoumari I, Papageorgiou O, Basileiadi A, Lafiatis I, Dimitriadou E, Kalpaka A, Kalkana C, Xanthopoulidis G, Adamopoulos I, Kaiafas P, Mpitzioni A, Goula A, Kontonis I, Alepi C, Anastasiadis A, Papadopoulou M, Maili P, Dionisopoulou D, Tsirka A, Makis A, Kostaridou S, Politou M, Papassotiriou I, Greek Haemoglobinopathies Study G. National registry of hemoglobinopathies in Greece: updated demographics, current trends in affected births, and causes of mortality. Ann Hematol 2019;98(1):55-66.
4. Forni GL, Gianesin B, Musallam KM, Longo F, Rosso R, Lisi R, Gamberini MR, Pinto VM, Graziadei G, Vitucci A, Bonetti F, Musto P, Piga A, Cappellini MD, Borgna-Pignatti C, Webthal p. Overall and complication-free survival in a large cohort of patients with beta-thalassemia major followed over 50 years. Am J Hematol 2023;98(3):381-387.
5. Gines P, Krag A, Abraldes JG, Sola E, Fabrellas N, Kamath PS. Liver cirrhosis. Lancet 2021;398(10308):1359-1376.
6. Carrat F, Fontaine H, Dorival C, Simony M, Diallo A, Hezode C, De Ledinghen V, Larrey D, Haour G, Bronowicki JP, Zoulim F, Asselah T, Marcellin P, Thabut D, Leroy V, Tran A, Habersetzer F, Samuel D, Guyader D, Chazouilleres O, Mathurin P, Metivier S, Alric L, Riachi G, Gournay J, Abergel A, Cales P, Ganne N, Loustaud-Ratti V, D'Alteroche L, Causse X, Geist C, Minello A, Rosa I, Gelu-Simeon M, Portal I, Raffi F, Bourliere M, Pol S, French ACOHc. Clinical outcomes in patients with chronic hepatitis C after direct-acting antiviral treatment: a prospective cohort study. Lancet 2019;393(10179):1453-1464.
7. Moon AM, Green PK, Rockey DC, Berry K, Ioannou GN. Hepatitis C eradication with direct-acting anti-virals reduces the risk of variceal bleeding. Aliment Pharmacol Ther 2020;51(3):364-373.
8. Mandorfer M, Simbrunner B. Prevention of First Decompensation in Advanced Chronic Liver Disease. Clin Liver Dis 2021;25(2):291-310.
9. Di Marco V, Bronte F, Cabibi D, Calvaruso V, Alaimo G, Borsellino Z, Gagliardotto F, Almasio PL, Capra M, Craxi A. Noninvasive assessment of liver fibrosis in thalassaemia major patients by transient elastography (TE) - lack of interference by iron deposition. Br J Haematol 2010;148(3):476-479.
10. Paparo F, Cevasco L, Zefiro D, Biscaldi E, Bacigalupo L, Balocco M, Pongiglione M, Banderali S, Forni GL, Rollandi GA. Diagnostic value of real-time elastography in the assessment of hepatic fibrosis in patients with liver iron overload. Eur J Radiol 2013;82(12):e755-761.
11. Ferraioli G, Lissandrin R, Tinelli C, Scudeller L, Bonetti F, Zicchetti M, Longo F, Murgia M, Bernuzzi S, Zecca M, Casula P, Piga A, Filice C. Liver stiffness assessed by transient elastography in patients with beta thalassaemia major. Ann Hepatol 2016;15(3):410-417.
12. Poustchi H, Eslami M, Ostovaneh MR, Modabbernia A, Saeedian FS, Taslimi S, George J, Malekzadeh R, Zamani F. Transient elastography in hepatitis C virus-infected patients with beta-thalassemia for assessment of fibrosis. Hepatol Res 2013;43(12):1276-1283.
13. Musallam KM, Cappellini MD, Daar S, Karimi M, El-Beshlawy A, Graziadei G, Magestro M, Wulff J, Pietri G, Taher AT. Serum ferritin level and morbidity risk in transfusion-independent patients with beta-thalassemia intermedia: the ORIENT study. Haematologica 2014;99(11):e218-221.

14. Angelucci E, Muretto P, Nicolucci A, Baronciani D, Erer B, Gaziev J, Ripalti M, Sodani P, Tomassoni S, Visani G, Lucarelli G. Effects of iron overload and hepatitis C virus positivity in determining progression of liver fibrosis in thalassemia following bone marrow transplantation. Blood 2002;100(1):17-21.
15. Musallam KM, Motta I, Salvatori M, Fraquelli M, Marcon A, Taher AT, Cappellini MD. Longitudinal changes in serum ferritin levels correlate with measures of hepatic stiffness in transfusion-independent patients with beta-thalassemia intermedia. Blood Cells Mol Dis 2012;49(3-4):136-139.
16. Maakaron JE, Cappellini MD, Graziadei G, Ayache JB, Taher AT. Hepatocellular carcinoma in hepatitis-negative patients with thalassemia intermedia: a closer look at the role of siderosis. Ann Hepatol 2013;12(1):142-146.
17. Deugnier Y, Turlin B, Ropert M, Cappellini MD, Porter JB, Giannone V, Zhang Y, Griffel L, Brissot P. Improvement in liver pathology of patients with beta-thalassemia treated with deferasirox for at least 3 years. Gastroenterology 2011;141(4):1202-1211, 1211 e1201-1203.
18. Hsu YC, Huang DQ, Nguyen MH. Global burden of hepatitis B virus: current status, missed opportunities and a call for action. Nat Rev Gastroenterol Hepatol 2023;20(8):524-537.
19. Polaris Observatory C. Global prevalence, cascade of care, and prophylaxis coverage of hepatitis B in 2022: a modelling study. Lancet Gastroenterol Hepatol 2023;8(10):879-907.
20. WHO. Hepatitis B. 9 April 2024. Available at: https://www.who.int/news-room/fact-sheets/detail/hepatitis-b. Accessed: 28 Nov 2024.
21. Hofstraat SHI, Falla AM, Duffell EF, Hahne SJM, Amato-Gauci AJ, Veldhuijzen IK, Tavoschi L. Current prevalence of chronic hepatitis B and C virus infection in the general population, blood donors and pregnant women in the EU/EEA: a systematic review. Epidemiol Infect 2017;145(14):2873-2885.
22. Bivegete S, McNaughton AL, Trickey A, Thornton Z, Scanlan B, Lim AG, Nerlander L, Fraser H, Walker JG, Hickman M, Vickerman P, Johnson H, Duffell E, Brooks-Pollock E, Christensen H. Estimates of hepatitis B virus prevalence among general population and key risk groups in EU/EEA/UK countries: a systematic review. Euro Surveill 2023;28(30).
23. Mardh O, Quinten C, Amato-Gauci AJ, Duffell E. Mortality from liver diseases attributable to hepatitis B and C in the EU/EEA - descriptive analysis and estimation of 2015 baseline. Infect Dis (Lond) 2020;52(9):625-637.
24. Mirmomen S, Alavian SM, Hajarizadeh B, Kafaee J, Yektaparast B, Zahedi MJ, Zand V, Azami AA, Hosseini MM, Faridi AR, Davari K, Hajibeigi B. Epidemiology of hepatitis B, hepatitis C, and human immunodeficiency virus infecions in patients with beta-thalassemia in Iran: a multicenter study. Arch Iran Med 2006;9(4):319-323.
25. Ocak S, Kaya H, Cetin M, Gali E, Ozturk M. Seroprevalence of hepatitis B and hepatitis C in patients with thalassemia and sickle cell anemia in a long-term follow-up. Arch Med Res 2006;37(7):895-898.
26. Wanachiwanawin W, Luengrojanakul P, Sirangkapracha P, Leowattana W, Fucharoen S. Prevalence and clinical significance of hepatitis C virus infection in Thai patients with thalassemia. Int J Hematol 2003;78(4):374-378.
27. Ramia S, Koussa S, Taher A, Haraki S, Klayme S, Sarkis D, Naman R. Hepatitis-C-virus genotypes and hepatitis-G-virus infection in Lebanese thalassaemics. Ann Trop Med Parasitol 2002;96(2):197-202.
28. Jaiswal SP, Chitnis DS, Jain AK, Inamdar S, Porwal A, Jain SC. Prevalence of hepatitis viruses among multi-transfused homogenous thalassaemia patients. Hepatol Res 2001;19(3):247-253.
29. Jamal R, Fadzillah G, Zulkifli SZ, Yasmin M. Seroprevalence of hepatitis B, hepatitis C, CMV and HIV in multiply transfused thalassemia patients: results from a thalassemia day care center in Malaysia. Southeast Asian J Trop Med Public Health 1998;29(4):792-794.
30. Lee WS, Teh CM, Chan LL. Risks of seroconversion of hepatitis B, hepatitis C and human immunodeficiency viruses in children with multitransfused thalassaemia major. J Paediatr Child Health 2005;41(5-6):265-268.
31. Singh H, Pradhan M, Singh RL, Phadke S, Naik SR, Aggarwal R, Naik S. High frequency of hepatitis B virus infection in patients with beta-thalassemia receiving multiple transfusions. Vox Sang 2003;84(4):292-299.
32. Farshadpour F, Taherkhani R, Farajzadeh H. Hepatitis B infection among beta-thalassemia major patients in Bushehr province of southern Iran. J Immunoassay Immunochem 2023;44(2):147-161.

33. Dodd RY, Crowder LA, Haynes JM, Notari EP, Stramer SL, Steele WR. Screening Blood Donors for HIV, HCV, and HBV at the American Red Cross: 10-Year Trends in Prevalence, Incidence, and Residual Risk, 2007 to 2016. Transfus Med Rev 2020;34(2):81-93.
34. O'Brien SF, Yi QL, Fan W, Scalia V, Goldman M, Fearon MA. Residual risk of HIV, HCV and HBV in Canada. Transfus Apher Sci 2017;56(3):389-391.
35. Lopez-Menchero C, Alvarez M, Fernandez P, Guzman M, Ortiz-de-Salazar MI, Arbona C. Evolution of the residual risk of HBV, HCV and HIV transmission through blood transfusion in the Region of Valencia, Spain, during a 15-year period (2003-2017). Blood Transfus 2019;17(6):418-427.
36. Romano L, Paladini S, Galli C, Raimondo G, Pollicino T, Zanetti AR. Hepatitis B vaccination. Hum Vaccin Immunother 2015;11(1):53-57.
37. Borgna-Pignatti C, Garani MC, Forni GL, Cappellini MD, Cassinerio E, Fidone C, Spadola V, Maggio A, Restivo Pantalone G, Piga A, Longo F, Gamberini MR, Ricchi P, Costantini S, D'Ascola D, Cianciulli P, Lai ME, Carta MP, Ciancio A, Cavalli P, Putti MC, Barella S, Amendola G, Campisi S, Capra M, Caruso V, Colletta G, Volpato S. Hepatocellular carcinoma in thalassaemia: an update of the Italian Registry. Br J Haematol 2014;167(1):121-126.
38. Fattovich G, Bortolotti F, Donato F. Natural history of chronic hepatitis B: special emphasis on disease progression and prognostic factors. J Hepatol 2008;48(2):335-352.
39. European Association for the Study of the Liver. EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. J Hepatol 2017;67(2):370-398.
40. Lin PC, Hsu WY, Lee PY, Hsu SH, Chiou SS. Insights into Hepatocellular Carcinoma in Patients with Thalassemia: From Pathophysiology to Novel Therapies. Int J Mol Sci 2023;24(16).
41. Mangia A, Bellini D, Cillo U, Laghi A, Pelle G, Valori VM, Caturelli E. Hepatocellular carcinoma in adult thalassemia patients: an expert opinion based on current evidence. BMC Gastroenterol 2020;20(1):251.
42. Wonke B, Hoffbrand AV, Brown D, Dusheiko G. Antibody to hepatitis C virus in multiply transfused patients with thalassaemia major. J Clin Pathol 1990;43(8):638-640.
43. WHO. Blood safety and availability. 2 June 2023. Available at: https://www.who.int/news-room/fact-sheets/detail/blood-safety-and-availability. Accessed: 28 Nov 2024.
44. European Association for the Study of the Liver. EASL recommendations on treatment of hepatitis C: Final update of the series(☆). J Hepatol 2020;73(5):1170-1218.
45. Di Marco V, Lo Iacono O, Capra M, Grutta S, Ciaccio C, Gerardi C, Maggio A, Renda D, Almasio P, Pisa R, et al. alpha-Interferon treatment of chronic hepatitis C in young patients with homozygous beta-thalassemia. Haematologica 1992;77(6):502-506.
46. Wonke B, Donohue SM, Hoffbrand AV, Scheuer PJ, Brown D, Dusheiko G. Recombinant alpha 2B interferon (IFN) in the treatment of chronic hepatitis C disease in thalassaemia major (TM). Bone Marrow Transplant 1993;12 Suppl 1:24-25.
47. Di Marco V, Lo Iacono O, Almasio P, Ciaccio C, Capra M, Rizzo M, Malizia R, Maggio A, Fabiano C, Barbaria F, Craxi A. Long-term efficacy of alpha-interferon in beta-thalassemics with chronic hepatitis C. Blood 1997;90(6):2207-2212.
48. Mangia A, Milligan S, Khalili M, Fagiuoli S, Shafran SD, Carrat F, Ouzan D, Papatheodoridis G, Ramji A, Borgia SM, Wedemeyer H, Losappio R, Perez-Hernandez F, Wick N, Brown RS, Jr., Lampertico P, Doucette K, Ntalla I, Ramroth H, Mertens M, Vanstraelen K, Turnes J. Global real-world evidence of sofosbuvir/velpatasvir as simple, effective HCV treatment: Analysis of 5552 patients from 12 cohorts. Liver Int 2020;40(8):1841-1852.
49. Berg T, Naumann U, Stoehr A, Sick C, John C, Teuber G, Schiffelholz W, Mauss S, Lohmann K, Konig B, Pangerl A, Niederau C. Real-world effectiveness and safety of glecaprevir/pibrentasvir for the treatment of chronic hepatitis C infection: data from the German Hepatitis C-Registry. Aliment Pharmacol Ther 2019;49(8):1052-1059.

50. Mangia A, Sarli R, Gamberini R, Piga A, Cenderello G, Piazzolla V, Santoro R, Caruso V, Quarta A, Ganga R, Copetti M, Forni G. Randomised clinical trial: sofosbuvir and ledipasvir in patients with transfusion-dependent thalassaemia and HCV genotype 1 or 4 infection. Aliment Pharmacol Ther 2017;46(4):424-431.
51. Flamm S, Mutimer D, Asatryan A, Wang S, Rockstroh J, Horsmans Y, Kwo PY, Weiland O, Villa E, Heo J, Gane E, Ryder SD, Welzel TM, Ruane PJ, Agarwal K, Ng TI, Xue Z, Lovell SS, Krishnan P, Kopecky-Bromberg S, Trinh R, Mensa FJ, Wyles DL. Glecaprevir/Pibrentasvir in patients with chronic HCV genotype 3 infection: An integrated phase 2/3 analysis. J Viral Hepat 2019;26(3):337-349.
52. Fahmy DM, Shokeir M, El Zeiny SM, Jonas MM, Abdallah A. Changes in Liver Stiffness and Noninvasive Fibrosis Scores in Egyptian Adolescents Successfully Treated with Ledipasvir-Sofosbuvir for Chronic Hepatitis C Virus Infection. J Pediatr 2021;231:110-116.
53. Origa R, Ponti ML, Filosa A, Galeota Lanza A, Piga A, Saracco GM, Pinto V, Picciotto A, Rigano P, Madonia S, Rosso R, D'Ascola D, Cappellini MD, D'Ambrosio R, Tartaglione I, De Franceschi L, Gianesin B, Di Marco V, Forni GL, Italy for T, hepatitis CASITeE. Treatment of hepatitis C virus infection with direct-acting antiviral drugs is safe and effective in patients with hemoglobinopathies. Am J Hematol 2017;92(12):1349-1355.
54. Sinakos E, Kountouras D, Koskinas J, Zachou K, Karatapanis S, Triantos C, Vassiliadis T, Goulis I, Kourakli A, Vlachaki E, Toli B, Tampaki M, Arvaniti P, Tsiaoussis G, Bellou A, Kattamis A, Maragkos K, Petropoulou F, Dalekos GN, Akriviadis E, Papatheodoridis GV. Treatment of chronic hepatitis C with direct-acting antivirals in patients with beta-thalassaemia major and advanced liver disease. Br J Haematol 2017;178(1):130-136.
55. Ponti ML, Comitini F, Murgia D, Ganga R, Canu R, Dessi C, Foschini ML, Leoni G, Morittu M, Perra M, Pilia MP, Casini MR, Zappu A, Origa R. Impact of the direct-acting antiviral agents (DAAs) on chronic hepatitis C in Sardinian patients with transfusion-dependent Thalassemia major. Dig Liver Dis 2019;51(4):561-567.
56. Friedman SL. Mechanisms of hepatic fibrogenesis. Gastroenterology 2008;134(6):1655-1669.
57. Cunningham MJ, Macklin EA, Neufeld EJ, Cohen AR, Thalassemia Clinical Research N. Complications of beta-thalassemia major in North America. Blood 2004;104(1):34-39.
58. Perifanis V, Tziomalos K, Tsatra I, Karyda S, Patsiaoura K, Athanassiou-Metaxa M. Prevalence and severity of liver disease in patients with b thalassemia major. A single-institution fifteen-year experience. Haematologica 2005;90(8):1136-1138.
59. Prati D, Maggioni M, Milani S, Cerino M, Cianciulli P, Coggi G, Forni GL, Magnano C, Meo A, Gramignoli R, Rebulla P, Fiorelli G, Cappellini MD, Cooleycare Cooperative G. Clinical and histological characterization of liver disease in patients with transfusion-dependent beta-thalassemia. A multicenter study of 117 cases. Haematologica 2004;89(10):1179-1186.
60. Jean G, Terzoli S, Mauri R, Borghetti L, Di Palma A, Piga A, Magliano M, Melevendi M, Cattaneo M. Cirrhosis associated with multiple transfusions in thalassaemia. Arch Dis Child 1984;59(1):67-70.
61. Garcia-Tsao G, Friedman S, Iredale J, Pinzani M. Now there are many (stages) where before there was one: In search of a pathophysiological classification of cirrhosis. Hepatology 2010;51(4):1445-1449.
62. Ellis EL, Mann DA. Clinical evidence for the regression of liver fibrosis. J Hepatol 2012;56(5):1171-1180.
63. Muretto P, Angelucci E, Lucarelli G. Reversibility of cirrhosis in patients cured of thalassemia by bone marrow transplantation. Ann Intern Med 2002;136(9):667-672.
64. D'Amico G, Garcia-Tsao G, Pagliaro L. Natural history and prognostic indicators of survival in cirrhosis: a systematic review of 118 studies. J Hepatol 2006;44(1):217-231.
65. Bosch J, Abraldes JG, Berzigotti A, Garcia-Pagan JC. The clinical use of HVPG measurements in chronic liver disease. Nat Rev Gastroenterol Hepatol 2009;6(10):573-582.
66. Papadopoulos N, Kountouras D, Malagari K, Tampaki M, Theochari M, Koskinas J. Characteristics and Prognosis of Hepatocellular Carcinoma in Multi-Transfused Patients with beta-Thalassemia. Experience of a Single Tertiary Center. Mediterr J Hematol Infect Dis 2020;12(1):e2020013.

67. Fattovich G, Stroffolini T, Zagni I, Donato F. Hepatocellular carcinoma in cirrhosis: incidence and risk factors. Gastroenterology 2004;127(5 Suppl 1):S35-50.
68. Voskaridou E, Ladis V, Kattamis A, Hassapopoulou E, Economou M, Kourakli A, Maragkos K, Kontogianni K, Lafioniatis S, Vrettou E, Koutsouka F, Papadakis A, Mihos A, Eftihiadis E, Farmaki K, Papageorgiou O, Tapaki G, Maili P, Theohari M, Drosou M, Kartasis Z, Aggelaki M, Basileiadi A, Adamopoulos I, Lafiatis I, Galanopoulos A, Xanthopoulidis G, Dimitriadou E, Mprimi A, Stamatopoulou M, Haile ED, Tsironi M, Anastasiadis A, Kalmanti M, Papadopoulou M, Panori E, Dimoxenou P, Tsirka A, Georgakopoulos D, Drandrakis P, Dionisopoulou D, Ntalamaga A, Davros I, Karagiorga M, Greek Haemoglobinopathies Study G. A national registry of haemoglobinopathies in Greece: deducted demographics, trends in mortality and affected births. Ann Hematol 2012;91(9):1451-1458.
69. Origa R, Gianesin B, Longo F, Di Maggio R, Cassinerio E, Gamberini MR, Pinto VM, Quarta A, Casale M, La Nasa G, Caocci G, Piroddi A, Piolatto A, Di Mauro A, Romano C, Gigante A, Barella S, Maggio A, Graziadei G, Perrotta S, Forni GL. Incidence of cancer and related deaths in hemoglobinopathies: A follow-up of 4631 patients between 1970 and 2021. Cancer 2023;129(1):107-117.
70. Borgna-Pignatti C, Vergine G, Lombardo T, Cappellini MD, Cianciulli P, Maggio A, Renda D, Lai ME, Mandas A, Forni G, Piga A, Bisconte MG. Hepatocellular carcinoma in the thalassaemia syndromes. Br J Haematol 2004;124(1):114-117.
71. Mancuso A, Sciarrino E, Renda MC, Maggio A. A prospective study of hepatocellular carcinoma incidence in thalassemia. Hemoglobin 2006;30(1):119-124.
72. Moukhadder HM, Halawi R, Cappellini MD, Taher AT. Hepatocellular carcinoma as an emerging morbidity in the thalassemia syndromes: A comprehensive review. Cancer 2017;123(5):751-758.
73. Kew MC. Hepatic iron overload and hepatocellular carcinoma. Liver Cancer 2014;3(1):31-40.
74. Marsella M, Ricchi P. Thalassemia and hepatocellular carcinoma: links and risks. J Blood Med 2019;10:323-334.
75. Ricchi P, Meloni A, Spasiano A, Costantini S, Pepe A, Cinque P, Filosa A. The impact of liver steatosis on the ability of serum ferritin levels to be predictive of liver iron concentration in non-transfusion-dependent thalassaemia patients. Br J Haematol 2018;180(5):721-726.
76. Anstee QM, Reeves HL, Kotsiliti E, Govaere O, Heikenwalder M. From NASH to HCC: current concepts and future challenges. Nat Rev Gastroenterol Hepatol 2019;16(7):411-428.
77. He LN, Chen W, Yang Y, Xie YJ, Xiong ZY, Chen DY, Lu D, Liu NQ, Yang YH, Sun XF. Elevated Prevalence of Abnormal Glucose Metabolism and Other Endocrine Disorders in Patients with beta-Thalassemia Major: A Meta-Analysis. Biomed Res Int 2019;2019:6573497.
78. European Association for the Study of the Liver. EASL Clinical Practice Guidelines: Management of hepatocellular carcinoma. J Hepatol 2018;69(1):182-236.
79. Karlsen TH, Sheron N, Zelber-Sagi S, Carrieri P, Dusheiko G, Bugianesi E, Pryke R, Hutchinson SJ, Sangro B, Martin NK, Cecchini M, Dirac MA, Belloni A, Serra-Burriel M, Ponsioen CY, Sheena B, Lerouge A, Devaux M, Scott N, Hellard M, Verkade HJ, Sturm E, Marchesini G, Yki-Jarvinen H, Byrne CD, Targher G, Tur-Sinai A, Barrett D, Ninburg M, Reic T, Taylor A, Rhodes T, Treloar C, Petersen C, Schramm C, Flisiak R, Simonova MY, Pares A, Johnson P, Cucchetti A, Graupera I, Lionis C, Pose E, Fabrellas N, Ma AT, Mendive JM, Mazzaferro V, Rutter H, Cortez-Pinto H, Kelly D, Burton R, Lazarus JV, Gines P, Buti M, Newsome PN, Burra P, Manns MP. The EASL-Lancet Liver Commission: protecting the next generation of Europeans against liver disease complications and premature mortality. Lancet 2022;399(10319):61-116.
80. Sangro B. EASL HCC guidelines update presented at the International Liver Congress (EASL) in Milan. 5-8 June 2024. Available at: https://easlcampus.eu/webcast/new-cpgs-complete-session. Accessed: 29 Nov 2024.

81. Tzartzeva K, Obi J, Rich NE, Parikh ND, Marrero JA, Yopp A, Waljee AK, Singal AG. Surveillance Imaging and Alpha Fetoprotein for Early Detection of Hepatocellular Carcinoma in Patients With Cirrhosis: A Meta-analysis. Gastroenterology 2018;154(6):1706-1718 e1701.
82. Fragatou S, Tsourveloudis I, Manesis G. Incidence of hepatocellular carcinoma in a thalassemia unit. Hemoglobin 2010;34(3):221-226.
83. Park HJ, Kim SY, Singal AG, Lee SJ, Won HJ, Byun JH, Choi SH, Yokoo T, Kim MJ, Lim YS. Abbreviated magnetic resonance imaging vs ultrasound for surveillance of hepatocellular carcinoma in high-risk patients. Liver Int 2022;42(9):2080-2092.
84. Brechot C, Gozuacik D, Murakami Y, Paterlini-Brechot P. Molecular bases for the development of hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC). Semin Cancer Biol 2000;10(3):211-231.
85. WHO. Guidelines for the prevention, diagnosis, care and treatment for people with chronic hepatitis B infection. March 2024. Available at: https://www.who.int/publications/i/item/9789240090903. Accessed: 29 Nov 2024.
86. Waziry R, Hajarizadeh B, Grebely J, Amin J, Law M, Danta M, George J, Dore GJ. Hepatocellular carcinoma risk following direct-acting antiviral HCV therapy: A systematic review, meta-analyses, and meta-regression. J Hepatol 2017;67(6):1204-1212.
87. Johnson PJ, Pirrie SJ, Cox TF, Berhane S, Teng M, Palmer D, Morse J, Hull D, Patman G, Kagebayashi C, Hussain S, Graham J, Reeves H, Satomura S. The detection of hepatocellular carcinoma using a prospectively developed and validated model based on serological biomarkers. Cancer Epidemiol Biomarkers Prev 2014;23(1):144-153.
88. Yang HI, Yuen MF, Chan HL, Han KH, Chen PJ, Kim DY, Ahn SH, Chen CJ, Wong VW, Seto WK, Group R-BW. Risk estimation for hepatocellular carcinoma in chronic hepatitis B (REACH-B): development and validation of a predictive score. Lancet Oncol 2011;12(6):568-574.
89. Papatheodoridis G, Dalekos G, Sypsa V, Yurdaydin C, Buti M, Goulis J, Calleja JL, Chi H, Manolakopoulos S, Mangia G, Gatselis N, Keskin O, Savvidou S, de la Revilla J, Hansen BE, Vlachogiannakos I, Galanis K, Idilman R, Colombo M, Esteban R, Janssen HL, Lampertico P. PAGE-B predicts the risk of developing hepatocellular carcinoma in Caucasians with chronic hepatitis B on 5-year antiviral therapy. J Hepatol 2016;64(4):800-806.
90. Ricchi P, Costantini S, Spasiano A, Cinque P, Esposito S, Filosa A. Hepatocellular carcinoma in patients with thalassemia in the post-DAA era: not a disappearing entity. Ann Hematol 2021;100(7):1907-1910.
91. Mazzaferro V, Romito R, Schiavo M, Mariani L, Camerini T, Bhoori S, Capussotti L, Calise F, Pellicci R, Belli G, Tagger A, Colombo M, Bonino F, Majno P, Llovet JM, Force HCCIT. Prevention of hepatocellular carcinoma recurrence with alpha-interferon after liver resection in HCV cirrhosis. Hepatology 2006;44(6):1543-1554.
92. Reig M, Forner A, Rimola J, Ferrer-Fabrega J, Burrel M, Garcia-Criado A, Kelley RK, Galle PR, Mazzaferro V, Salem R, Sangro B, Singal AG, Vogel A, Fuster J, Ayuso C, Bruix J. BCLC strategy for prognosis prediction and treatment recommendation: The 2022 update. J Hepatol 2022;76(3):681-693.
93. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, de Oliveira AC, Santoro A, Raoul JL, Forner A, Schwartz M, Porta C, Zeuzem S, Bolondi L, Greten TF, Galle PR, Seitz JF, Borbath I, Haussinger D, Giannaris T, Shan M, Moscovici M, Voliotis D, Bruix J, Group SIS. Sorafenib in advanced hepatocellular carcinoma. N Engl J Med 2008;359(4):378-390.
94. Kudo M, Finn RS, Qin S, Han KH, Ikeda K, Piscaglia F, Baron A, Park JW, Han G, Jassem J, Blanc JF, Vogel A, Komov D, Evans TRJ, Lopez C, Dutcus C, Guo M, Saito K, Kraljevic S, Tamai T, Ren M, Cheng AL. Lenvatinib versus sorafenib in first-line treatment of patients with unresectable hepatocellular carcinoma: a randomised phase 3 non-inferiority trial. Lancet 2018;391(10126):1163-1173.
95. Yau T, Park JW, Finn RS, Cheng AL, Mathurin P, Edeline J, Kudo M, Harding JJ, Merle P, Rosmorduc O, Wyrwicz L, Schott E, Choo SP, Kelley RK, Sieghart W, Assenat E, Zaucha R, Furuse J, Abou-Alfa GK, El-Khoueiry AB, Melero I, Begic D, Chen G, Neely J, Wisniewski T, Tschaika M, Sangro B. Nivolumab versus sorafenib in advanced hepatocellular carcinoma (CheckMate 459): a randomised, multicentre, open-label, phase 3 trial. Lancet Oncol 2022;23(1):77-90.

96. Finn RS, Qin S, Ikeda M, Galle PR, Ducreux M, Kim TY, Kudo M, Breder V, Merle P, Kaseb AO, Li D, Verret W, Xu DZ, Hernandez S, Liu J, Huang C, Mulla S, Wang Y, Lim HY, Zhu AX, Cheng AL, Investigators IM. Atezolizumab plus Bevacizumab in Unresectable Hepatocellular Carcinoma. N Engl J Med 2020;382(20):1894-1905.
97. Kulkarni AV, Tevethia H, Kumar K, Premkumar M, Muttaiah MD, Hiraoka A, Hatanaka T, Tada T, Kumada T, Kakizaki S, Vogel A, Finn RS, Rao PN, Pillai A, Reddy DN, Singal AG. Effectiveness and safety of atezolizumab-bevacizumab in patients with unresectable hepatocellular carcinoma: a systematic review and meta-analysis. EClinicalMedicine 2023;63:102179.
98. Sangro B, Chan SL, Kelley RK, Lau G, Kudo M, Sukeepaisarnjaroen W, Yarchoan M, De Toni EN, Furuse J, Kang YK, Galle PR, Rimassa L, Heurgue A, Tam VC, Van Dao T, Thungappa SC, Breder V, Ostapenko Y, Reig M, Makowsky M, Paskow MJ, Gupta C, Kurland JF, Negro A, Abou-Alfa GK, investigators H. Four-year overall survival update from the phase III HIMALAYA study of tremelimumab plus durvalumab in unresectable hepatocellular carcinoma. Ann Oncol 2024;35(5):448-457.
99. Abou-Alfa GK, Lau G, Kudo M, Chan SL, Kelley RK, Furuse J, Sukeepaisarnjaroen W, Kang YK, Van Dao T, De Toni EN, Rimassa L, Breder V, Vasilyev A, Heurgue A, Tam VC, Mody K, Thungappa SC, Ostapenko Y, Yau T, Azevedo S, Varela M, Cheng AL, Qin S, Galle PR, Ali S, Marcovitz M, Makowsky M, He P, Kurland JF, Negro A, Sangro B. Tremelimumab plus Durvalumab in Unresectable Hepatocellular Carcinoma. NEJM Evid 2022;1(8):EVIDoa2100070.
100. Frenette C. How to Choose Second-Line Treatment for Hepatocellular Carcinoma. Gastroenterol Hepatol (N Y) 2021;17(9):431-434.
101. Mancuso A, Perricone G. Time to define a new strategy for management of hepatocellular carcinoma in thalassaemia? Br J Haematol 2015;168(2):304-305.
102. Restivo Pantalone G, Renda D, Valenza F, D'Amato F, Vitrano A, Cassara F, Rigano P, Di Salvo V, Giangreco A, Bevacqua E, Maggio A. Hepatocellular carcinoma in patients with thalassaemia syndromes: clinical characteristics and outcome in a long term single centre experience. Br J Haematol 2010;150(2):245-247.

06 GROWTH ABNORMALITIES, ENDOCRINE, AND BONE DISEASE

Authors: **Maddalena Casale[1], Marina Baldini[2], Andrea Giusti[3], Anna Grandone[4], Maurizio Poggi[5]**

[1.] Centre of Paediatric Haematology and Oncology, Department of Woman, Child and General and Specialised Surgery, University of Campania Luigi Vanvitelli, Naples, Italy

[2.] Unit of Medicine and Metabolic Disease, Department of Internal Medicine, IRCCS Fondazione Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

[3.] Division of Internal Medicine and Metabolic Bone Diseases, Department of Internal Medicine and Cardiology, Villa Scassi Hospital, Genoa, Italy

[4.] Centre of Paediatric Endocrinology, Department of Woman, Child and General and Specialised Surgery, Naples, Italy

[5.] Endocrine Metabolic Unit, Department of Internal Medicine, Sant'Andrea Hospital, Rome, Italy

Competing interest: None to disclose.

1. INTRODUCTION

Endocrinopathies currently represent the four leading causes of complications in transfusion-dependent β-thalassaemia (TDT) patients; with rates 2-3 times higher than cardiac and hepatic disorders [1, 2]. The risk of developing a new endocrinopathy within 5 years is around 10%, even in patients with satisfactory ferritin, liver iron concentration (LIC), and cardiac T2* values [3]. This is primarily attributed to the early toxic effect of iron on endocrine glands which cannot be detected by conventional assays and hence limits the ability to predict new endocrinological disorders. A risk assessment chart has recently been proposed, based on parameters that revealed to be predictive of endocrinopathy development on longitudinal follow up [3]. Such risk prediction tools, however, require further refinement and validation before wide implementation can be recommended, with the aim of preventing future morbidity development; similar to what has been achieved with the use of magnetic resonance imaging (MRI) values for cardiac and hepatic iron overload as predictors of future organ dysfunction.

Another priority that needs to be addressed is achieving equitable progress in the management of endocrinological complications around the world, in light of the large disparity in endocrinopathy rates reported in TDT patients living in low- vs high-income countries, which is primarily attributed to limited access and organisation of healthcare services [3, 4].

This Chapter provides an organisational model, based on a first diagnosis level, using simple and unexpensive tests that can be implemented at all centres caring for TDT patients. The second level of diagnosis and treatment should be restricted to patients who present with abnormal first level tests, through the involvement of a consultant endocrinologist and/or networking with other centres with expertise along with a clear definition of the anticipated patient journey. The hub and spoke model for the management of endocrine complications in TDT patients has the potential to ensure early diagnosis for all patients around the world, while centralising complex cases that require expert staff and specific diagnostic tests, and thus limiting costs and disparities in access to care.

2. HEIGHT AND GROWTH DISORDERS

2.1. Epidemiology

Short stature is frequent among patients with TDT, with prevalence varying between 8% and 75%. Over time, growth failure can manifest from the first years of life to the teenage period [5]. The pathophysiology of growth disorders in haemoglobinopathies is multifactorial, and includes chronic anaemia, iron overload, and adverse events to iron chelation therapy [5, 6]. Therefore, active monitoring of growth in all children with TDT is warranted.

2.2. Clinical presentation and diagnosis

In patients with TDT it is recommended to perform screening for short stature from the start of care at the treating centre, with serial reassessments every 6 months. A period of observation and the evaluation of growth velocity over at least 6-12 months is required, before diagnosis of a growth disorder. Standardised clinical growth velocity charts with 5th and 95th percentiles should be used for boys and girls (https://www.cdc.gov/growthcharts/cdc-charts.htm). Monitoring of growth and development include evaluation of standing and sitting height charted against growth centiles, pubertal stage according to Tanner's tables, body mass index (BMI), proportions of the various skeletal segments and any dysmorphic notes, presence of lesions similar to rickets affecting the carpus and knees, and the presence of platyspondyly. It is necessary to request measurement of the parents' height to evaluate the genetic height potential/target, which is calculated with the formula: height of mother + height of father +13 cm [if male] or -13 cm [if female] / 2 [7]. Clinical observations requiring careful assessment are:

- Severe short stature (stature ≤ -2.5 standard deviations [SD]).
- Stature ≤ -1.5 SD compared to the family target and growth rate ≤ -2 SD or ≤ -1.5 SD after 2 consecutive years.
- Stature ≤ -2 SD with growth rate ≤ -1 SD evaluated at least 6 months apart or stature reduction of 0.5 SD in one year in children over 2 years old.
- If no short stature is present, growth rate ≤ -2 SD in one year or ≤ -1.5 SD in two consecutive years.

Laboratory tests to be performed in case of short stature include: pretransfusion haemoglobin level, iron indices, inflammation indices, liver and kidney function, electrolytes, blood gas analysis, total proteins with electrophoresis, chemical-physical examination of urine, screening for celiac disease (anti-transglutaminase antibodies [ATA] ± anti-gliadin antibodies [AGA] depending on the age of the subject with concomitant assessment of IgA), thyroid function (free thyroxine [FT4], thyroid-stimulating hormone [TSH]), phospho-calcium metabolism, and insulin-like growth factor 1 (IGF-1).

When other causes of short stature have been excluded, a growth hormone (GH) deficiency should be investigated through GH secretion provocation tests. The GH stimulus test is performed according to standardised protocols. Dynamic tests used in the paediatric age are growth-hormone-releasing hormone (GHRH)-arginine, glucagon, or clonidine tests. The manufacturing company interrupted the production of the GHRH analogue in 2008 in the USA and in 2023 in Europe [8], so this test is becoming unavailable worldwide [9]. At the moment, the available products for GH dynamic testing are mainly glucagon or clonidine.

2.3. Management and monitoring

Optimizing the transfusion regimen, maintaining a pretransfusion haemoglobin value between 9.5 and 10.5 g/dL [5], and a satisfactory iron balance are crucial approaches to prevent short stature in children with TDT [5, 6].

In case of short stature, any endocrinological disorders potentially associated with growth failure (hypothyroidism, glucose metabolism disorders, delayed pubertal development) and any nutritional, vitamin, or micronutrient deficiency (deficiency of folate, vitamin D, vitamin A, zinc) must be corrected. In children with a confirmed diagnosis of GH deficiency, recombinant human GH (rhGH) therapy should be initiated at a dose of 0.025-0.035 mg/kg/day in a single subcutaneous evening administration, to mimic the metabolic effects of the physiological secretion of GH. The response to replacement therapy is highly variable, probably linked to different severity of GH deficiency, compliance with the replacement treatment, and different individual patient responses [10].

2.4. Other considerations and unmet needs

The diagnostic cut offs for GH deficiency are still controversial, and affected by the use of sex steroids, the starting dose of rhGH, and definition of the therapeutic response [11-14].

3. DISORDERS OF PUBERTAL DEVELOPMENT

3.1. Epidemiology

Pubertal development disorders represent the most frequent endocrinological problem in paediatric patients with TDT, and can manifest with variable severity, from pubertal delay to the arrest of pubertal progression to hypogonadism [15]. Hypogonadotropic hypogonadism is the most frequent complication [16]. Prevalence and severity of hypogonadism in patients with TDT vary widely in different case series depending on the age group analysed, thalassaemia genotype, and adequacy of the management of iron overload and associated complications [16]. According to data from 29 centres in 2004, hypogonadism had a prevalence of 38% in females and 43% in males among 3817 adolescents and adults with TDT [17]. However, in a more recent cohort study from a high-income country, the overall crude risk of delayed puberty/hypogonadism in TDT patients under 18 years of age was approximately 7% [3].

3.2. Clinical presentation and diagnosis

Delayed pubertal development is characterised by the complete absence of signs of pubertal development at the age of 13 years in girls and 14 years in boys. Pubertal arrest is characterised by the lack of progression of pubertal development for a period of one year. In this case, testicular size stops at 6-8 mL in boys and breast size at stage B3 in girls; growth velocity is also severely impaired [18].

In the general paediatric population, the most frequent form of pubertal delay is represented by constitutional delay in growth and puberty (CDGP) [19]; characterised by growth delay in developmental age and delayed puberty in adolescence with spontaneous resolution within 18 years of age, especially in patients with chronic diseases. This condition represents a diagnosis of exclusion with respect to three different disorders: hypergonadotropic hypogonadism (identified in 7% of males and 25% of females with delayed puberty, respectively), permanent

hypogonadotropic hypogonadism (9% of males and 20% of females with delayed puberty, respectively), transient or functional hypogonadotropic hypogonadism, due to delayed maturation of the hypothalamic-pituitary-gonadal axis secondary to an underlying pathological condition (20% of cases of delayed puberty) [20]. Many authors place the pubertal delay of TDT in the latter category [19, 21, 22], although forms of hypergonadotropic hypogonadism due to gonadal damage can be detected, even if more rarely [16].

Familial evaluation for a history of pubertal delay is necessary. In fact, up to 75% of patients with CDGP have a positive family history of pubertal delay [23, 24]. Furthermore, in TDT patients, reassessment of the transfusion regimen and adequacy of iron chelation therapy is important to contextualise pubertal disorders.

The diagnostic process for delayed puberty includes clinical examination and auxological evaluation, with measurements of weight, height, and pubertal stage according to Tanner, evaluation of testicular volume in males, reconstruction of the growth curve with previous measurements and calculation of the height growth rate/year. Most subjects with CDGP present with a slowing of height growth in the prepubertal period with consequent short stature or deflection of the growth curve. In subjects with isolated hypogonadotropic hypogonadism, height growth is constant and normal during childhood, while in the pubertal period, a deflection of height occurs due to the lack of pubertal spurt [20]. This trend in the growth curve is the one most reported in patients with TDT from the 2000s onwards [5]. Bone age assessment is also recommended, preferably using the Greulich and Pyle method or the Tanner Whitehouse III radius-ulna-short bone (RUS) protocol.

The diagnostic work up includes measurement of luteinising hormone (LH), follicle stimulating hormone (FSH), oestrogen in females, and total testosterone in males; in addition to the measurement of IGF-1 and thyroid function. High levels of gonadotropins indicate gonadal damage, which is very rare in TDT. Low levels of LH and FSH are found in both CDGP and hypogonadotropic hypogonadism, whether functional or permanent. The contextual interpretation of growth velocity is useful to proceed with a definitive diagnosis [21].

In the presence of low gonadotropins and a growth velocity in the prepubertal range, it is useful to perform a gonadotropin-releasing hormone (GnRH) test. A poor response or low LH and FSH levels confirms the diagnosis of hypogonadotropic hypogonadism. However, patients with CDGP or hypogonadotropic hypogonadism can both have a prepubertal response [21]. The human chorionic gonadotropin (hCG) test has also been used to differentiate CDGP from hypogonadotropic hypogonadism. Peak testosterone levels are lower in patients with hypogonadotropic hypogonadism than in patients with CDGP [21]. In case of low gonadotropins, a brain MRI (T2) with study of the hypothalamic-pituitary region is recommended [11, 21]. Thus, low values of gonadotropins and growth velocity lower than the prepubertal range suggest functional or permanent hypogonadotropic hypogonadism or multiple pituitary deficits, for which a brain MRI should be performed with study of the hypothalamic-pituitary region [21]. Finally, pelvic ultrasound in females allows evaluation of the size of the uterus and ovaries [11].

3.3. Management and monitoring

In patients with TDT and delayed pubertal development, it is crucial to optimise transfusion regimens and iron chelation therapy considering the impact of iron overload on the hypothalamic-pituitary-gonadal axis, and data indicating lower rates of pubertal delay especially with oral iron chelators [25, 26].

Induction of pubertal development with hormone therapy must be considered according to a comprehensive patient evaluation including age, extent of iron overload, chronic liver disease, and psychological aspects related to the failure to start or arrest of pubertal development. Since at the onset of pubertal delay the clinical presentation and hormonal status do not allow a distinction between a self-limited pubertal delay and a hypogonadotropic hypogonadism, in males, it is recommended to start with a 3-6 month cycle of testosterone (enanthate, cypionate, propionate) at a low dose (50 mg every 4 weeks with administration into the muscle), followed by re-evaluation with the possibility of performing a second cycle with a dose increase of 25-50 mg for another 3-6 months [27]. If the patient presents with an increase in testicular volume, therapy should be stopped and pubertal progression should be clinically monitored, since this may be indicative of a simple pubertal delay. Failure to progress in pubertal development indicates a condition of hypogonadotropic hypogonadism and treatment should be resumed with low doses and with a subsequent gradual increase in testosterone dose of 50 mg every 6-12 months. Once the dosage reaches 100-150 mg per month, administration should be switched to every 15 days up to the adult dose which is gradually reached over 3 years. Pubertal induction with testosterone in males also allows identification of patients with hypogonadotropic hypogonadism, in whom an increase in testicular volume is not observed in response to therapy, unlike patients with simple pubertal delay [28]. In males with confirmed hypogonadotropic hypogonadism, one treatment option is the induction of pubertal development with gonadotropins (FSH and hCG), to allow maturation and increase in testicular volume, then switching to maintenance therapy with testosterone. According to some authors, this approach would be preferable because it is more physiologically acceptable by patients; however, there is no evidence of an unfavourable effect of induction of puberty with testosterone on the development of spermatogenetic tissue and on future fertility [29].

In females, hormone therapy involves an induction phase with low doses of oestrogens, followed by a reassessment after suspension of therapy. In case of failure of pubertal progression, treatment with oestrogens is resumed with gradual increases in dose. The complete induction cycle lasts approximately 2-3 years. At the onset of menstrual flow, it is necessary to add a progestin for 10 days per month from the 17th to the 26th day of the cycle. The use of 17-beta oestradiol transdermal 25 µg patches is recommended. Initial therapy is 3.1-6.2 µg/day (1/8-1/4 of a patch), to be increased by 3.1-6.2 µg/day after 6 months. The patch should be applied continuously and changed twice a week (every 3.5 days). The subsequent increases are 3.1-6.2 µg up to a dose of 25 µg (1 whole patch). If the use of the transdermal formulation is not possible, the use of oral ethinyloestradiol 10 µg tablets is suggested, starting with 2.5 µg (1/4 tablet), increasing to 5 µg per day (1/2 tablet) after 6-12 months. Progestin therapy should be applied for 10-12 days per month to avoid endometrial hyperplasia. The drugs used are medroxyprogesterone acetate 5 mg once a day, norethisterone 5 mg, or utrogestan 200 mg once a day.

It is necessary to monitor the onset of possible treatment-related side effects, with evaluation of blood pressure and lipid profile. Due to the reported risk of thromboembolic episodes, particular

attention should be paid to patients with a history of thrombophilia even before starting therapy. Thrombophilia screening with a coagulation profile and measurement of homocysteine levels before starting hormone therapy allows identification of a predisposition to such events and is therefore suggested. Monitoring of bone health with dual-energy X-ray absorptiometry (DXA) is also suggested when an evaluation with appropriate reference values is possible.

In patients with delayed puberty in whom hormone therapy is not undertaken, clinical, laboratory, and instrumental monitoring is recommended with checks every 3-6 months for reassessment of the pubertal stage, auxological parameters and growth velocity, levels of sexual hormones (LH, FSH, and total testosterone), as well as bone age once a year. The aim of re-evaluation is to establish whether there is a spontaneous progression of puberty or, on the contrary, there is a need for hormonal therapy.

4. FEMALE HYPOGONADISM

4.1. Epidemiology

Hypogonadism (in females or males) is the most frequent endocrine complication in adult patients with TDT, which has been consistently attributed to iron overload secondary to regular transfusions. Elevated serum ferritin levels (<2500 ng/mL) are associated with a 2.75-fold increased risk of hypogonadism than levels <1000 ng/mL [30]. The origin is central or hypothalamic-pituitary in most cases, due to the particular sensitivity of the pituitary to iron overload, as demonstrated by MRI reports of pituitary hemosiderosis since childhood [16, 31]. In fact, pituitary iron and volume have been shown to predict hypogonadism in transfusional iron overload in TDT patients [32].

The prevalence varies among studies, with reported values higher than 50% in historical series of TDT; while in more recent cohorts the figures are lower, though still considerable [3, 33, 34]. In analogy with other endocrine complications, early and proper iron chelation therapy especially with oral or combinatorial regimens is the most efficacious measure to prevent or even reverse hypogonadism in patients with TDT [35-39].

4.2. Clinical presentation and diagnosis

In female patients, hypogonadism manifests with menses irregularities, principally primary or secondary amenorrhoea, depending on the appearance at puberty or in adult life. Primary amenorrhoea is defined as absence of menarche by the age of 16 years and is discussed in the previous section on 'Disorders of Pubertal Development'. Secondary amenorrhoea is absence of menses for ≥3 months in a woman with previously regular cycles, or for ≥6 months in a woman with previously irregular cycles. The occurrence of <9 menstrual cycles/year configurates oligomenorrhoea.

Given the impact of hypogonadism on quality of life, fertility, and mortality, a regular screening of all premenopausal patients is mandatory, by assessing the menstrual rhythm every 6 months and promptly providing diagnostic and therapeutic follow-up if indicated.

The recommended first level tests in case of oligo/amenorrhoea are assessments of FSH, LH, and oestradiol serum levels. A pelvic ultrasound is also required, and it is recommended to exclude other possible causes of oligo/amenorrhoea such as hyperprolactinaemia, hyperandrogenism, hypothyroidism, and pregnancy [36, 40]. Since the hypothalamic-pituitary-gonadal axis is

compromised in TDT, the dynamic stimulation of gonadotropins with luteinizing hormone-releasing hormone (LHRH) is not useful.

If serum oestradiol is low in association with high gonadotropins, the diagnosis is hypergonadotropic or primary hypogonadism (gonadal failure); conversely, hypogonadotropic hypogonadism (hypothalamic-pituitary dysfunction) is characterised by low concentrations of oestradiol associated with reduced or inappropriately normal gonadotropins. Hypogonadism might only be one aspect of a pituitary defect with multiple deficiencies; thus, the endocrine evaluation must be expanded to the other endocrine axes (morning cortisol, morning adrenocorticotropic hormone [ACTH], FT4, TSH, and IGF-1). Pituitary imaging by MRI with contrast medium (unless contraindicated) is also recommended, to exclude pathologies/masses but also taking in account the prognostic value of the size of the gland [16, 31].

4.3. Management and monitoring

If untreated, hypogonadism with premenopausal onset is burdened with multiple health risks, including low bone density with increased fracture risk and early cardiovascular disease. Appropriate therapy replacing physiological levels of gonadal hormones (hormone replacement therapy [HRT]) reduces these risks and is therefore recommended for all affected patients, unless a specific contraindication is present.

HRT in females includes two components, oestrogen and cyclic progestin; ideally, treatment should mimic physiologic ovarian function until the average age of menopause [36, 40-42]. It is possible to get closer to this goal using 17-beta oestradiol (the prevailing form of endogenous oestrogen), available in oral and transdermal formulations; the latter route of administration being the best choice as it avoids the first hepatic pass and has a lower impact on angiotensinogen and coagulation factors synthesis, which reduces the thrombotic risk [41-43]. Furthermore, it has been demonstrated that transdermal oestradiol is more efficacious than oral combined contraceptives in preserving bone health [44, 45].

In women with an intact uterus, the association of a progestin, inducing regular withdrawal bleedings, protects the endometrium from hyperplasia, and potentially from related cancer. It is recommended to prescribe this component for about 12-14 days a month, with a sequential cyclic scheme. Natural micronised progesterone is easily absorbed by oral (200 mg/day) or intravaginal routes and is preferable to other synthetic progestogens, as fewer effects on lipid metabolism and thromboembolic risk have been described [44, 46, 47]. Hysterectomised women do not need the progestin component.

Due to the ease of administration, oral combined contraceptives (COC) are often better accepted by patients than the above mentioned sequential option. Issues associated with their use mainly concern the higher risk of serious health events (luckily rare) such as venous thromboembolism and breast cancer. The safest choice is commercial COC containing oestradiol (rather than ethyniloestradiol) associated to levonorgestrel, nomegestrol acetate, or micronised progesterone as the progestin component [44, 45]. Minor side effects of HRT are reported in Table 1.

Table 1. *Side effects of hormone replacement therapy.*

General
• Breast tenderness • Face melasma • Fluid retention • Weight gain • Headache • Mood changes • Unscheduled bleeding • Blood pressure increase • Worsening of glucose tolerance • Increase in liver enzymes or bilirubin • Dyslipidaemia • Venous thromboembolism • Cardio/cerebrovascular events
For transdermal formulations
• Local skin irritation; discomfort at remotion • Patch detachment (in summer, after physical exercise)

Experience is lacking in the TDT population with oestetrol, a native oestrogen produced exclusively by the foetal liver, recently approved as the oestrogenic component of a COC. The advantages described in the literature with the use of oestetrol include safety and tolerability, with lower impact on breast, lipids, and haemostasis parameters (Table 2).

Table 2. *Characteristics of different oestrogens.*

Parameter	Oestradiol	Ethinyloestradiol	Oestetrol
Origin	Natural, principally ovaries	Synthetic	Natural, foetus liver
Bioavailability (oral)	High if micronised or esterified	High	High
Dose in combined contraceptives	1.5-2 mg	15-30 mcg	14.2 mg
Half-life (hours)	35	12	28
Impact on liver	+	++	+

Oestroprogestin replacement treatment contraindications are summarised in Table 3. In patients recognizably bearing thrombotic risk factors, it is suggested to evaluate the risk-benefit ratio of HRT and discuss the possible association of an anticoagulant / antiaggregant drug with a specialist in coagulopathies, especially considering that β-thalassaemia has been recognised with a hypercoagulable state (see Chapter 4). A useful ais in the decision process can be the United States Medical Eligibility Criteria (US MEC) for Contraceptive Use, highlighting medical eligibility criteria categories for different hormone formulations [48].

All patients with irregular menses (primary and secondary amenorrhoea, oligomenorrhoea) should be referred to an endocrinologist as well as the gynaecologist for diagnostic assessment and initial treatment, thereafter, followed annually.

Table 3. *Contraindications to hormone replacement therapy.*

General
• Vaginal bleeding of undiagnosed cause • Ongoing, suspected, or previous breast or endometrial carcinoma • Ongoing, suspected, or previous venous or arterial thrombosis • Migraine with aura • Severe microvascular complications of diabetes • Severe uncontrolled hypertension • Severe chronic liver disease • Severe allergic reaction to any component of the therapy

The parameters to monitor on oestroprogestin HRT are blood pressure, liver and kidney function, blood lipids, and glucose (Table 4). Moreover, we suggest performing annually a gynaecological examination with pelvic ultrasound, bringing forward the investigations in the event of unexpected vaginal bleeding to rule out any pelvic pathology, especially endometrial hyperplasia or cancer. For endometrial and breast cancer screening, it is recommended to follow the age-appropriate local guidelines. In hypothyroid patients on replacement therapy, it is mandatory to check FT4 and TSH serum levels every 3 months after the beginning of HRT, because the need of levothyroxine may be increased by oestrogens.

4.4. Other considerations and unmet needs

The prescription of HRT to hypogonadic patients with additional risk factors of thrombosis, such as protein C/S or antithrombin deficiency, factor V Leiden, or prothrombin mutations may be a problem. In this case, it is suggested to share the decision-making process with a specialist in coagulopathies. In every patient, to reduce the risks of thromboembolic events, it is recommended to maintain a proper lifestyle, including a healthy diet, regular physical exercise, and avoiding smoking and alcohol.

Table 4. *Monitoring of adult hypogonadal patients on hormone replacement therapy.*

General
• Physical examination • Family and personal history of venous thromboembolism • Gynaecological examination • Pelvic and abdominal ultrasound • Blood pressure • FSH, LH and 17-beta oestradiol levels • Basal glucose and insulin with HOMA-IR assessment (oral glucose tolerance test in selected cases) • Renal function • Liver function • Fasting lipids • TSH levels • DXA of lumbar spine and femur (every 18-24 months)

Abbreviations: FSH, follicle-stimulating hormone; LH, luteinizing hormone; HOMA-IR, homeostatic model assessment for insulin resistance; TSH, thyroid-stimulating hormone; DXA, dual-energy X-ray absorptiometry.

5. MALE HYPOGONADISM

5.1. Epidemiology

As mentioned in the previous section, hypogonadism is the endocrine complication with the highest prevalence in TDT patients, reaching up to 80% in some studies [22], while in a recent series of patients at puberty, a 23.6% prevalence and a negative correlation between ferritin and pituitary volume were described [31]. The wide heterogeneity in epidemiological data depends mostly on the clinical characteristics of the cohorts studied, in terms of age at the beginning of transfusion and iron chelation therapy, average age at evaluation, efficacy and compliance to chelation therapy, and the type of chelating agent used [3, 49-51].

Hypogonadism in males can be classified in accordance with the level of affection of the hypothalamic-pituitary-gonadal axis. The gonadotrophs cells are more sensitive to pathologic insults (anaemia, iron overload) than other anterior pituitary stems, and hypogonadism is most commonly hypogonadotropic (central or secondary). However, it can also result from testicular defects (primary hypogonadism), and less frequently the testes and the hypothalamus–pituitary unit (mixed hypogonadism) can both be affected (Table 5). It is essential to establish the hypogonadism origin, as it has implications on clinical evaluation and treatment [22, 52].

Table 5. *Causes of hypogonadism in men with transfusion-dependent β-thalassaemia.*

Primary (testicular or hypergonadotropic)
• Iron overload • Anorchia (congenital or acquired) • Ectopic or undescended testis • Testicular cancer • Orchitis • Drugs
Secondary (central or hypogonadotropic)
• Iron overload (isolated or multiple hormonal defects) • Hyperprolactinemia • Radiotherapy • Trauma • Vascular

5.2. Clinical presentation and diagnosis

The clinical presentation of hypogonadism differs according to the age of onset and the severity of androgen deficiency [22]. When testosterone deficiency starts in the prepubertal period, it manifests with delayed or arrested puberty (see previous section on 'Disorders of Pubertal Development'). In the adult patient, sexual characteristics are completely developed and the most frequent manifestations are related to the sexual sphere, such as decreased libido, reduced morning erections, and erectile dysfunction. Less specific symptoms commonly associated to androgen deficiency include fatigue, irritability, poor concentration, reduced psychophysical performance, and mood alterations [53]. Slowing down of beard and body hair growth, reduced muscle strain and size, and bone demineralisation occur lately (Table 6).

Table 6. *Symptoms and signs of hypogonadism in the adult with transfusion-dependent β-thalassaemia.*

Specific
• Libido reduction/loss • Erectile dysfunction • Reduced spontaneous/morning erections • Flushing • Body hair loss • Gynaecomastia
Non-specific
• Fatigue • Depressed mood • Overweight/obesity • Low bone mass • Sarcopaenia

Clinical examination of testicular and penile size as well as of sexual secondary characteristics is recommended to evaluate the androgen status. In adult-onset hypogonadism, body proportions are normal, contrary to patients with pre-pubertal hypogonadism or delayed puberty where a eunuchoid body shape can be found, defined as an upper segment/lower segment ratio <0.92 and arm-span to height ratio >1.0. Digital rectal examination is also recommended in patients aged >40 years; it allows both confirmation of the diagnosis of hypogonadism (if the prostate volume is reduced) and to exclude abnormalities that contraindicate testosterone replacement treatment.

The high prevalence of hypogonadism and the low specificity of many symptoms and signs in the adult age dictate the need to screen all TDT patients at least once a year from puberty. Assessment should include clinical history and evaluation (age and course of puberty, sexual life, gonads, and secondary sex characters) as well as serum levels of testosterone and gonadotropins (FSH, LH) [49]. Since serum concentrations exhibit day-to-day and diurnal variations and peak in the morning, testosterone levels should be tested on two different occasions, on blood collected between 7 and 11 am (or within 3 hours from awakening) after an overnight fast.

Equilibrium dialysis is recognised as the most accurate method for total testosterone measurement, but the commercial immunoassays are mostly reliable for clinical use. Serum total testosterone level is considered absolutely normal if >350 ng/dL (12 nmol/l), absolutely pathologic if <230 ng/dL (<8 nmol/L); for intermediate values, the estimation (or calculation) of free testosterone is recommended. Moreover, the evaluation of free testosterone should always be considered in TDT patients, since iron overload and liver disease can interfere with sex hormone binding globulin (SHBG) levels. Due to the low accuracy and reliability of the commercial immunoassays, the direct estimation of the free fraction is not recommended; it is preferable to derive it from a formula including total testosterone, albumin, SHBG (Free & Bioavailable Testosterone Calculator is available at: https://www.issam.ch/freetesto.htm). Although no validated thresholds are available from the literature, free testosterone values <64 pg/mL increase the likelihood to correctly identify hypogonadism and may reasonably be considered pathologic [53-55]. Taking into account the considerable interassay and interlaboratory variability in testosterone measurements and the different cut-off values indicated by various guidelines [27], clinicians are encouraged to refer to their own laboratory reference ranges. The diagnosis of hypogonadism in a male adult patient requires the association of suggestive signs and symptoms with low testosterone levels, confirmed by two different samples correctly collected.

Patients with low concentrations of testosterone associated with high levels of gonadotropins are considered to have hypergonadotropic or primary hypogonadism (gonadal failure); in this case, testicular ultrasound is recommended. When low testosterone is associated with reduced or inappropriately normal levels of gonadotropins, the diagnosis is hypogonadotropic hypogonadism, due to hypothalamic-pituitary dysfunction; which requires assessment of other hormonal axes (morning cortisol, morning ACTH, FT4, TSH, IGF-1, prolactin) and a pituitary MRI with contrast medium (unless contraindicated), since patients might have multiple deficiencies that require management [40]. Furthermore, MRI-assessed pituitary volume might predict central hypogonadism, as a lower volume was described in hypogonadic TDT patients, and a

potential reversibility of hypogonadism through iron chelation optimisation and intensification has been suggested [31, 32, 35-39, 51, 56].

With regards to the consequences of hypogonadism on fertility, spermatogenic failure is a common consequence. Aspects related to fertility in male hypogonadism are discussed in Chapter 7.

5.3. Management and monitoring

All TDT patients with suspected hypogonadism should be referred to the endocrinologist for diagnosis, treatment planning, and monitoring, at least once a year.

Unless contraindicated, testosterone replacement therapy should be proposed to all patients with signs and symptoms suggestive of deficiency associated with consistent and unequivocal low morning testosterone concentrations. Replacement with testosterone aims to maintain secondary sexual characteristics and to correct symptoms, thereby improving psychophysical performance and quality of life. Furthermore, it allows halting the detriment of muscle and bone [40, 49].

In hypogonadal patients who plan fertility in the near term (6-12 months), the initiation of testosterone replacement is not advised as it could impair spermatogenesis; alternatively, gonadotropin therapy can be proposed if indicated (see Chapter 7). For the same reason, if the patient is in the fertile age, a semen analysis is recommended before starting testosterone.

Testosterone therapy is contraindicated in men with breast or prostate cancer, a palpable prostatic mass or nodule, or a prostate-specific antigen (PSA) level >4 ng/mL or >3 ng/mL combined with a high risk of prostate cancer (e.g., a first-degree relative with prostate cancer) (Table 7). We suggest assessing the risk of prostate cancer before starting testosterone in men aged >40 years; prostate examination and monitoring are generally not needed in younger patients, as the risk of prostate cancer is very low [50].

Table 7. *Contraindications to testosterone replacement therapy.*

General
• Plans of paternity in the next 6-12 months • Nodule/palpable prostatic mass or prostate cancer • PSA >4 ng/mL or PSA >3 ng/mL associated with high risk of prostate cancer (first-degree relative affected) • Severe untreated obstructive sleep apnoea • Severe obstructive disorders of the lower urinary tract • Cardiac decompensation • Breast carcinoma • Recent stroke or myocardial infarction (last 6 months)

Abbreviations: PSA, prostate-specific antigen.

Further contraindications to testosterone therapy include untreated severe obstructive sleep apnoea syndrome, severe obstructive symptoms of the lower urinary tract, uncontrolled heart failure, and recent (previous 6 months) stroke or myocardial infarction. In males with recognised thrombophilia, we suggest sharing the decision-making process with a specialist in coagulopathies and to discuss with the patient the potential benefits and risks of testosterone replacement therapy.

It is recommended to start replacement therapy with testosterone enanthate or propionate or with transdermal testosterone gel; longer lasting preparations should be avoided in patients who might need a fast change of the dose and can be better reserved to those who have already reached the steady state (Table 8). Treatment should start with a low dosage of testosterone, followed by gradual increase and adjustment based on clinical and hormonal responses as well side effects. The target serum concentrations of testosterone during treatment are in the mid-normal range.

Table 8. *Formulations available for testosterone replacement therapy.*

Parameter	Administration route	Advantages	Disadvantages
Enantate	Intramuscular Every 2-3 weeks	Short duration of side effects	Serum level fluctuations
Undecanoate	Intramuscular Every 10-14 weeks	No serum level fluctuations	Long duration of side effects
Testosterone gel	Transdermic Daily	No serum level fluctuations	Skin irritation, transfer to the partner

Replacement treatment in hypogonadal men is associated with improvement in libido, erectile function, and sexual activity, as well as increase in body hair and muscle and bone strength. Drug-related adverse events are rare with replacement doses of testosterone (Table 9); with skin oiliness or acne and breast tenderness being the most common ones. Although some case reports suggested an increased risk for venous thromboembolism in patients with pre-existing thrombophilia, there is no conclusive evidence that testosterone replacement in hypogonadal men with TDT is associated with a significant increase in venous thromboembolic or cardiovascular risk [57-64].

In hypogonadal men who have started testosterone therapy, it is recommended to test serum testosterone every 3 months until the target values are reached, afterwards every 6-12 months. Regular clinical evaluation of the patient is crucial to assess clinical response, compliance with the therapeutic regimen, and possible side effects.

In patients >40 years, it is recommended to monitor the risk of prostate cancer through PSA level and digital rectal examination, according to local guidelines. Patients with a progressive increase in PSA or with absolute PSA values >4 ng/mL or >3 ng/mL in the presence of a high risk for prostate cancer (a first-degree relative with prostate cancer) can be referred to a urologist [53, 54].

Table 9. *Side effects of testosterone replacement therapy.*

General
• Acne, seborrhoea • Gynaecomastia • Breast or prostate cancer progression • Impaired spermatogenesis • Worsening of sleep apnoea • Congestive heart
Formulation-specific

• Intramuscular administration
  - Local pain
  - Fluctuations in mood, libido, stamina (short-lived preparations)
  - Cough after injection (undecanoate)
• Transdermic administration
  - Local reaction

5.4. Other considerations and unmet needs

The cut-off levels of total and free testosterone still represent an area of uncertainty. One of the most controversial points concern the values suggested for starting replacement therapy, especially when deciding treatment in older patients or in the presence of cardiovascular morbidities or thrombophilia. These patients need to be carefully counselled before starting replacement therapy.

6. HYPOTHYROIDISM

6.1. Epidemiology

Hypothyroidism is one of the most frequent and relevant endocrine disorders in patients affected by haemoglobinopathies, especially TDT [65]. Prevalence of thyroid function disorders varies in different clinical studies, depending on age of the population studied, country of origin, adequacy of iron chelation therapy, and distinction in reporting overt vs subclinical disease [51, 66, 67]. The prevalence of thyroid dysfunction, mostly hypofunction or hypothyroidism, is reported in up to 20% of patients with TDT [68]. The disease generally occurs between the second and third decades of life and tends to increase with aging. Prevalence seems to be higher in females. It Is also directly correlated with inadequate treatment of iron overload management, thus underlining once again the importance of optimising iron chelation therapy to preserve thyroid function and global endocrine health [3, 30, 69]. Oral and combinatorial iron chelation regimens not only preserve but were also shown to reverse thyroid dysfunction (e.g., subclinical hypothyroidism) [3, 35, 36, 38, 39, 70].

Beyond iron overload, there are no clear predisposing factors related to the onset of thyroid disease in TDT, apart from family history. There are also some conflicting results regarding the impact of hepatitis C virus infection [71]. The impact of some drugs, such as amiodarone, on thyroid function must always be carefully evaluated in patients with TDT [72]. Patients with TDT

are at higher risk of secondary hypothyroidism, related to hypothalamic or pituitary damage from iron overload [73], unlike the general population where this manifestation is very rare. For all these reasons, it is essential that the evaluation of thyroid function and the diagnosis of thyroid dysfunction are shared within the multidisciplinary care team.

6.2. Clinical presentation and diagnosis

Symptoms and clinical signs of hypothyroidism include asthenia, reduced physical and psychological performance, constipation, depressed mood, and reduced quality of life. Unfortunately, most of these features may be associated with a diagnosis of thalassaemia itself, making regular screening of thyroid function mandatory in all patients with TDT [73].

The onset of thyroid dysfunction in TDT is usually slowly progressive, starting with subclinical hypothyroidism characterised by normal levels of FT4 and elevated TSH levels [74]. Subclinical hypothyroidism may progress towards a form of overt hypothyroidism (high TSH and low FT4 levels) or regress, even to normalisation of thyroid function, in particular if an improvement in iron overload is achieved or if drugs harmful for thyroid function are suspended. In primary hypothyroidism (overt and subclinical), assessment of anti-thyroperoxidase (anti-TPO) antibody assay is recommended to exclude concomitant chronic autoimmune thyroiditis, although rare in patients with thalassaemia, as well as thyroid ultrasound to evaluate the morphology of the gland.

Central (secondary) hypothyroidism is rarer, although there is an increasing number of new diagnoses, especially in older TDT patients. The diagnosis of central hypothyroidism is indicated by low FT4 levels and low or inappropriately normal TSH levels [73, 75]. Therefore, screening for thyroid dysfunction in subjects with TDT must always include the simultaneous evaluation of TSH and FT4 (Table 10), starting from the age of 9 years, unlike the general population, where only TSH measurement is indicated [73].

Table 10. *Thyroid function tests interpretation.*

Test	Normal range*	Overt hypothyroidism	Subclinical hypothyroidism	Suspected central hypothyroidism
TSH	0.38-4.2 mU/L	High	High	Low or inappropriately normal
FT4	0.89-1.7 ng/dL	Low	Normal	Low

**Reference range are indicative and may vary based on laboratory methods and kits adopted.*
Abbreviations: TSH, thyroid-stimulating hormone; FT4, free thyroxine.

In case of central hypothyroidism, it is necessary to exclude the coexistence of other deficits in hypothalamic-pituitary function (hypogonadism, hypoadrenalism, and impairment of the GH/IGF-1 axis) through the evaluation of the other hormonal axes and MRI of the hypothalamic-pituitary region if not contraindicated [73, 75].

6.3. Management and monitoring

Replacement therapy for primary hypothyroidism consists or oral administration of synthetic L-thyroxine, starting with low doses (25-50 mcg/day), to be further reduced (12.5 mcg/day) in case of pre-existing cardiac disease. Hormonal monitoring is required after two months of starting therapy and then every 6 months, with the aim of maintaining FT4 level within the normal range and TSH level <4 mU/L, while avoiding overdose with TSH suppression (i.e., TSH <0.5 mU/L) which increases the risk of atrial fibrillation and osteoporosis, both being already highly prevalent in TDT patients [73, 76, 77].

Treatment of overt hypothyroidism is mandatory in all patients [74, 78], with the aim of maintaining the TSH level within the normal range for age and avoiding over-treatment. Subclinical hypothyroidism often benefits from therapy, particularly in young adults, when TSH level >10 mU/L and/or in the presence of subjective symptoms [79]. However, the choice of initiating therapy must be evaluated on a case-by-case basis. In untreated patients with subclinical hypothyroidism, thyroid function must be monitored every 6 months, for early identification of progression to overt hypothyroidism [80]. Treatment for subclinical hypothyroidism is required in all patients seeking pregnancy or during pregnancy, with close hormonal monitoring (every 4 weeks) with the aim of maintaining TSH levels <2.5 mU/L before conception. During pregnancy, L-thyroxine dosage must be adjusted on the basis of the TSH value, with close hormonal monitoring (monthly test until the 20th week, then at least one more test between the 26th and 32nd weeks). After childbirth, the L-thyroxine dosage should be reduced to pre-conceptional levels and then adjusted based on the TSH and FT4 levels measured approximately 6 weeks after childbirth [81]. Treatment of subclinical hypothyroidism that was diagnosed in mid/advanced adulthood appears to be unbeneficial in older patients (over 70 years) and is contraindicated in patients over 80 years [80, 82, 83]. However, hormonal monitoring is always indicated for the possible evolution to overt hypothyroidism, which requires treatment at any age.

The aim of therapy in central hypothyroidism is normalisation of FT4 levels, since TSH levels cannot be used to assess for the response to therapy [75]. Formulation and dosage are similar to those indicated for primary hypothyroidism. In case of concomitant presence of hypocorticosurrenalism, corticosteroid replacement therapy must always be started before L-thyroxine supplementation.

Concomitant hormonal supplementation, such as with oestrogens and GH, may require a dosage increase of L-thyroxine. On the contrary, a reduction in the dosage of L-thyroxine should be considered if oestrogen or GH replacement therapy is stopped, or patients enter menopause.

Closer thyroid function monitoring (every 3 months) is required for patients on treatment with drugs detrimental to thyroid function, such as amiodarone.

In all patients with TDT, an assessment with thyroid ultrasound is recommended at diagnosis, to be repeated if abnormalities are detected (enlargement of the thyroid or palpable thyroid nodules). Thyroid nodules should be managed in accordance with local guidelines for the general population [73, 84, 85].

6.4. Other considerations and unmet needs

Although starting thyroid function screening at 9 years of age is suggested, the exact time to start screening is still controversial. Moreover, the indications for starting thyroid hormone supplementation in patients with subclinical hypothyroidism are still debated. Central hypothyroidism, which could possibly be more frequent in TDT than the general population due to hypothalamic-pituitary iron overload, should be kept in mind to avoid improper diagnosis and management.

Differentiated thyroid cancer cases are reported in recent literature but longitudinal evaluation that could give a real estimation about incidence of this emerging disease are limited [86, 87].

7. GLUCOSE METABOLISM DISORDERS

7.1. Epidemiology

Glucose metabolism disorders are also among the most frequent endocrine disorders in patients affected by TDT [70]; typically occurring in the second decade of life. Pre-existing iron overload, liver dysfunction, high BMI, and a family history for diabetes have been reported as risk factors for the development of the disease in TDT patients [30, 88].

Despite improved management in chelation and transfusion regimens, and the global reduction in the prevalence of endocrinopathies [3], some studies still report rates of glucose metabolism disorders as high as 24%, with variability between studies attributed to stark differences in study cohorts, management of iron overload, and access to high standards of care [89].

Early detection and treatment of glucose metabolism disorders are mandatory, as they affect cardiovascular and kidney functions, which could already be impaired in TDT patients [67, 90]. Furthermore, impaired glucose metabolism may highly impact general well-being and quality of life, especially considering the challenges posed by monitoring and care of these disorders [91].

7.2. Clinical presentation/progression and diagnosis

Impaired fasting glycaemia (IFG) and impaired glucose tolerance (IGT) are generally asymptomatic. TDT patients can develop the same symptoms of diabetes as the general population; however, diabetic ketoacidosis is rarer.

Screening for glucose metabolism disorders is recommended starting from 10 years of age [73]. However, the most accurate and feasible method is still a matter of debate. Performing oral glucose testing (OGTT) annually in all patients with TDT even in the presence of normal fasting glycaemia appears challenging in clinical practice, and indeed, it was reported that OGTT is not routinely performed as a screening test [92]. Fasting blood glucose is the most effective and simple method to screen patients regularly. The OGTT can be performed in cases of impaired fasting blood sugar levels or in conditions of severe or persistent iron overload or presence of other risk factors such as obesity [73].

The homeostasis model assessment for insulin resistance (HOMA-IR) index (fasting glucose in mmol/L x fasting insulin in mU/L, divided by 22.5) is a marker of insulin resistance. It has some limitations related to the absence of standardisation in insulin measurement and the lack of data demonstrating its predictive value for therapy response. However, since the HOMA-IR index can

be easily performed and the assessment of insulin resistance is a relevant feature to be detected, it is commonly indicated as a screening test in TDT patients [73]. Several online calculators exist (e.g., https://www.siditalia.it/homa-ir-index; with automatic calculation of the HOMA-IR index by entering fasting blood sugar in mg/dL and fasting insulin in µIU/ml).

Plasma concentration of C-peptide is recommended in patients with diabetes to complete the diagnosis, particularly in patients with catabolic signs (weight loss) and high glycaemia, to identify patients in the phase of insufficiency/exhaustion of pancreatic insulin secretion who have low/undetectable C-peptide.

The diagnostic criteria for glucose metabolism disorders are the same as in the general population, except for haemoglobin A1C (HbA1C), which must not be used in TDT patients. Monthly monitoring of fructosamine is indicated instead, although it is still not validated, not widely available, and has some methodological limitations especially in patients with hyperbilirubinemia. Some groups are working to validate and use albumin glycated serum values [93, 94], but no definitive recommendations can be made for TDT patients yet.

The diagnostic criteria of diabetes are:

- Glycaemia >200 mg/dL in presence of typical symptoms of the disease (polyuria, polydipsia, weight loss) on single random serum evaluation.
- Fasting blood glycaemia >126 mg/dL in at least two evaluations.
- Glycaemia >200 mg/dL two hours after OGTT.

IFG is defined as glycaemia between 100 and 126 mg/dL in fasting assessment while IGT is defined as glycaemia between 140 and 200 mg/dL during OGTT.

7.3. Management and monitoring

For TDT patients with a diagnosed glucose metabolism disorder, a healthy lifestyle (balanced diet, quality of sleep, regular physical activity, and smoking cessation) is mandatory.

Pharmacological treatment of diabetes mellitus (type 2) in patients with TDT must be individualised. Metformin is the first-choice oral hypoglycaemic drug used at the early stages of diabetes, before insulin dependence occurs [73, 95]. In patients treated with metformin, it is useful to check vitamin B12 concentrations (because its absorption can be reduced), particularly in case of worsening anaemia, and to have dose adjustment in case of renal dysfunction.

Other drugs can be added to metformin, including insulin, when necessary. The presence of typical symptoms of diabetes, persistent hyperglycaemia, hypercatabolic signs (weight loss), and low/undetectable C-peptide are findings of severe impairment of pancreatic insulin secretory capacity and indicate the need to start insulin treatment [96]. The recommended insulin therapy scheme is the basal/bolus regimen, as for non-thalassaemic patients with diabetes [97].

Continuous subcutaneous insulin infusion through an insulin pump is recommended in selected cases, such as in patients who fail to have good management of diabetes despite intensified and optimised multiple dose injection therapy, those who have severe or nocturnal hypoglycaemia, in paediatric patients with high insulin-sensitivity, in patients younger than two years, and in case of compromised lifestyle with multiple dose injection therapy [73].

New classes of drugs such as glucagon-like peptide-1 (GLP-1) receptor agonists, sodium-glucose cotransporter-2 (SGLT-2) inhibitors, and peptidyl peptidase-4 (DPP-4) inhibitors have showed greater efficacy not only in glucose metabolism control but also in cardiovascular and kidney protection in patients with type 2 diabetes. In particular, in patients with high cardiovascular risk, cardiac decompensation, or renal disease SGLT-2 inhibitors and GLP-1 agonists are recommended in non-thalassaemic patients, due to their proven cardiovascular benefit [97]. Unfortunately, no efficacy and safety data are available in patients with haemoglobinopathies and no specific recommendations can be made about these new drugs in this patient population.

In pregnant women, fasting blood glucose levels should be checked every month starting conception while OGTT should be done between 24 and 28 weeks of gestation, similar to the general population and following the specific criteria for the diagnosis of gestational diabetes, although without considering HbA1C which can be falsely abnormal in TDT patients [73].

In TDT patients with diabetes, an educational programme to improve patients' ability to perform adequate self-monitoring of blood glucose and to prevent and manage hypoglycaemia-related events, especially during insulin therapy, is crucial [90]. As for the general population, regular checks for micro- and macroangiopathic complications are recommended in TDT, especially considering the specific fragilities and comorbidities in TDT patients which require a holistic approach by all multidisciplinary team [98]. Importantly, adequate management of iron overload through oral or combinatorial regimens in TDT patients was shown to stabilise or reverse glucose metabolism disorders [37-39, 70].

7.4. Other considerations and unmet needs

As previously mentioned, methods of screening for glucose metabolism disorders and the use and validity of the HOMA-IR index and fructosamine have controversial aspects in TDT, while safety and efficacy data for new drugs (such as GLP-1 agonists or SGLT-2 inhibitors) in TDT are still awaited.

8. HYPOCORTICOSURRENALISM

8.1. Epidemiology

Hypocorticosurrenalism is an endocrine complication characterised by reduced corticosteroid production from adrenal disease (primary hypocorticosurrenalism) or hypothalamus/pituitary disease (secondary hypocorticosurrenalism) [73, 99]. In TDT patients, iron damage of the pituitary gland is probably more relevant than adrenal cortex disease.

Studies published so far report a prevalence rate ranging from 4% to 50%, due to differences in study cohorts and diagnostic methods [100, 101]. Diagnosis and monitoring of disease progression are major challenges in TDT patients, since the diagnostic criteria are only partially reliable, and markers of disease progression are not always fully applicable in patients with TDT [102].

8.2. Clinical presentation and diagnosis

Symptoms and clinical signs of hypocorticosurrenalism (asthaenia, reduced physical performance and sense of well-being, hypotension) overlap with those of TDT, so the diagnosis cannot be suspected relying on clinical features alone. A specific sign of suspicion of hypocorticosurrenalism is the onset of hyponatraemia and/or hypoglycaemia, which are not normally observed in patients with TDT [102].

Patients with undiagnosed hypocorticosurrenalism are at risk of developing an adrenergic crisis, a severe and life-threatening acute event characterised by hypotension, dyselectrolytaemia, and hypoglycaemia due to the inability to increase circulating levels of cortisol in stress conditions such as infections, acute events, or surgical interventions [103, 104].

Low levels of serum cortisol at 8 am, correctly assessed and in the presence of clinical signs and symptoms such as hypotension, asthaenia, fatigue, and dyselectrolytaemia, suggest the diagnosis of hypocorticosurrenalism and a stimulus test is required to confirm the diagnosis. Suggested dynamic tests are the ACTH test (low dose: 1 mcg or high dose: 250 mcg) or the glucagon test, to be chosen according to personnel experience and product availability at the centre [105]. Interestingly, glucagon is used to test both the adrenergic and GH axes; so, the need to rule out both hypocorticosurrenalism and GH deficiency may make the glucagon test the preferred one. A history of severe iron overload or poor compliance to iron chelation therapy, pre-existing multiple endocrinopathies especially hypothalamic-pituitary disorders, are relevant risk factors for the development of hypocorticosurrenalism [73, 102].

8.3. Management and monitoring

Treatment for hypocorticosurrenalism consists of the administration of glucocorticoids, in replacement doses [106]. Adrenal production is influenced by age, gender, and body composition with average values of approximately 5-8 mg/m2 per day, corresponding to an oral dose of about 15-25 mg per day of hydrocortisone or 20-35 mg of cortisone acetate in adults. As with other endocrine disorders, therapy must be individualised, especially based on the clinical response (quality of life, improvement of asthaenia and mood), blood pressure values, and serum electrolyte levels. Overtreatment must be avoided, especially in TDT patients considering the effects of corticosteroid use on bone tissue and metabolism [73].

To better reproduce the physiological adrenal function, therapy should be administered in two daily doses with a first administration at awakening followed by a second dose (approximately. 50% of the first one) early in the afternoon. Sometimes, some patients could experience better results with therapies divided in three daily doses with a third dose in the late afternoon, but not too close to evening rest time [40]. Usual treatment schedules consist of hydrocortisone, 10-15 mg/day or cortisone acetate 25-37.5 mg/day.

Patients and family members should be properly educated on risk factors to avoid and on how to promptly recognise an adrenergic crisis, such as doubling or tripling the usual dose in case of intercurrent disease, fever, vomiting, gastroenteritis, drop in blood pressure, or stressful events. During an acute crisis or in case of vomiting or diarrhoea, parenteral hydrocortisone therapy, usually intramuscular, is required [73].

Replacement therapy monitoring requires the assessment of symptoms and feelings of well-being, blood pressure, weight, and signs of overdosing. Laboratory monitoring involves measurement of serum sodium and potassium. In patients on cortisol replacement therapy, measurement of cortisol levels is not indicated [40, 107].

8.4. Other considerations and unmet needs

Currently, the most controversial point is the preferred stimulus test (low dose or high dose ACTH, glucagon) as reliability of the different tests still needs to be validated in TDT. Furthermore, cortisol levels used to define normality range in the general population are not fully applicable in TDT patients. Based on clinical experience, the authors recommend performing the stimulus test in all TDT patients with a serum cortisol level ≤10 mg/dL. In patients with serum cortisol values between 10 and 15 mg/dL, correctly measured and confirmed on two different assessments, the dynamic test should be considered on the basis of clinical signs and symptoms, history of iron overload, and the presence of other deficits in hypothalamic-pituitary function.

9. HYPOPARATHYROIDISM

9.1 Epidemiology

Hypoparathyroidism typically occurs in the second decade of life, with a highly variable prevalence rates reported in different studies, ranging from 0.3% up to 32%. Younger patients, especially those born after 1980, seem to be at lower risk of developing hypoparathyroidism, likely reflecting the detrimental impact of iron overload which is mitigated by improved iron chelation [3, 30, 38, 67, 70, 108].

9.2. Clinical presentation and diagnosis

Parathyroid hormone (PTH) plays a crucial role in serum calcium homeostasis. PTH deficiency impairs calcium bone resorption and intestinal calcium absorption, with the final result of hypocalcaemia and chronic hyperphosphataemia [109-111].

Hypoparathyroidism in patients with TDT is a chronic condition that develops progressively, so signs and symptoms can be very mild and overlap with those of the underlying disease, justifying the need for regular screening. However, chronic hypoparathyroidism can be associated with arrythmias such as QT prolongation; neuromuscular disorders such as paraesthesia of the face and extremities, muscle pain, and weakness; neuropsychiatric and cognitive disorders such as anxiety, depression, emotional lability, and concentration difficulty (brain fog); pathological calcifications in the kidneys (stones and nephrocalcinosis), brain (especially calcifications of the basal ganglia), joints, skin, eyes, and vessels [109, 110].

First level screening tests to rule out hypoparathyroidism include assessment of serum calcium and phosphorus. For the diagnosis of hypocalcaemia, ionised serum calcium is reported to be the most accurate assessment, but technical issues and lack of validation limit its use worldwide. Instead, assessment of serum calcium corrected for albumin is reliable and feasible to diagnose hypocalcaemia [109], based on the formula: corrected calcium = measured calcium + [(4.0 - albumin) x 0.8] [111, 112].

Postoperative hypoparathyroidism is due to surgical removal of the parathyroid glands or their postoperative devascularisation leading to a functional damage. The diagnostic criteria of postoperative hypoparathyroidism include a calcium concentration <8 mg/dL (<2 mmol/L) associated with a low or inappropriately normal PTH level (<15 ng/L) [110, 113]. We propose the same cut-off values for diagnosis of hypoparathyroidism in TDT as well, since the permanent functional damage to the parathyroid glands due to the toxic effect of iron overload is similar to the permanent vascular damage reported in postoperative hypoparathyroidism.

9.3. Management and monitoring

The ultimate goals of hypoparathyroidism treatment are the correction of hypocalcaemia, hyperphosphataemia, hypercalciuria, while avoiding hypercalcaemia, stones, and calcifications in the kidneys and other sites. The aim is to keeping serum calcium level within the low limits of normal range and the serum calcium-phosphorus ratio <55 mg^2/dL^2 (4.4 $mmol^2/L^2$) [109].

The occurrence of variable symptoms such as facial paraesthesia, muscle spasms and cramps, tetanic crises, laryngospasm, and convulsions may suggest an acute-onset hypocalcaemia requiring emergency intravenous administration of calcium gluconate therapy, integrated with oral administration of calcium and calcitriol [109, 111, 114].

Calcium carbonate is the first choice for calcium supplementation, because it contains 40% elemental calcium and is the most inexpensive. Calcium citrate, which is more expensive and contains 20% elemental calcium, can be used in case of achlorhydria or during treatment with proton-pump inhibitors or if side effects of calcium carbonate develop (e.g., constipation) [111].

The dosage of supplemental calcium needs to be tailored considering that higher single doses are associated with a higher risk of side effects without improvement in absorption. The initial therapeutic approach can be a daily dose of 1 to 2.5 grams, divided in 2 or 3 daily administrations, starting from a single dose of 500 mg of calcium; which can be tailored based on clinical and laboratory assessments [111, 112].

In hypoparathyroidism, renal conversion of 25-hydroxy (25OH) vitamin D to its active form is impaired and administration of the active form of vitamin D, calcitriol (1,25-dihydroxycholecalciferol), instead of vitamin D2 or D3 (ergocalciferol or cholecalciferol) is required. The use of higher doses of calcitriol is indicated to reduce the calcium dosages necessary to prevent hypocalcaemia [109, 111]. Calcitriol dosage can be increased by 0.25 micrograms, according to serum calcium assessment.

Although calcium and calcitriol supplementation prevent hypocalcaemia and other effects of hypoparathyroidism, PTH deficiency impairs renal calcium absorption leading to hypercalciuria with the risk of kidney stones, nephrocalcinosis, and renal failure [111]. Monitoring urinary calcium levels is of uttermost importance, with the ultimate goal of keeping urinary calcium levels within the normal range (<300 mg/day for men, <250 mg/day for women, and <4 mg/kg/day in general).

Hypercalciuria can be treated with thiazide diuretics which increase renal tubular reabsorption of calcium. Hydrochlorothiazide is a widely used drug, administered at a dose of 25-50 mg, in one or two daily doses. Besides serum calcium, during therapy with thiazide diuretics, it is required to monitor potassium and magnesium levels which may decrease due to their increased renal excretion. To prevent or treat hypokalaemia secondary to prolonged use of thiazide diuretics, amiloride can be used [111, 112].

Phosphorus binders along with a low phosphate diet are recommended in cases of sustained hyperphosphataemia (>6.5 mg/dL) not controlled by calcium supplementation [109]. Although generally calcium therapy reduces phosphorus levels, a low-phosphate diet should be recommended due to the PTH deficit [111].

Severe chronic hypoparathyroidism is characterised by inadequate control of hypocalcaemia despite high doses of calcium (>2.5 grams a day) and calcitriol (>1.5 micrograms a day), development of hypercalciuria and secondary complications (urinary stones, nephrocalcinosis, reduction of glomerular filtration), hyperphosphataemia, gastrointestinal absorption disorders, and reduced quality of life.

The US Food and Durg Administration (FDA) (2015) and European Medicines agency (EMA) (2017) approved the use of PTH intact analogue 1-84 for the treatment of hypoparathyroidism not controlled by conventional therapy, in the absence of contraindications (hypersensitivity to the active substance or to one of the excipients, ongoing or previous skeletal radiotherapy, malignant neoplasms of the skeleton or bone metastases, increased risk of osteosarcoma such as patients with Paget disease or with genetic bone diseases, and unexplained increase in bone alkaline phosphatase).

Around 70% of patients treated with PTH intact analogue 1-84 show an efficacy response (>50% reduction in oral calcium or calcitriol dose, normalised or stable calcium levels compared to baseline) without serious adverse events after 5 years of therapy [115, 116]. However, some authors do not recommend the use of PTH analogues in patients with hypoparathyroidism for several reasons such as the daily subcutaneous administration, the high costs, the need to continue therapy with calcium and vitamin D, and lack of data on the evolution of long-term complications. Furthermore, preclinical studies in mice showed an increased risk of osteosarcoma during the use of PTH analogues, although this risk is not confirmed in long-term studies in humans [109, 111, 117].

9.4. Other considerations and unmet needs

There is a suggestion that concomitant supplementation with calcitriol and oral formulations of vitamin D2 (ergocalciferol) or D3 (cholecalciferol) can help keep the serum level of 25OH vitamin D at the lower limits of normal with beneficial effects on skeletal and other body tissues. However, there is a substantial lack of scientific evidence to recommend the routine use of such combination [111].

10. GROWTH HORMONE DEFICIENCY IN ADULTS

10.1. Epidemiology

GH deficiency in adult patients with TDT is an emerging problem and it is reported in 8% and up to 44% of patients, depending on study cohort characteristics [3, 67, 70, 118, 119].

10.2. Clinical presentation and diagnosis

Signs and symptoms of GH deficiency in adults may overlap with features of TDT, such as reduced muscle strength and bone mass, along with general worsening of quality of life. Other metabolic effects of GH deficiency, such as an increase in visceral fat and altered lipid metabolism that is not otherwise explainable, may raise the clinical suspicion although their profile in TDT patients is not known [120].

IGF-1 level is the first biochemical screening test for GH deficiency. However, around 50% of thalassaemia patients have low IGF-1 values (2 SD below average values for healthy individuals) [119], which is primarily attributed to hepatopathy (due to iron accumulation and/or viral hepatitis), chronic anaemia, increase in inflammatory cytokines, malnutrition, and GH resistance. Thus, there is no current agreement on the ideal screening approach for GH deficiency in TDT. The authors suggest an annual evaluation of IGF-1 in all patients with TDT, starting from the second decade of life. In case of significantly reduced IGF-1 levels (2 SD below average values for healthy individuals) [121], a stimulus test for GH is indicated.

The reference GH stimulus test is the insulin tolerance test (ITT), but technical issues and safety concerns related to the potential of inducing severe hypoglycaemia limited its use in the USA, while in Europe it is still recommended [122]. A feasible and widely used test was the arginine test combined with the GH-RH analogue (GHRH-arginine test), which has been recommended as an alternative test for the detection of GH deficiency [120]. However, as mentioned in earlier sections, the manufacturing company interrupted its production in 2008 in the USA and in 2023 in Europe [8], so this test is no longer available worldwide [9]. Two alternative tests are now also available.

Macimorelin is a synthetic GH-releasing peptide approved in 2017 by the US FDA [123] and in 2019 by the EMA [124] for diagnostic use in adults with suspected GH deficiency. Data on diagnostic performance, safety, feasibility, and comparability to the ITT [125] and the GHRH-arginine tests [126], led the American Association of Clinical Endocrinologists to recommend the use of macimorelin as an alternative test for GH deficiency [9]. However, it is expensive with poor access in many countries and with various potential drug interactions. Moreover, a temporary interruption of marketing in the USA limited the widespread use of this test [8].

There is renewed interest in the use of glucagon stimulation test which has been considered as a possible alternative to GHRH-arginine and IIT testing, although its use is currently limited because of some disadvantages including intramuscular administration, the length of the test, the need for several samples, and relatively common side effects such as nausea, vomiting, and headaches ranging from <10% to 34% in frequency. Strengths include reproducibility, limited cost, safety, limited contraindications, and lack of influence by gender [122]. However, shortages similarly reported for this product preclude its wide implementation [8]. At the moment, it is not possible to recommend a specific test, considering the different availability of products and variable positions of scientific societies and institutions around the world. Local experience and resources are crucial in deciding which test to use, although implementation of procedures to perform provocation tests at the care centre may be demanding and challenging [67].

In case of GH deficiency, it is recommended to check overall pituitary function and to perform MRI of the hypothalamic-pituitary region.

10.3. Management and monitoring

The goal of therapy in adults with GH deficiency is improvement of cardiovascular function, physical exercise capacity, and quality of life, as well as increasing lean body mass and bone mineral density (BMD). GH replacement with rhGH is administered once a day, in the evening, subcutaneously. A common dose-dependent side effect is liquid retention; so, it is recommended to start with a low dose (0.2 mg/day in males and 0.3 mg/day in females) to be tailored based on clinical response, side effects, and IGF-1 levels which need to be maintained in the lower limits of the normal range for age and gender [40, 120]. However, this goal may be difficult to achieve in patients with TDT and a very low baseline IGF1 level.

10.4. Other considerations and unmet needs

GH deficiency is an emerging problem in patients with TDT that may impact numerous functions, particularly the cardiovascular system which may already be impaired due to the underlying disease. However, there are currently no clear or validated indications for screening, final diagnosis, or therapeutic monitoring in this patient population.

11. BONE DISEASE

11.1. Epidemiology

Bone disease has become a major healthcare concern in patients with TDT and non-transfusion-dependent β-thalassaemia (NTDT) due to the progressive aging of the thalassaemic population [127]. Thalassaemia-associated bone disease is a complex and multifactorial condition (Table 11), with various genetic and acquired factors involved in its pathogenesis [30, 91, 127].

Table 11. *Factors contributing to reduced bone mineral density and fragility fractures in transfusion-dependent β-thalassaemia. Modified with permission from [127].*

Genetic variants predisposing to reduced BMD and fragility fractures	
Collagen type Ia1 (COLIA 1) gene (*Sp1* polymorphism)	
Vitamin D receptor (*FokI* and *BsmI* polymorphisms)	
Acquired factors contributing to reduced BMD and fragility fractures	
Primary disease	Bone marrow expansion (ineffective erythropoiesis)
	Iron overload
Secondary factors	Endocrine • Hypogonadism (delayed puberty, secondary hypogonadism) • Growth hormone and insulin-like growth factor-1 deficiency • Diabetes mellitus • Thyroid and parathyroid dysfunction
	Nutritional • Vitamin D deficiency • Zinc, vitamin C, and vitamin K deficiency
	Reduced physical activity
	Chronic liver disease
	Renal tubular disease (renal phosphate wasting and hypercalciuria)
	Iron chelators (hypercalciuria)
	Sarcopaenia and falls

Abbreviations: BMD, bone mineral density.

Abnormalities in BMD and microarchitecture occur early in children and adolescents, due to an imbalance in bone remodelling, leading to suboptimal peak bone mass. With aging, bone loss and deterioration of bone microarchitecture progression produces an increase in fracture risk, causing fragility fractures and spine deformities [91, 128, 129].

Even with effective iron chelation and optimal transfusion regimens, the reported prevalence of osteoporosis and fragility fracture in TDT/NTDT patients is extremely high, with some reports describing a prevalence of bone disease in up to 90% and fragility fractures in up to 40% of β-thalassaemia patients [91, 127, 129-131]. A recent meta-analysis described a higher pooled prevalence of fragility fractures in patients with β-thalassaemia (17%, 95% confidence interval [CI]:16%-19%) and TDT (18%, 95%CI: 16%-19%), compared to patients with α-thalassaemia (4%, 95%CI: 2%-6%) and NTDT (7%, 95%CI: 4%-10%) [129].

Fragility fractures are more frequent in patients aged 20 years or older compared to younger subjects, with a different distribution of fractures according to site and age class: upper extremities fractures occur more frequently in adolescents and young adults, while lower extremities and spine fractures increase with age [127, 132].

11.2. Clinical presentation and diagnosis

According to the World Health Organization (WHO), osteoporosis is defined as "a disease characterised by low bone mass and microarchitectural deterioration of bone tissue, leading to enhanced bone fragility and a consequent increase in fracture risk" [133]. Thus, there are no clinical manifestations of osteoporosis until a patient has a fracture.

Signs and symptoms associated with thalassaemia-related bone disease correspond to direct or indirect manifestations of fragility fractures. These include [134]: height loss, back pain, and kyphosis as a consequence of vertebral fractures; dyspnoea and constipation in severe thoracic kyphosis; and significant pain associated with vertebral and non-vertebral fractures, even if asymptomatic, clinically inconspicuous vertebral fractures are frequent.

If not appropriately treated, bone mass and microarchitecture deterioration progress and fragility fractures occur. After a first major osteoporotic fracture (MOF), the risk of a subsequent MOF is dramatically increased (imminent fracture risk), while it thereafter decreases with time although it remains higher than the baseline population risk [135]. Cumulative vertebral and non-vertebral fractures produces a significant reduction in quality of life and may lead to disability and overall increased mortality in vulnerable and multimorbid patients.

The standard evaluation of TDT patients at risk of osteoporosis and fragility fractures should be based on [91, 127]:

- Measurement of BMD by DXA.
- Assessment of fracture probability to identify patients at high risk of fracture for whom pharmacological treatment is appropriate.
- Identification of potential modifiable risk factors and secondary causes of bone fragility, such as vitamin D deficiency or hypogonadism.

Annual checks of BMD by DXA starting in adolescence, or earlier (starting 10 years) if feasible, is warranted. Healthy subjects with baseline BMD values within the reference range (according

to Z-score), may undergo less frequent measuring (e.g., every two or three years) according to the clinical course of the disease. DXA is the gold standard for the measurement of BMD. It is a non-invasive technique and can be performed at the hip (femoral neck [FN] and total hip [TH]), lumbar spine (LS), forearm, and total body less head (TBLH). According to the official positions of the International Society for Clinical Densitometry, the LS and TBLH are the preferred skeletal sites for bone mineral content and BMD measurements in children and adolescents [136]. In adults, the LS, FN, and TH are the favoured sites for BMD assessment, while the forearm should be considered under particular circumstances [137]. In TDT patients, the FN-BMD has been shown as the most appropriate and informative skeletal site for long-term follow-up [138].

BMD measurements are expressed in comparison to established normative data, as SD scores. The scores represent the number of SDs from the expected normal values of BMD. The age-matched SD score is commonly referred as the Z-score, while the young normal SD score has been labelled as the T-score. The WHO has defined the diagnostic threshold for postmenopausal osteoporosis on the basis of the T-score, defining osteoporosis with a BMD 2.5 or more SDs below young normal (T-score ≤ −2.5). Therefore, the T-score should be considered for BMD reporting in postmenopausal women and in men aged 50 years and older [137]. In TDT females before menopause and in males younger than 50 years, it is better to consider the Z-score rather than the T-score (this is particularly important for children and adolescents), with a Z-score of -2.0 or lower defined as 'below the expected range for age' [136, 137].

Assessment of fracture probability is based on integration of information about fracture risk provided by clinical risk factors (CRFs) for fracture and BMD [127], by using algorithms developed to estimate the probability of hip fracture and MOF, such as the Fracture Risk Assessment Tool (known as FRAX, https://frax.shef.ac.uk/FRAX/). Traditional CRFs considered include age, gender, BMI, prior fragility fracture, parental history of hip fracture, current smoking, ever use of glucocorticoids, rheumatoid arthritis, other causes of secondary osteoporosis, and alcohol intake of three or more units daily; but they may vary depending on the assessment tool used including also, for example, the risk of falls. In patients aged 40 years and older, CRFs assessment should be performed at each patient visit, and at least once a year (with BMD assessment by DXA).

All TDT patients should undergo, at baseline and possibly on a yearly basis, a laboratory diagnostic workup to identify conditions and risk factors, related to thalassaemia or not, that may potentially contribute to bone loss and increase fracture risk [127]. Laboratory testing should include the measurement of serum calcium, phosphate, creatinine, albumin and protein electrophoresis, PTH, 25-OH vitamin D, 24-hour urinary calcium, creatinine, and phosphate excretion, as well as laboratory tests to assess gonadal function. The evaluation of markers of bone turnover (MBTs) should not be considered in routine clinical practice and should be interpreted by a 'bone specialist' [139]. Finally, targeted laboratory tests should also be considered in the presence of signs or symptoms indicating a specific disease.

Additional or more invasive testing may be performed in specific situations, following the baseline assessment, and under the supervision of a skilled 'bone specialist'. These may include [127]: peripheral quantitative computer tomography, quantitative morphometry of the vertebrae on lateral views of the spine using conventional X-ray or DXA, or bone histomorphometry after tetracycline double labelling.

11.3. Management and monitoring

Due to its complexity, patients presenting with thalassaemia-associated bone disease require a comprehensive and multidisciplinary approach with the contribution of several healthcare providers [127]. The therapeutic approach should include general non-pharmacologic measures, and pharmacological treatment for patients at high risk of fracture.

General measures for osteoporosis and fracture prevention include an adequate control of anaemia and iron overload (experience with oral iron chelation indicted prevention or reversal of bone disease [38, 70]), prompt identification and treatment of underlying comorbid conditions (e.g., diabetes), healthy nutrition, regular physical exercise, avoidance of detrimental lifestyle factors (discourage smoking and alcohol consumption), appropriate dietary calcium intake (800-1500 mg/day depending on age and gender), vitamin D supplementation and, when indicated, hormone replacement therapy for hypogonadism [127]. Clinical conditions potentially associated with bone loss and increased fracture risk should be treated and removed when possible (e.g., diabetes, GH deficiency, hypogonadism). Due to the progressive aging of the thalassaemic population, sarcopenia and falls have also become a major clinical concern, being associated with increased risk of fracture and disability [130]. Therefore, intervention to prevent falls and to improve muscle strength, mass, and physical performance should be implemented in all patients at risk.

Vitamin D treatment and/or supplementation plays a key role in the management of TDT patients, given the highly reported prevalence of marked vitamin D deficiency (25OH vitamin D <10 ng/mL) or insufficiency (25OH vitamin D <30 ng/mL) in this population. Table 12 depicts recommendations for 25OH vitamin D assessment and cholecalciferol supplementation in adults with TDT [91, 140]. As shown, cholecalciferol should be the treatment of choice, daily oral doses are preferred, and assessment of risk factors for vitamin D deficiency should drive the therapeutic approach [140].

The experience with pharmacological agents in the management of thalassaemia-related bone disease and fracture prevention is mainly confined to bisphosphonates and denosumab [127, 141, 142], with randomised-controlled trials (RCTs) demonstrating their efficacy on surrogate and secondary outcomes (BMD and MBTs). Very limited clinical data are available about the use of teriparatide and strontium ranelate (no more available), while there are currently not published data with abaloparatide and romosozumab treatment.

Alendronate (10 mg daily, oral), neridronic acid (100 mg every three months, intravenous), and zoledronic acid (4 mg every three or six months, intravenous) have been shown, in well-designed RCTs, to significantly reduce bone turnover and significantly increase BMD versus placebo/no-therapy [127]. Moreover, both neridronic acid and zoledronic acid demonstrated reduction in bone and back pain, improving quality of life. A recent RCT of denosumab (60 mg every six months, subcutaneous) versus placebo demonstrated beneficial effects on bone turnover, BMD, and pain comparable to those reported with bisphosphonates [142]. The small samples included, the short duration of the studies (1 year to 3 years), and the low incidence of fragility fractures did not allow their potential efficacy on fracture prevention to be established. However, it should be emphasised that RCTs of bisphosphonates and denosumab in thalassaemic patients produced improvements in the BMD comparable to those reported in postmenopausal women, for whom anti-fracture efficacy have been established [127]. In all studies, these pharmacological agents demonstrated to be safe and well-tolerated, leading only expected and self-limiting adverse events [143].

As noted, experience with teriparatide (20 mcg daily, subcutaneous, for a maximum of 24 months) is limited to case reports/case series, which reported improvements in BMD, but a high frequency of adverse events (muscle and bone pain) leading to discontinuation [144].

Table 12. *Recommendations for 25-hydroxy vitamin D assessment and cholecalciferol supplementation in adults with transfusion-dependent β-thalassaemia. Modified with permission from [91] and [140].*

Basal 25OHD value*	Cumulative therapeutic dose†	Daily maintenance dose‡	Frequency of 25OHD assessment
Optimal 30-50 ng/mL (75-100 nmol/L)	None	800-1,000 IU	Not necessary
Insufficiency <30 ng/mL (75 nmol/L)	300,000 IU	800-1,600 IU	Annually
Marked deficiency <10 ng/mL (25 nmol/L)	300,000-600,000 IU	1000-2,000 IU	Every 6 months

**Serum 25OHD should be measured at baseline in all adults with transfusion-dependent β-thalassaemia.*
† The cumulative therapeutic dose should be distributed over a period of 1-3 months, with oral daily doses of 3,000-10,000 IU/day recommended.
‡ The recommended daily dose should be defined on the basis of baseline 25OHD and the presence of risk factors for vitamin D deficiency (age, inadequate sun exposure, obesity, pregnancy and breast feeding, vegan diet, anorexia nervosa, chronic renal failure, cancer, type II diabetes, intestinal malabsorption and bariatric surgery, or drugs that interfere with vitamin D metabolism).
Abbreviations: 25-hydroxy vitamin D, 25OHD.

11.4. Other considerations and unmet needs

Recently, radiofrequency echographic multi spectrometry (REMS) has been proposed as an innovative radiation-free, portable technology, which can be used for the assessment of BMD in alternative to DXA [145]. Although there are no published studies investigating the potential benefits of this technology in patients with thalassaemia, the non-ionising radiation emitted, its ability to overcome overestimation of BMD measurements by DXA in specific conditions (e.g., spine osteoarthritis), its relatively low cost, and the possibility to also measure bone quality (in addition to the quantitative measure of BMD by DXA), represent potential advantages to support the use of REMS as an alternative to DXA.

In light of recent reports describing the occurrence of osteonecrosis of the jaw or atypical subtrochanteric/femoral shaft fractures in thalassaemic patients while on bisphosphonate therapy, and considering that there is no experience of bisphosphonate treatment greater than 3 years, it is advisable t to not exceed 36 months of bisphosphonate therapy in patients with thalassaemia-associated osteoporosis, and eventually to reconsider the treatment (with bisphosphonates or denosumab) after a period of 'drug-off holiday' and on the basis of individual fracture risk [127].

KEY POINTS AND RECOMMENDATIONS

1. Recommendations for height and growth disorders.

- **First level assessment:**
  - The recommended screening tests for growth disorders in patients with transfusion-dependent β-thalassaemia (TDT) include complete clinical and auxological evaluation (weight, height, body mass index [BMI], height when sitting, growth rate, Tanner stage), plotting all values on appropriate centile growth velocity charts for age and sex and in relationship to familial/genetic target, performed every six months, starting from patient first access at the treatment centre, until adult height achievement and completion of pubertal development (**Grade A, Class I**).
- **Criteria for diagnosis:**
  - The diagnosis of a height growth disorder should be based on the following criteria (**Grade A, Class I**):
    - Severe short stature (stature ≤ -2.5 standard deviations [SD]).
    - Stature ≤ -1.5 SD compared to the family target and growth rate ≤ -2 SD or ≤ -1.5 SD after 2 consecutive years.
    - Stature ≤ -2 SD with growth rate ≤ -1 SD evaluated at least 6 months apart or stature reduction of 0.5 SD in one year in children over 2 years old.
    - Growth rate ≤ -2 SD in one year or ≤ -1.5 SD in two consecutive years, even if stature is normal.
- **Confirmatory, stimulus, or second level tests:**
  - In case of a growth disorder, it is recommended to evaluate disease management (reassess pretransfusion haemoglobin and iron indices) and to perform a general assessment (phlogosis indicators, hepatic and renal function, electrolytes, blood gases, total proteins with electrophoresis, physical and chemical examination of urine, screening for celiac disease, free thyroxine (FT4), thyroid-stimulating hormone (TSH), calcium, phosphorus, alkaline phosphatase, parathyroid hormone, and vitamin D) (**Grade C, Class I**).
  - If the above tests are normal and/or growth disorders persist, it is recommended to perform insulin-like growth factor 1 (IGF-1) measurement and dynamic tests to evaluate growth hormone secretion (**Grade C, Class I**).
  - A diagnosis of growth hormone (GH) deficiency should be made when GH peak is <8 ng/mL on two different stimulus tests (glucagon and clonidine), performed on different days (**Grade C, Class 1**).
- **Management and monitoring:**
  - Before starting therapy with recombinant human GH (rhGH), it is recommended to evaluate glucose metabolism, thyroid and pituitary function (adrenocorticotropic hormone [ACTH], cortisol, FT4, and TSH; and in puberty luteinising hormone [LH], follicle stimulating hormone [FSH], and total testosterone/oestradiol) and to carry out magnetic resonance imaging (MRI) of the hypothalamic-pituitary region (**Grade A, Class I**).
  - It is recommended to optimise transfusion therapy, iron chelation therapy, and nutritional status, and to correct endocrine diseases if present (hypothyroidism, impaired glucose homeostasis, pubertal delay) (**Grade A, Class I**).

- For patients diagnosed with GH deficiency, the recommended rhGH dose is 0.025-0.035 mg/kg/day (0.160-0.40 mg/kg/week) subcutaneously in a single evening dose (**Grade A, Class I**).
- For patients treated with rhGH, it is recommended to monitor patients with auxological evaluation, IGF1, and glycemia every 6 months; assessment of lipid profile, other pituitary hormones, thyroid function, and bone age every year (**Grade A, Class I**).
- In the transition age, it is recommended to repeat the dynamic tests in patients treated with rhGH therapy in childhood, at least one month after rhGH treatment interruption, in order to evaluate the need to continue (or not) the therapy at an age-appropriate dosage (**Grade B, Class I**).
- Referral to paediatric endocrinologist, if available, is recommended for patients with growth disorders, suspected GH deficiency or other endocrinopathy, confirmed GH deficiency and/or other hormone deficiency for initiating and monitoring treatment (**Grade C, Class I**).

2. Recommendations for disorders of pubertal development.

- **First level assessment:**
  - The recommended screening tests for disorders of pubertal development in TDT are the measurements of testicular volume, growth rate, and Tanner stage to be performed every six months, starting from the normal age of puberty, until the completion of pubertal growth (**Grade A, Class I**).
- **Criteria for diagnosis:**
  - A diagnosis of a disorder of pubertal development should be made in male patients showing no signs of pubertal development (testicular volume <4 mL) after 14 years of age and in patients with lack of pubertal progression for a period of 6-12 months after a spontaneous beginning of puberty. In female patients, it should be based on lack of thelarche after 13 years of age, or on lack of pubertal progression for a period of 6-12 months after a spontaneous beginning of puberty, or with non-appearance of menarche 4 years after the appearance of thelarche, or with non-appearance of menarche by the age of 16 (**Grade A, Class I**).
- **Confirmatory, stimulus, or second level tests:**
  - In case of disorder of pubertal development, it is recommended to perform FT4, TSH, prolactin, IGF-1, LH, FSH, bone age assessment, as well as total testosterone in male patients and 17-beta oestradiol and pelvic ultrasound in female patients (**Grade A, Class I**).
  - In all patients with failure to begin pubertal development, by the age of 13 years in females and 14 years in males, a short course of 3-6 months of hormone therapy is recommended (oestrogens in females and testosterone in males), followed by a treatment-free period with clinical and laboratory re-evaluation, in order to differentiate constitutional growth and puberty delay (CGPD) from hypogonadotropic hypogonadism (**Grade A, Class I**).
  - In male patients with pubertal delay, the recommended pubertal induction therapy is testosterone enanthate, propionate, or cypionate in depot formulation to be administered every 4 weeks with an initial dose of 50 mg, for a period of 3-6 months,

after the chronological age of 14 years and a bone age of at least 12 years (**Grade A, Class I**). In case of non-response to the first cycle, it is suggested to consider a second cycle of treatment with a dose increase of 25-50 mg (**Grade C, Class IIa**).

- In female patients with pubertal delay, the recommended pubertal induction therapy is low dose 17-beta oestradiol, preferably transdermal formulation, after the chronological age of 13 years and bone age of at least 11 years (**Grade A, Class I**).
- The diagnosis of A diagnosis of CGPD in male or female patients with a disorder of pubertal development should be made in view of low levels of LH, FSH, and sex steroids with a recovery of pubertal progression after 1 or 2 cycles of pubertal induction with low-dose hormones (**Grade A, Class I**).
- The diagnosis of hypogonadotropic hypogonadism in male or female patients with a disorder of pubertal development should be made in view of reduced growth rate, low levels of LH, FSH, and sex steroids with lack of spontaneous pubertal progression after 1 or 2 cycles of pubertal induction with low-dose hormones (**Grade A, Class I**).
- In case of diagnosis of hypogonadotropic hypogonadism, it is recommended to perform a pituitary MRI to complete the diagnosis (**Grade A, Class I**).

- **Management and monitoring:**
  - In case of diagnosis of CGPD with spontaneous beginning of puberty, a wait and see strategy is recommended in concert with the patient and his/her parents, with periodic clinical and laboratory reassessment to be carried out every 3-6 months (**Grade A, Class I**).
  - In case of diagnosis of hypogonadotropic hypogonadism, it is recommended to start therapy no earlier than the chronological age of 14 years or bone age of 12 years in males, and no earlier than the chronological age of 13 years or bone age of 11 years in females (**Grade A, Class I**).
  - In male patients with hypogonadotropic hypogonadism, it is recommended to increase the testosterone dose gradually until reaching the adult dose for 3-4 years (**Grade A, Class I**).
  - In male patients with hypogonadotropic hypogonadism, it is suggested to consider the use of gonadotropins, as an alternative to testosterone, only for the induction of testicular growth, starting from the age of 14 years (**Grade C, Class IIb**).
  - In female patients with hypogonadotropic hypogonadism, it is recommended to add to a progesterone preparation at the appearance of induced menstrual flow or 24-36 months after the beginning of oestrogen therapy (**Grade A, Class I**).
  - In male patients on testosterone therapy, it is recommended to monitor the progression of puberty stage, height growth, levels of circulating total testosterone, and possible therapy side effects at least every 3-6 months, and bone age once a year (**Grade A, Class I**).
  - In female patients on oestrogen therapy, it is recommended to monitor the progression of puberty stage, height growth, dimensions of uterus and ovaries with an ultrasound, and possible therapy side effects at least every 3-6 months, and of bone age once a year (**Grade A, Class I**).
  - It is recommended to refer all patients with pubertal delay to a paediatric endocrinologist both for diagnosis and management (**Grade C, Class I**).
  - It is recommended to optimise transfusion and iron chelation therapy to prevent delated puberty in TDT patients (**Grade C, Class I**).

3. Recommendations for female hypogonadism.

- **First level assessment:**
  - The recommended screening test for hypogonadism in female patients is the assessment of menses rhythm every 6 months, through the evaluation of the patients' menstrual diary (**Grade B, Class I**).
  - A diagnosis of oligomenorrhoea should be made in case of <9 menstrual cycles/year, primary amenorrhoea in case of failure to reach menarche by the age of 16 years, and secondary amenorrhoea in case of absence of menses for ≥3 months in a woman who previously had regular menses or absence of menses for ≥6 months in women who previously had irregular menses (**Grade A, Class I**).
- **Confirmatory tests:**
  - In case of amenorrhoea or oligomenorrhoea, it is recommended to perform the following tests to complete the diagnosis: FSH, LH, and oestradiol serum levels and pelvic ultrasound. In addition, hyperprolactinaemia, hyperandrogenism, hypothyroidism, and pregnancy must be excluded by appropriate tests (**Grade A, Class I**).
- **Criteria for diagnosis:**
  - A diagnosis of hypogonadism should be made in adult female patients with oligo/amenorrhoea associated with low oestradiol levels, confirmed by at least two different tests (**Grade A, Class I**).
  - A diagnosis of hypergonadotropic or primary hypogonadism (ovary disease) should be made when low serum oestradiol is associated with high gonadotropin levels (**Grade A, Class I**).
  - A diagnosis of hypogonadotropic hypogonadism (hypothalamic/pituitary disease) should be made when low serum oestradiol is associated with reduced or inappropriately normal gonadotropin levels (**Grade A, Class I**).
  - Patients with primary or secondary amenorrhoea or oligomenorrhoea should be referred to an endocrinologist as well as a gynaecologist, if available, for initial diagnostic assessment and treatment, then once a year for follow-up (**Grade C, Class I**).
- **Second level tests:**
  - In patients diagnosed with hypogonadotropic hypogonadism, it is recommended to expand hormonal assays to assess morning cortisol, morning ACTH, FT4, TSH, and IGF-1, and to perform pituitary MRI with a contrast agent (unless contraindicated) (**Grade A, Class I**).
- **Management and monitoring:**
  - It is recommended to refer hypogonadic patients to the gynaecologist before beginning hormone replacement therapy (HRT), then once a year for follow-up, if available (**Grade C, Class I**).
  - It is recommended to start HRT in all patients with a diagnosis of hypogonadism in the premenopausal age, if not contraindicated (**Grade A, Class I**).
  - It is recommended to use the combination of oestradiol (ideally transdermal) and a cyclic progestin for HRT; cyclic progestin is not necessary in hysterectomised patients (**Grade A, Class I**).
  - If thrombotic risk factors are present, it is suggested to have shared decision-making on the risk-benefit ratio of HRT and on the potential use of an anticoagulants or antiaggregants with a specialist in coagulopathies (**Grade C, Class IIa**).

- During oestroprogestin HRT, it is recommended to perform the following assessments: blood pressure, liver and kidney function, blood lipids and glycemia; annual gynaecological examination with pelvic ultrasound, to be brought forward if unexpected vaginal bleeding appears, according to the age-appropriate guidelines (**Grade A, Class I**).
- In case of hypothyroidism on levothyroxine therapy, it is recommended to check FT4 and TSH levels every 3 months after the beginning of HRT (**Grade A, Class I**).
- It is recommended to optimise transfusion and iron chelation therapy (oral or combinatorial regimens) to prevent or potentially reverse hypogonadism in TDT patients (**Grade C, Class I**).

4. Recommendations for male hypogonadism.

- **First level assessment:**
  - The recommended screening for hypogonadism in male patients includes medical history, clinical evaluation, assays of serum testosterone, FSH, and LH (**Grade A, Class I**).
  - All adult TDT patients should undergo screening once a year, to be brought forward if suggestive symptoms appear (**Grade C, Class I**).
- **Criteria for diagnosis:**
  - A diagnosis of hypogonadism should be made in case of suggestive signs and symptoms associated with low testosterone levels, confirmed by two different tests collected correctly (**Grade A, Class I**).
  - A diagnosis of hypogonadotropic hypogonadism (hypothalamic/pituitary disease) should be made in case of low serum testosterone associated with reduced or inappropriately normal gonadotropin levels (**Grade A, Class I**).
  - A diagnosis of hypergonadotropic or primary hypogonadism (testicular disease) should be made in case of low serum testosterone associated with high gonadotropin levels (**Grade A, Class I**).
  - Adult patients with hypogonadism should be referred to an endocrinologist, if available, for initial diagnostic assessment and treatment, then once a year for follow-up (**Grade C, Class I**).
- **Second level tests:**
  - In patients diagnosed with primary hypogonadism, a testicular ultrasound is recommended (**Grade A, Class I**).
  - In patients diagnosed with hypogonadotropic hypogonadism, it is recommended to expand hormonal assays to assess morning cortisol, morning ACTH, FT4, TSH, IGF-1, prolactin, and to perform pituitary MRI with a contrast agent (unless contraindicated) (**Grade A, Class I**).
- **Management and monitoring:**
  - In the absence of contraindications, it is recommended to initiate replacement therapy with testosterone to all patients with clinical manifestations of androgen deficiency and confirmed low testosterone levels (**Grade A, Class I**).
  - Before starting treatment, it is recommended to: examine the prostate and breasts, explore cardiovascular and venous thromboembolism risk factors, and perform a semen analysis (in patients in the fertile age) (**Grade A, Class I**).

- In patients operated for prostate cancer with negative follow up >1 year, the risks can be discussed with the urologist and only patients at low risk of recurrence should be treated (preoperative prostate-specific antigen [PSA] <10 ng/mL, Gleason <8; stage pT1-2) (**Grade A, Class I**).
- In patients with cardiovascular disease undergoing testosterone replacement therapy, it is recommended to optimise secondary prevention measures (**Grade A, Class I**).
- In patients with recognised factors of thrombotic risk, it is recommended to evaluate the risk-benefit ratio of testosterone therapy with a specialist in coagulopathies, if available (**Grade C, Class I**).
- It is recommended to avoid testosterone replacement therapy in the following cases (**Grade A, Class I**):
  - Plans of paternity in the next 6-12 months.
  - Nodule/palpable prostatic mass or prostate cancer.
  - PSA >4 ng/mL or PSA > 3 ng/mL associated with high risk of prostate cancer (first-degree relative affected).
  - Severe untreated obstructive sleep apnoea.
  - Severe obstructive disorders of the lower urinary tract.
  - Cardiac decompensation.
  - Breast carcinoma.
  - Recent stroke or myocardial infarction (last 6 months).
- It is recommended to start treatment with testosterone enanthate or propionate or testosterone transdermal gel administered at low dosages, and to adjust the dose according to clinical and hormonal responses (**Grade C, Class I**).
- It is recommended to reserve longer lasting preparations for patients who have reached target testosterone levels, avoiding their use in those who might need a rapid dosage change and in older patients (**Grade C, Class I**).
- It is recommended to test serum testosterone every 3 months until normal values are reached, then every 6 months (**Grade C, Class I**).
- It is recommended to monitor heart symptoms and compensation and maintain testosterone serum levels within mid-normal range, in patients with chronic cardiac failure or cardiovascular disease (**Grade C, Class I**).
- It is recommended to monitor PSA, starting from the age of 40 years, and to monitor the risk of prostate cancer in compliance with local guidelines (**Grade A, Class I**).
- Patients with a progressive increase in PSA during testosterone replacement therapy or with absolute PSA values >4 ng/mL or > 3 ng/mL in the presence of a high risk for prostate cancer (a first-degree relative with prostate cancer) can be referred to the urologist (**Grade C, Class IIa**).
- It is recommended to optimise transfusion and iron chelation therapy (oral or combinatorial regimens) to prevent or potentially reverse hypogonadism in TDT patients (**Grade C, Class I**).

5. Recommendations for hypothyroidism.

- **First level assessment:**
  - The recommended screening tests for hypothyroidism are the measurement of serum FT4 and TSH levels (**Grade A, Class I**).
  - Screening for hypothyroidism can start from 9 years of age, once a year, in all patients with normal thyroid function and every six months in patients with suboptimal iron overload management (**Grade A, Class IIa**).
  - It is suggested to perform screening for hypothyroidism in women who seek pregnancy or are planning to undergo assisted fertilisation, to be repeated during pregnancy once a month until the 20th week and once again between the 26th and the 32nd weeks of pregnancy (**Grade A, Class IIa**).
  - It is recommended to perform screening for hypothyroidism every three months in patients that are receiving drugs interfering with thyroid function, in particular amiodarone (**Grade A, Class I**).
- **Criteria for diagnosis:**
  - A diagnosis of primary subclinical hypothyroidism should be made in case of normal FT4 and high TSH levels, confirmed with repeat testing after 2-3 months (**Grade A, Class I**).
  - A diagnosis of primary overt hypothyroidism should be made in case of low FT4 and high TSH levels (**Grade A, Class I**).
  - A diagnosis of central (secondary) hypothyroidism (hypothalamic-pituitary dysfunction) should be made in case of low FT4 and low or inappropriately normal TSH levels, confirmed at least on two tests and after excluding possible interferences (**Grade A, Class I**).
- **Second level tests:**
  - In patients with primary hypothyroidism, it is recommended to assess at diagnosis anti-thyroperoxidase (anti-TPO) antibody levels and to perform a thyroid ultrasound, to be repeated according to clinical indication (**Grade A, Class I**).
  - In patients with central hypothyroidism, it is recommended to perform at diagnosis the following tests: morning cortisol, morning ACTH, LH, FSH, prolactin, IGF-1, as well as testosterone in males and oestradiol in females; and an MRI of the hypothalamus-pituitary region, unless contraindicated (**Grade A, Class I**).
- **Management and monitoring:**
  - It is recommended to start therapy in patients with primary overt hypothyroidism with L-thyroxine at low dose (25-50 mcg/day), to be further reduced (12.5 mcg/day) in patients with pre-existing cardiac disease, with the aim of maintaining FT4 level within the normal range and TSH level <4 μU/ml, while avoiding overdose with TSH suppression (i.e., TSH <0.5 mU/L) (**Grade A, Class I**).
  - In patients with primary overt or subclinical hypothyroidism on L-thyroxine supplementation, it is recommended to monitor FT4 and TSH levels every 6 weeks until TSH normalisation and then every 6 months (**Grade A, Class I**).
  - It is recommended to start therapy in patients with central hypothyroidism with L-thyroxine at low dose (25-50 mcg/day), to be further reduced (12.5 mcg/day) in patients with pre-existing cardiac disease, with the aim of maintaining FT4 level within the normal range (**Grade A, Class I**).

- In patients with central hypothyroidism on L-thyroxine supplementation, it is recommended to monitor FT4 levels every 4 weeks until FT4 normalisation and then every 6 months (**Grade A, Class I**).
- It is suggested to consider L-thyroxine therapy in patients with subclinical hypothyroidism on a case by case basis, particularly in young adult patients with TSH >10 mU/L and/or in the presence of subjective symptoms, with the aim of maintaining TSH level <4 mU/mL, while avoiding overdose with TSH suppression (i.e., TSH <0.5 mU/L) (**Grade A, Class IIa**).
- Treatment for subclinical hypothyroidism is required in all patients seeking pregnancy, with close hormonal monitoring (every 4 weeks) with the aim of maintaining TSH levels <2.5 mU/L before conception. (**Grade C, Class I**).
- During pregnancy, it is recommended to start L-thyroxine therapy in case of TSH >2.5 mU/L, monitoring FT4 and TSH levels once a month until the 20th week and once between the 26th and the 32nd weeks with subclinical hypothyroidism, with the aim of maintaining FT4 level within the normal range and TSH level <2.5 mU/L in the first and second trimesters and between 2.5 and 3 mU/L during the third trimester. After childbirth, the L-thyroxine dosage should be reduced to pre-conceptional levels and then adjusted based on the TSH and FT4 levels measured approximately 6 weeks after childbirth (**Grade C, Class I**).
- All patients should be referred to the endocrinologist at first diagnosis of hypothyroidism, with difficult-to-compensate hypothyroidism, and with structural alterations of the thyroid at ultrasound. Referral is also recommended in patients seeking pregnancy, pregnant patients, patients treated with drugs interfering with thyroid function, cardiopathic, and fragile patients (**Grade C, Class I**).
- It is recommended to optimise transfusion and iron chelation therapy (oral or combinatorial regimens) to prevent or potentially reverse hypothyroidism in TDT patients (**Grade C, Class I**).

6. Recommendations for glucose metabolism disorders.

- **First level assessment:**
  - The recommended screening tests for glucose metabolism disorders are the measurement of fasting blood glucose levels and/or blood glucose during oral glucose tolerance test (OGTT), to be performed starting from 10 years of age, at least every 2 years from 10 to 18 years of age, and annually thereafter (**Grade B, Class I**).
  - It is suggested to use the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) index as a guide to evaluate insulin resistance, to be calculated starting from 10 years of age, at least every 2 years from 10 to 18 years of age, and annually thereafter (**Grade C, Class IIa**).
  - It is suggested to conduct periodic assessment of serum fructosamine measurement (**Grade C, Class IIa**).
  - For pregnant women, it is recommended to assess fasting blood glucose level every month from the beginning of pregnancy and OGTT at 24-28 weeks of gestation, following the specific criteria for gestational diabetes (**Grade B, Class I**).
  - It is recommended to not consider glycosylated haemoglobin A1C (HbA1C) measurement for screening of glucose metabolism disorders in patients with TDT, unlike the general population (**Grade B, Class I**).

- **Criteria for diagnosis:**
  - A diagnosis of diabetes should be made in case of blood glycaemia >200 mg/dL in the presence of typical symptoms of diabetes (polyuria, polydipsia, weight loss) on a single random serum evaluation, fasting glycaemia >126 mg/dL on at least two evaluations, or glycemia >200 mg/dL two hours after OGTT (**Grade A, Class I**).
  - A diagnosis of impaired fasting glycemia (IFG) should be made in case of fasting glycaemia between 100 and 126 mg/dL (**Grade A, Class I**).
  - A diagnosis of impaired glucose tolerance (IGT) should be made in case of glycaemia between 140 and 200 mg/dL during OGTT (**Grade A, Class I**).
- **Confirmatory, stimulus tests, or second level tests:**
  - It is recommended to perform OGTT in patients with impaired fasting glucose found at least on two occasions (**Grade A, Class I**).
  - It is recommended to assess serum plasma C-peptide to complete the diagnosis in patients with a glucose metabolism disorder (**Grade C, Class I**).
- **Management and monitoring:**
  - In patients with glucose metabolism disorders, a healthy lifestyle is recommended (appropriate diet, regular physical activity, smoking cessation, adequate amount of sleep) (**Grade C, Class I**).
  - In patients with IFG and IGT, therapy with metformin is recommended, especially in case of insulin resistance (**Grade C, Class I**).
  - In patients with diabetes, therapy with metformin or insulin is recommended, depending on the presence of symptoms, catabolic signs, and glycaemia and C-peptide levels. Sn early start of insulin therapy should be considered in case of hypercatabolism (weight loss), persistent high glucose levels, and low/undetectable C-peptide (**Grade C, Class I**).
  - A basal-bolus insulin scheme through multiple daily dose injections is recommended, while continuous subcutaneous insulin infusion through a pump is recommended in selected cases (**Grade C, Class I**).
  - Patient education on blood glucose self-monitoring, prevention, as well as recognition and treatment of hypoglycaemia is recommended (**Grade C, Class I**).
  - Assessment of micro- and macroangiopathic complications is recommended, as indicated in diabetes guidelines for the general population (**Grade C, Class I**).
  - It is recommended to optimise transfusion and iron chelation therapy (oral or combinatorial regimens) to prevent or potentially reverse glucose metabolism disorders in TDT patients (**Grade C, Class I**).

7. Recommendations for hypocorticosurrenalism.

- **First level assessment:**
  - The suggested screening test for hypocorticosurrenalism is the measurement of serum sodium, potassium, ACTH and cortisol levels at 8 a.m., starting from adolescence and once a year (**Grade C, Class IIa**).
- **Criteria for diagnosis:**
  - A diagnosis of hypocorticosurrenalism should be made in patients with morning cortisol values <3 mcg/dL (80 nmol/L) (**Grade C, Class I**).

- A diagnosis of hypocorticosurrenalism should be excluded in patients with morning cortisol values >15 mcg/dL (400 nmol/L) (**Grade C, Class I**).

- **Confirmatory, stimulus, or second level tests:**
  - In patients with confirmed values of serum cortisol between 3 and 10 mcg/dL (80 -275 nmol/L) in the absence of interfering drugs and in patients with values between 10 and 15 mcg/dL (275-400 nmol/L) with previous history of iron overload and co-existence of other deficiencies of the hypothalamic-pituitary function, it is suggested to perform a stimulus test with ACTH, low dose (1 mcg intravenously) or standard dose (250 mcg intravenously), or glucagon, according to the experience and product availability at the centre and the need to test for other axes disorders (possible with glucagon test) (**Grade C, Class IIa**).
  - A diagnosis of hypocorticosurrenalism should be made in patients with a cortisol peak <15-18 mcg/dL (400- 500 nmol/L) at stimulus tests (ACTH or glucagon) (**Grade A, Class I**).
  - A diagnosis of secondary hypocorticosurrenalism should be made in patients with reduced cortisol and reduced or inappropriately normal ACTH levels (**Grade A, Class I**).
  - In patients with secondary hypocorticosurrenalism, it is recommended to perform a pituitary MRI with contrast agent (unless contraindicated) and tests of the other hormone axes (FSH, LH, FT4, TSH, prolactin, testosterone in males, oestradiol in females, and IGF-1) (**Grade A, Class I**).
  - A diagnosis of primary hypocorticosurrenalism should be made in patients with reduced cortisol and high ACTH levels (**Grade A, Class I**).
  - In patients with primary hypocorticosurrenalism, it is recommended to perform adrenal imaging by MRI or computed tomography (**Grade A, Class I**).

- **Management and monitoring:**
  - In patients with a diagnosis of hypocorticosurrenalism, it is recommended to start cortisone replacement therapy with oral administration of hydrocortisone (10-15 mg/day) or cortisone acetate in adults (25-37.5 mg/day) divided in 2-3 daily doses, with a higher dose in the morning upon awakening (**Grade A, Class I**).
  - In patients with hypocorticosurrenalism on replacement therapy, it is recommended to carry out clinical monitoring and assessment of sodium, potassium, and glycaemia at least every 6 months (**Grade A, Class I**).
  - It is recommended to educate patients and family members on prevention and recognition of adrenal crisis and provide the patient with a card certifying the disease (**Grade A, Class I**).
  - It is suggested to refer all patients with suspicion of hypocorticosurrenalism to the endocrinologist, especially adults with history of iron overload and other endocrinological disorders (**Grade C, Class IIa**).

8. Recommendations for hypoparathyroidism.

- **First level assessment:**
  - The recommended screening tests for hypoparathyroidism are the measurement of serum calcium, corrected for albumin level, and serum phosphorus, to be performed once a year starting from 10 years of age (**Grade C, Class I**).

- **Confirmatory, stimulus, or second level tests:**
  - In case of hypocalcaemia (serum calcium corrected for albumin <8 mg/dL), it is recommended to perform the following tests to complete the diagnosis: parathyroid hormone (PTH), serum magnesium, creatinine, 25-hydroxy (OH) vitamin D, and 24-hour urine calcium (**Grade A, Class I**).
- **Criteria for diagnosis:**
  - A diagnosis of hypoparathyroidism should be made in case of hypocalcaemia (serum calcium corrected for albumin <8 mg/dL), in combination with reduced or inappropriately normal PTH levels, found on at least two assessments (**Grade A, Class I**).
- **Management and monitoring:**
  - It is recommended to start therapy in patients with hypocalcaemia symptoms and/or serum calcium levels corrected for albumin value <8 mg/dL (<2 mmol/L) with calcium supplementation (calcium carbonate as first choice or calcium citrate, in case of side effects, proton-pump inhibitors therapy or achlorhydria), at a daily dose of 1.0-2.5 g/day in the adult patient, divided into 2-3 doses (**Grade A, Class I**).
  - Supplementation with the active form of vitamin D, calcitriol (1,25-dihidroxicolecalciferol) is recommended, at a daily dose of 0.25 to 2 micrograms, divided into two daily doses (**Grade A, Class I**).
  - It is suggested to add oral supplementation of vitamin D at the dose of 400-800 IU per day (**Grade C, Class IIb**).
  - It is suggested to consider the use of PTH analog drugs in cases of severe chronic hypoparathyroidism not compensated by traditional therapy, unless contraindicated (**Grade C, Class IIb**).
  - It is recommended to perform clinical monitoring (assessment of hypo/hypercalcaemia symptoms) as well as laboratory monitoring with the following tests: calcium levels corrected for albumin, phosphorus and magnesium levels, creatinine and estimated glomerular filtration rate every 3-6 months, 24-hour urine calcium and creatinine level once a year if the patient with hypoparathyroidism is stable; and more frequently (weekly or monthly) at the start of therapy, in case of dose change, or poor compliance with therapy (**Grade A, Class I**).
  - It is recommended to keep calcium level at the lower limits of the normal range, phosphorus level at the upper limits of the normal range, and the calcium-phosphate ratio <55 mg^2/dL^2 (4.4 $mmol^2/L^2$), to avoid hypercalciuria, nephrolithiasis, and nephrocalcinosis (**Grade A, Class I**).
  - It is recommended to perform renal and urinary tract ultrasound in patients with hypoparathyroidism, which can be done along with any planned abdominal ultrasound required for patients with TDT (**Grade C Class I**).
  - It is recommended to refer all patients with hypoparathyroidism to the endocrinologist at diagnosis, for therapy planning and monitoring, to be followed every 6-12 months thereafter (**Grade C Class I**).
  - It is recommended to optimise transfusion and iron chelation therapy (oral or combinatorial regimens) to prevent or potentially reverse hypoparathyroidism in TDT patients (**Grade C, Class I**).

9. Recommendations for growth hormone deficiency in adults.

- **First level assessment:**
  - The suggested screening test for GH deficiency is the measurement of IGF-1 level, to be performed once a year starting from 25 years of age or earlier in case of severe iron overload and other pituitary deficits (**Grade C, Class IIa**).
  - Assessment of GH basal level is not recommended (**Grade A, Class I**).
- **Confirmatory, stimulus, or second level tests:**
  - In case of low IGF-1 level (<2 SD), it is suggested to perform a stimulus test with macimorelin or glucagon, according to local experience and product availability (**Grade C, Class IIa**).
- **Criteria for diagnosis:**
  - A diagnosis of GH deficiency should be made in case of GH peak ≤1 mcg/L with a glucagon stimulus test (if BMI <25 kg/m^2, the GH cut-off value is ≤3 mcg/L); or in case of GH peak ≤5.1 mcg/L with a macimorelin stimulus test (**Grade C, Class I**).
  - In case of GH deficiency, it is recommended to check overall pituitary function and to perform MRI of the hypothalamic-pituitary region, unless contraindicated (**Grade A, Class I**).
- **Management and monitoring:**
  - In patients with GH deficiency, it is recommended to start therapy with biosynthetic GH at a low dose (0.2-0.4 mg/day) in the presence of an appropriate clinical context, according to the parameters indicated, and in the absence of contraindications (**Grade C, Class I**).
  - It is recommended to not start therapy with biosynthetic GH in case of active malignant neoplasm or a proliferative diabetic retinopathy (**Grade A, Class I**).
  - At the start therapy with biosynthetic GH, it is recommended to retest the adrenal function and adjust replacement therapy with glucocorticoids in patients with pre-existing hypocorticosurrenalism (**Grade A, Class I**).
  - After the start therapy with biosynthetic GH, it is recommended to perform clinical monitoring (evaluation of BMI, blood pressure, abdominal circumference, side effects), metabolic monitoring (glycaemia, lipid profile), and IGF-1 level after 2 months, then every 6 months (**Grade A, Class I**).
  - It is recommended to test thyroid function after 6 months from the start of therapy or after the change of biosynthetic GH dose (**Grade A, Class I**).

10. Recommendations for bone disease.

- **First level assessment:**
  - The recommended screening test for bone disease is the measurement of bone mineral density (BMD) by dual-energy X-ray absorptiometry (DXA), to be performed from adolescence, or earlier (from 10 years) if feasible, every 12-24 months, according to clinical evolution (**Grade C, Class I**).
  - It is recommended to perform DXA at the lumbar spine (LS) and total body less head (TBLH) in children and adolescents; it is recommended to assess BMD at the LS, femoral neck (FN), and total hip (TH) in adults (**Grade C, Class I**).

- **Confirmatory, stimulus, or second level tests:**
  - In case of bone disease, additional or more invasive diagnostic tests, such as peripheral quantitative computer tomography, quantitative morphometry of the vertebrae or bone histomorphometry after tetracycline double labelling, could be performed only in specific situations and under the supervision of a skilled 'bone specialist' (**Grade C, Class IIb**).
- **Criteria for diagnosis:**
  - A diagnosis of osteoporosis should be made in case of BMD T-score ≤ –2.5 SD in post-menopausal women and in men aged ≥50 years old; and a diagnosis of bone mass below the expected range for age should be made in females before menopause and in males ≤ 50 years old in case of Z-score ≤ -2 SD (**Grade A, Class I**).
  - It is recommended to assess the absolute risk of fracture using the Fracture Risk Assessment Tool (FRAX) by integrating the information about fracture risk provided by clinical risk factors (CRFs) for fracture and BMD, starting from the age of ≥40 years and once a year (**Grade C, Class I**).
- **Management and monitoring:**
  - It is recommended to manage thalassaemia-associated bone disease in a multidisciplinary team with a comprehensive approach, starting from adolescence (**Grade B, Class I**).
  - It is recommended to maintain an adequate dietary calcium intake according to age and gender (800-1500 mg daily) in all TDT patients, irrespective of the presence of bone disease; calcium supplementation is recommended only in the presence of a critically low dietary calcium intake (**Grade C, Class I**).
  - It is recommended to attain and maintain a serum 25OH vitamin D value above 30 ng/mL (optimal range 30-50 ng/mL) in all TDT patients, irrespective of the presence of bone disease, by prescribing supplementation with cholecalciferol according to Table 12, preferring a daily oral dose (**Grade A, Class I**).
  - It is suggested to limit the use of calcifediol or active metabolites of vitamin D to specific clinical conditions, such as chronic kidney disease or severe liver disease or in males with hypogonadism not responsive to cholecalciferol (**Grade C, Class IIa**).
  - It is recommended to start bisphosphonates or denosumab treatment in all patients with high fracture risk, according to FRAX or other validated algorithms, by integrating information of clinical risk factors and DEXA (**Grade B, Class I**).
  - It is recommended to start bisphosphonates or denosumab treatment in all patients presenting with a fragility fracture, possibly within 12 weeks to 16 weeks after the fracture (**Grade A, Class I**).
  - In patients with thalassaemia-associated bone disease and normal kidney function, it is recommended to start with an oral or intravenous bisphosphonate (alendronate, neridronate, or zoledronic acid) considering a treatment course of 2 or 3 years (**Grade B, Class I**).
  - In patients with thalassaemia-associated bone disease and chronic kidney disease with a glomerular filtration rate of 30 mL/min or higher, it is suggested to start with an oral or intravenous bisphosphonate (alendronate, neridronate, or zoledronic acid) considering a treatment course of 2 or 3 years, with close monitoring of the kidney function (**Grade C, Class IIa**).

- In patients with thalassaemia-associated bone disease and chronic kidney disease with a glomerular filtration rate of 29 mL/min or lower, it is suggested to start with denosumab, after a comprehensive evaluation of risks/benefits and under the supervision of a skilled 'bone specialist', with a close monitoring (every 1 to 3 months) of serum calcium, phosphate, parathyroid hormone, 25OH vitamin D, and kidney function (**Grade C, Class IIa**).
- In patients with normal kidney function or with chronic kidney disease and a glomerular filtration rate of 30 mL/min or higher, presenting with a new fragility fracture under bisphosphonate treatment, it is suggested to consider discontinuing bisphosphonate and starting denosumab therapy (**Grade B, Class IIa**).
- In patients at high risk of fracture presenting with a new fragility fracture during bisphosphonate/denosumab therapy, it is suggested to consider a combination treatment course with teriparatide and denosumab (**Grade C, Class IIa**).
- It is suggested to undertake an evaluation of risks/benefits of continuing treatment after three years of bisphosphonate therapy, considering a 'drug-free holiday', in order to minimise the risk of rare adverse events such as osteonecrosis of the jaw or atypical femoral fracture (**Grade C, Class IIa**).
- It is recommended to follow a treatment course with bisphosphonate after discontinuation of denosumab, to avoid a faster reduction of BMD (rebound effect) (**Grade A, Class I**).
- Adequate control of anaemia and iron overload is recommended to mitigate bone marrow expansion and iron toxicity to the bone (**Grade A, Class I**).
- Physical activity and healthy lifestyle (discontinue smoking and alcohol intake) is recommended in all patients (**Grade C, Class I**).
- In patients with hypogonadism, hormone replacement therapy is recommended according to dedicated recommendations (**Grade A, Class I**).
- It is suggested to consider referral to a fracture liaison service and/or 'bone specialist' in patients with recurrent fractures and/or severe bone disease (**Grade B, Class IIa**).
- It is recommended to assess lumbar spine and femoral BMD in the long-term follow up of patients with thalassaemia-related bone disease, considering the femoral neck is the most reliable site for the monitoring (**Grade B, Class I**).
- It is recommended to undertake, at baseline and once a year, a laboratory diagnostic workup (measurement of serum calcium, phosphate, creatinine, albumin and protein electrophoresis, PTH, 25OH vitamin D, 24-hour urinary calcium, creatinine, and phosphate excretion, and laboratory tests to assess gonadal function) to identify conditions and risk factors that may potentially contribute to bone loss and increase fracture risk (**Grade C, Class I**).
- It is recommended to promptly identify and treat diabetes and/or other secondary causes of osteoporosis and fracture mentioned in Table 11 (**Grade C, Class I**).
- During treatment with bisphosphonates or denosumab, it is suggested to refer patients for a dental check-up at baseline and once a year to prevent osteonecrosis of the jaw (**Grade C, Class IIb**).
- In patients undergoing bisphosphonate or denosumab treatment over three years, it is suggested to perform bilateral X-ray of the subtrochanteric region and femoral shaft, to exclude the presence of an incomplete atypical fracture (**Grade C, Class IIb**).

- In patients undergoing bisphosphonate or denosumab treatment presenting with prodromal symptoms such as dull or aching pain in the groin or thigh, it is recommended to perform bilateral X-rays of the subtrochanteric region and femoral shaft, to exclude the presence of an incomplete atypical fracture (**Grade C, Class I**).
- In all patients undergoing bisphosphonate or denosumab treatment presenting with an incomplete or complete atypical fracture, it is recommended to discontinue bisphosphonate or denosumab treatment, and eventually consider teriparatide therapy (**Grade B, Class I**).

REFERENCES

1. Jobanputra M, Paramore C, Laird SG, McGahan M, Telfer P. Co-morbidities and mortality associated with transfusion-dependent beta-thalassaemia in patients in England: a 10-year retrospective cohort analysis. Br J Haematol 2020;191(5):897-905.
2. Forni GL, Gianesin B, Musallam KM, Longo F, Rosso R, Lisi R, Gamberini MR, Pinto VM, Graziadei G, Vitucci A, Bonetti F, Musto P, Piga A, Cappellini MD, Borgna-Pignatti C, Webthal p. Overall and complication-free survival in a large cohort of patients with beta-thalassemia major followed over 50 years. Am J Hematol 2023;98(3):381-387.
3. Casale M, Forni GL, Cassinerio E, Pasquali D, Origa R, Serra M, Campisi S, Peluso A, Renni R, Cattoni A, De Michele E, Allo M, Poggi M, Ferrara F, Di Concilio R, Sportelli F, Quarta A, Putti MC, Notarangelo LD, Sau A, Ladogana S, Tartaglione I, Picariello S, Marcon A, Sturiale P, Roberti D, Lazzarino AI, Perrotta S. Risk factors for endocrine complications in transfusion-dependent thalassemia patients on chelation therapy with deferasirox: a risk assessment study from a multi-center nation-wide cohort. Haematologica 2022;107(2):467-477.
4. Sharma R, Seth A, Chandra J, Gohain S, Kapoor S, Singh P, Pemde H. Endocrinopathies in adolescents with thalassaemia major receiving oral iron chelation therapy. Paediatr Int Child Health 2016;36(1):22-27.
5. Chatterjee R, Bajoria R. Critical appraisal of growth retardation and pubertal disturbances in thalassemia. Ann N Y Acad Sci 2010;1202:100-114.
6. Casale M, Marsella M, Ammirabile M, Spasiano A, Costantini S, Cinque P, Ricchi P, Filosa A. Predicting factors for liver iron overload at the first magnetic resonance in children with thalassaemia major. Blood Transfus 2019;17(3):165-170.
7. Tanner JM, Goldstein H, Whitehouse RH. Standards for children's height at ages 2-9 years allowing for heights of parents. Arch Dis Child 1970;45(244):755-762.
8. Cuboni D, Caputo M, Ghigo E, Aimaretti G, Gasco V. Once upon a time: the glucagon stimulation test in diagnosing adult GH deficiency. J Endocrinol Invest 2024;47(7):1621-1631.
9. Yuen KCJ, Biller BMK, Radovick S, Carmichael JD, Jasim S, Pantalone KM, Hoffman AR. American Association of Clinical Endocrinologists and American College of Endocrinology Guidelines for Management of Growth Hormone Deficiency in Adults and Patients Transitioning from Pediatric to Adult Care. Endocr Pract 2019;25(11):1191-1232.
10. Low LC, Kwan EY, Lim YJ, Lee AC, Tam CF, Lam KS. Growth hormone treatment of short Chinese children with beta-thalassaemia major without GH deficiency. Clin Endocrinol (Oxf) 1995;42(4):359-363.
11. De Sanctis V, Soliman AT, Elsedfy H, Skordis N, Kattamis C, Angastiniotis M, Karimi M, Yassin MA, El Awwa A, Stoeva I, Raiola G, Galati MC, Bedair EM, Fiscina B, El Kholy M. Growth and endocrine disorders in thalassemia: The international network on endocrine complications in thalassemia (I-CET) position statement and guidelines. Indian J Endocrinol Metab 2013;17(1):8-18.
12. Murray PG, Dattani MT, Clayton PE. Controversies in the diagnosis and management of growth hormone deficiency in childhood and adolescence. Arch Dis Child 2016;101(1):96-100.
13. Ngim CF, Lai NM, Hong JY, Tan SL, Ramadas A, Muthukumarasamy P, Thong MK. Growth hormone therapy for people with thalassaemia. Cochrane Database Syst Rev 2020;5(5):CD012284.
14. Collett-Solberg PF, Ambler G, Backeljauw PF, Bidlingmaier M, Biller BMK, Boguszewski MCS, Cheung PT, Choong CSY, Cohen LE, Cohen P, Dauber A, Deal CL, Gong C, Hasegawa Y, Hoffman AR, Hofman PL, Horikawa R, Jorge AAL, Juul A, Kamenicky P, Khadilkar V, Kopchick JJ, Kristrom B, Lopes MLA, Luo X, Miller BS, Misra M, Netchine I, Radovick S, Ranke MB, Rogol AD, Rosenfeld RG, Saenger P, Wit JM, Woelfle J. Diagnosis, Genetics, and Therapy of Short Stature in Children: A Growth Hormone Research Society International Perspective. Horm Res Paediatr 2019;92(1):1-14.
15. Kyriakou A, Skordis N. Thalassaemia and aberrations of growth and puberty. Mediterr J Hematol Infect Dis 2009;1(1):e2009003.

16. Srisukh S, Ongphiphadhanakul B, Bunnag P. Hypogonadism in thalassemia major patients. J Clin Transl Endocrinol 2016;5:42-45.
17. De Sanctis V, Eleftheriou A, Malaventura C, Thalassaemia International Federation Study Group on G, Endocrine Complications in T. Prevalence of endocrine complications and short stature in patients with thalassaemia major: a multicenter study by the Thalassaemia International Federation (TIF). Pediatr Endocrinol Rev 2004;2 Suppl 2:249-255.
18. Dye AM, Nelson GB, Diaz-Thomas A. Delayed Puberty. Pediatr Ann 2018;47(1):e16-e22.
19. Abitbol L, Zborovski S, Palmert MR. Evaluation of delayed puberty: what diagnostic tests should be performed in the seemingly otherwise well adolescent? Arch Dis Child 2016;101(8):767-771.
20. Howard SR, Dunkel L. Management of hypogonadism from birth to adolescence. Best Pract Res Clin Endocrinol Metab 2018;32(4):355-372.
21. Palmert MR, Dunkel L. Clinical practice. Delayed puberty. N Engl J Med 2012;366(5):443-453.
22. De Sanctis V, Soliman AT, Yassin MA, Di Maio S, Daar S, Elsedfy H, Soliman N, Kattamis C. Hypogonadism in male thalassemia major patients: pathophysiology, diagnosis and treatment. Acta Biomed 2018;89(2-S):6-15.
23. Sedlmeyer IL, Palmert MR. Delayed puberty: analysis of a large case series from an academic center. J Clin Endocrinol Metab 2002;87(4):1613-1620.
24. Wehkalampi K, Widen E, Laine T, Palotie A, Dunkel L. Patterns of inheritance of constitutional delay of growth and puberty in families of adolescent girls and boys referred to specialist pediatric care. J Clin Endocrinol Metab 2008;93(3):723-728.
25. Al-Rimawi HS, Jallad MF, Amarin ZO, Obeidat BR. Hypothalamic-pituitary-gonadal function in adolescent females with beta-thalassemia major. Int J Gynaecol Obstet 2005;90(1):44-47.
26. Broccia MV, Vergier J, Benoit A, Huguenin Y, Lambilliotte A, Castex MP, Gourdon S, Ithier G, Kebaili K, Rohrlich P, Pondarre C, Chamouine A, Simon P, Kpati KPA, Allali S, Baron-Joly S, Bayart S, Billaud N, Brousse V, Dumesnil C, Garnier N, Guichard I, Joseph L, Kamdem A, Maitre J, Mathey C, Paillard C, Phulpin A, Renard C, Stoven C, Touati M, Trochu C, Nafissi SM, Badens C, Szepetowski S, Thuret I. Pubertal development of transfusion-dependent thalassemia patients in the era of oral chelation with deferasirox: results from the French registry. Haematologica 2024;109(7):2271-2276.
27. Salonia A, Rastrelli G, Hackett G, Seminara SB, Huhtaniemi IT, Rey RA, Hellstrom WJG, Palmert MR, Corona G, Dohle GR, Khera M, Chan YM, Maggi M. Paediatric and adult-onset male hypogonadism. Nat Rev Dis Primers 2019;5(1):38.
28. Mason KA, Schoelwer MJ, Rogol AD. Androgens During Infancy, Childhood, and Adolescence: Physiology and Use in Clinical Practice. Endocr Rev 2020;41(3).
29. Chioma L, Papucci G, Fintini D, Cappa M. Use of testosterone gel compared to intramuscular formulation for puberty induction in males with constitutional delay of growth and puberty: a preliminary study. J Endocrinol Invest 2018;41(2):259-263.
30. Belhoul KM, Bakir ML, Saned MS, Kadhim AM, Musallam KM, Taher AT. Serum ferritin levels and endocrinopathy in medically treated patients with beta thalassemia major. Ann Hematol 2012;91(7):1107-1114.
31. Nayak AM, Choudhari A, Patkar DP, Merchant RH. Pituitary Volume and Iron Overload Evaluation by 3T MRI in Thalassemia. Indian J Pediatr 2021;88(7):656-662.
32. Noetzli LJ, Panigrahy A, Mittelman SD, Hyderi A, Dongelyan A, Coates TD, Wood JC. Pituitary iron and volume predict hypogonadism in transfusional iron overload. Am J Hematol 2012;87(2):167-171.
33. Ehsan L, Rashid M, Alvi N, Awais K, Nadeem O, Asghar A, Sajjad F, Fatima M, Qidwai A, Hussain S, Hasan E, Brown N, Altaf S, Hasan B, Kirmani S. Clinical utility of endocrine markers predicting myocardial siderosis in transfusion dependent thalassemia major. Pediatr Blood Cancer 2018;65(10):e27285.
34. Bordbar M, Bozorgi H, Saki F, Haghpanah S, Karimi M, Bazrafshan A, Zekavat OR. Prevalence of endocrine disorders and their associated factors in transfusion-dependent thalassemia patients: a historical cohort study in Southern Iran. J Endocrinol Invest 2019;42(12):1467-1476.

35. Mahwi TO, Rashid ZG, Ahmed SF. Hypogonadism among patients with transfusion-dependent thalassemia: a cross-sectional study. Ann Med Surg (Lond) 2023;85(7):3418-3422.
36. Evangelidis P, Venou TM, Fani B, Vlachaki E, Gavriilaki E, on behalf of the International Hemoglobinopathy Research Network. Endocrinopathies in Hemoglobinopathies: What Is the Role of Iron? Int J Mol Sci 2023;24(22).
37. Farmaki K, Tzoumari I, Pappa C. Oral chelators in transfusion-dependent thalassemia major patients may prevent or reverse iron overload complications. Blood Cells Mol Dis 2011;47(1):33-40.
38. Poggi M, Sorrentino F, Pugliese P, Smacchia MP, Daniele C, Equitani F, Terlizzi F, Guitarrini MR, Monti S, Maffei L, Losardo A, Pasin M, Toscano V. Longitudinal changes of endocrine and bone disease in adults with beta-thalassemia major receiving different iron chelators over 5 years. Ann Hematol 2016;95(5):757-763.
39. Farmaki K, Tzoumari I, Pappa C, Chouliaras G, Berdoukas V. Normalisation of total body iron load with very intensive combined chelation reverses cardiac and endocrine complications of thalassaemia major. Br J Haematol 2010;148(3):466-475.
40. Fleseriu M, Hashim IA, Karavitaki N, Melmed S, Murad MH, Salvatori R, Samuels MH. Hormonal Replacement in Hypopituitarism in Adults: An Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab 2016;101(11):3888-3921.
41. De Sanctis V, Soliman AT, Elsedfy H, Albu A, Al Jaouni S, Anastasi S, Bisconte MG, Canatan D, Christou S, Daar S, Di Maio S, El Kholy M, Khater D, Elshinawy M, Kilinc Y, Mattei R, Mosli HH, Quota A, Roberti MG, Sobti P, Yaarubi SA, Canpisi S, Kattamis C. Review and Recommendations on Management of Adult Female Thalassemia Patients with Hypogonadism based on Literature Review and Experience of ICET-A Network Specialists. Mediterr J Hematol Infect Dis 2017;9(1):e2017001.
42. De Sanctis V, Soliman AT, Daar S, Di Maio S, Yassin MA, Canatan D, Vives Corrons JL, Elsedfy H, Kattamis A, Kattamis C. The experience of a tertiary unit on the clinical phenotype and management of hypogonadism in female adolescents and young adults with transfusion dependent thalassemia. Acta Biomed 2019;90(1):158-167.
43. Jameson JL. Endocrinology: adult and pediatric. 7th ed. Philadelphia, PA: Elsevier Saunders, 2016.
44. Sullivan SD, Sarrel PM, Nelson LM. Hormone replacement therapy in young women with primary ovarian insufficiency and early menopause. Fertil Steril 2016;106(7):1588-1599.
45. Swee DS, Javaid U, Quinton R. Estrogen Replacement in Young Hypogonadal Women-Transferrable Lessons From the Literature Related to the Care of Young Women With Premature Ovarian Failure and Transgender Women. Front Endocrinol (Lausanne) 2019;10:685.
46. Garcia-Saenz M, Ibarra-Salce R, Pozos-Varela FJ, Mena-Ureta TS, Flores-Villagomez S, Santana-Mata M, De Los Santos-Aguilar RG, Uribe-Cortes D, Ferreira-Hermosillo A. Understanding Progestins: From Basics to Clinical Applicability. J Clin Med 2023;12(10).
47. Hipolito Rodrigues MA, Gompel A. Micronized progesterone, progestins, and menopause hormone therapy. Women Health 2021;61(1):3-14.
48. Curtis KM, Tepper NK, Jatlaoui TC, Berry-Bibee E, Horton LG, Zapata LB, Simmons KB, Pagano HP, Jamieson DJ, Whiteman MK. U.S. Medical Eligibility Criteria for Contraceptive Use, 2016. MMWR Recomm Rep 2016;65(3):1-103.
49. De Sanctis V, Soliman AT, Elsedfy H, Di Maio S, Canatan D, Soliman N, Karimi M, Kattamis C. Gonadal dysfunction in adult male patients with thalassemia major: an update for clinicians caring for thalassemia. Expert Rev Hematol 2017;10(12):1095-1106.
50. Bhasin S, Brito JP, Cunningham GR, Hayes FJ, Hodis HN, Matsumoto AM, Snyder PJ, Swerdloff RS, Wu FC, Yialamas MA. Testosterone Therapy in Men With Hypogonadism: An Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab 2018;103(5):1715-1744.
51. Carsote M, Vasiliu C, Trandafir AI, Albu SE, Dumitrascu MC, Popa A, Mehedintu C, Petca RC, Petca A, Sandru F. New Entity-Thalassemic Endocrine Disease: Major Beta-Thalassemia and Endocrine Involvement. Diagnostics (Basel) 2022;12(8):1921.

52. Corona G, Goulis DG, Huhtaniemi I, Zitzmann M, Toppari J, Forti G, Vanderschueren D, Wu FC. European Academy of Andrology (EAA) guidelines on investigation, treatment and monitoring of functional hypogonadism in males: Endorsing organization: European Society of Endocrinology. Andrology 2020;8(5):970-987.
53. Morgentaler A, Traish A, Hackett G, Jones TH, Ramasamy R. Diagnosis and Treatment of Testosterone Deficiency: Updated Recommendations From the Lisbon 2018 International Consultation for Sexual Medicine. Sex Med Rev 2019;7(4):636-649.
54. Morgentaler A, Traish AM, Khera M. A Critique of the AUA Guidelines on Testosterone Deficiency. J Sex Med 2020;17(4):561-564.
55. Salonia A, Bettocchi C, Capogrosso P, Carvalho J, Corona G, Dinkelman-Smit M, Hatzichristodoulou G, Jones TH, Kadioglu A, Martinez-Salamanca J, Minhas S, Serefoglu EC, Verze P. EAU Guidelines on Sexual and Reproductive Health. . Arnhem, The Netherlands: EAU Guidelines Office, 2024.
56. Bozdag M, Bayraktaroglu S, Aydinok Y, Calli MC. MRI assessment of pituitary iron accumulation by using pituitary-R2 in beta-thalassemia patients. Acta Radiol 2018;59(6):732-739.
57. Baillargeon J, Urban RJ, Morgentaler A, Glueck CJ, Baillargeon G, Sharma G, Kuo YF. Risk of Venous Thromboembolism in Men Receiving Testosterone Therapy. Mayo Clin Proc 2015;90(8):1038-1045.
58. Martinez C, Suissa S, Rietbrock S, Katholing A, Freedman B, Cohen AT, Handelsman DJ. Testosterone treatment and risk of venous thromboembolism: population based case-control study. BMJ 2016;355:i5968.
59. Glueck CJ, Prince M, Patel N, Patel J, Shah P, Mehta N, Wang P. Thrombophilia in 67 Patients With Thrombotic Events After Starting Testosterone Therapy. Clin Appl Thromb Hemost 2016;22(6):548-553.
60. De Sanctis V, Daar S, Soliman AT, Elsedfy H, Khater D, Di Maio S. Does Testosterone Replacement Therapy Promote an Augmented Risk of Thrombotic Events in Thalassemia Major Male Patients with Hypogonadism? Indian J Endocrinol Metab 2017;21(4):636-637.
61. De Sanctis V, Soliman AT, Daar S, Di Maio S. Adverse events during testosterone replacement therapy in 95 young hypogonadal thalassemic men. Acta Biomed 2019;90(2):228-232.
62. Shores MM. Testosterone treatment and cardiovascular events in prescription database studies. Asian J Androl 2018;20(2):138-144.
63. Cannarella R, Gusmano C, Leanza C, Garofalo V, Crafa A, Barbagallo F, Condorelli RA, Vignera S, Calogero AE. Testosterone replacement therapy and vascular thromboembolic events: a systematic review and meta-analysis. Asian J Androl 2024;26(2):144-154.
64. European Medicines Agency. No consistent evidence of an increased risk of heart problems with testosterone medicines. 21 November 2024. Available at: https://www.ema.europa.eu/en/news/no-consistent-evidence-increased-risk-heart-problems-testosterone-medicines. Accessed: 08 Dec 2024.
65. Venou TM, Barmpageorgopoulou F, Peppa M, Vlachaki E. Endocrinopathies in beta thalassemia: a narrative review. Hormones (Athens) 2024;23(2):205-216.
66. De Sanctis V, Soliman AT, Canatan D, Yassin MA, Daar S, Elsedfy H, Di Maio S, Raiola G, Corrons JV, Kattamis C. Thyroid Disorders in Homozygous beta-Thalassemia: Current Knowledge, Emerging Issues and Open Problems. Mediterr J Hematol Infect Dis 2019;11(1):e2019029.
67. De Sanctis V, Soliman AT, Canatan D, Tzoulis P, Daar S, Di Maio S, Elsedfy H, Yassin MA, Filosa A, Soliman N, Mehran K, Saki F, Sobti P, Kakkar S, Christou S, Albu A, Christodoulides C, Kilinc Y, Al Jaouni S, Khater D, Alyaarubi SA, Lum SH, Campisi S, Anastasi S, Galati MC, Raiola G, Wali Y, Elhakim IZ, Mariannis D, Ladis V, Kattamis C. An ICET-A survey on occult and emerging endocrine complications in patients with beta-thalassemia major: Conclusions and recommendations. Acta Biomed 2019;89(4):481-489.
68. Haghpanah S, Hosseini-Bensenjan M, Sayadi M, Karimi M, de Sanctis V, Ramzi M, Mousavi MR. The Prevalence of Hypothyroidism among Patients With beta-Thalassemia: A Systematic Review and Meta-Analysis of Cross-Sectional Studies. Hemoglobin 2021;45(5):275-286.

69. Chirico V, Lacquaniti A, Salpietro V, Luca N, Ferrau V, Piraino B, Rigoli L, Salpietro C, Arrigo T. Thyroid dysfunction in thalassaemic patients: ferritin as a prognostic marker and combined iron chelators as an ideal therapy. Eur J Endocrinol 2013;169(6):785-793.
70. Casale M, Citarella S, Filosa A, De Michele E, Palmieri F, Ragozzino A, Amendola G, Pugliese U, Tartaglione I, Della Rocca F, Cinque P, Nobili B, Perrotta S. Endocrine function and bone disease during long-term chelation therapy with deferasirox in patients with beta-thalassemia major. Am J Hematol 2014;89(12):1102-1106.
71. Haghpanah S, Jelodari S, Karamifar H, Saki F, Rahimi R, De Sanctis V, Dehbozorgian J, Karimi M. The frequency of hypothyroidism and its relationship with HCV positivity in patients with thalassemia major in southern Iran. Acta Biomed 2018;89(1):55-60.
72. Bartalena L, Bogazzi F, Chiovato L, Hubalewska-Dydejczyk A, Links TP, Vanderpump M. 2018 European Thyroid Association (ETA) Guidelines for the Management of Amiodarone-Associated Thyroid Dysfunction. Eur Thyroid J 2018;7(2):55-66.
73. Casale M, Baldini MI, Del Monte P, Gigante A, Grandone A, Origa R, Poggi M, Gadda F, Lai R, Marchetti M, Forni GL. Good Clinical Practice of the Italian Society of Thalassemia and Haemoglobinopathies (SITE) for the Management of Endocrine Complications in Patients with Haemoglobinopathies. J Clin Med 2022;11(7).
74. Jonklaas J, Bianco AC, Bauer AJ, Burman KD, Cappola AR, Celi FS, Cooper DS, Kim BW, Peeters RP, Rosenthal MS, Sawka AM, American Thyroid Association Task Force on Thyroid Hormone R. Guidelines for the treatment of hypothyroidism: prepared by the american thyroid association task force on thyroid hormone replacement. Thyroid 2014;24(12):1670-1751.
75. Persani L, Brabant G, Dattani M, Bonomi M, Feldt-Rasmussen U, Fliers E, Gruters A, Maiter D, Schoenmakers N, van Trotsenburg ASP. 2018 European Thyroid Association (ETA) Guidelines on the Diagnosis and Management of Central Hypothyroidism. Eur Thyroid J 2018;7(5):225-237.
76. Leng O, Razvi S. Hypothyroidism in the older population. Thyroid Res 2019;12:2.
77. Lage MJ, Espaillat R, Vora J, Hepp Z. Hypothyroidism Treatment Among Older Adults: Evidence from a Claims Database. Adv Ther 2020;37(5):2275-2287.
78. Garber JR, Cobin RH, Gharib H, Hennessey JV, Klein I, Mechanick JI, Pessah-Pollack R, Singer PA, Woeber KA, American Association of Clinical E, American Thyroid Association Taskforce on Hypothyroidism in A. Clinical practice guidelines for hypothyroidism in adults: cosponsored by the American Association of Clinical Endocrinologists and the American Thyroid Association. Endocr Pract 2012;18(6):988-1028.
79. Bekkering GE, Agoritsas T, Lytvyn L, Heen AF, Feller M, Moutzouri E, Abdulazeem H, Aertgeerts B, Beecher D, Brito JP, Farhoumand PD, Singh Ospina N, Rodondi N, van Driel M, Wallace E, Snel M, Okwen PM, Siemieniuk R, Vandvik PO, Kuijpers T, Vermandere M. Thyroid hormones treatment for subclinical hypothyroidism: a clinical practice guideline. BMJ 2019;365:l2006.
80. Pearce SH, Brabant G, Duntas LH, Monzani F, Peeters RP, Razvi S, Wemeau JL. 2013 ETA Guideline: Management of Subclinical Hypothyroidism. Eur Thyroid J 2013;2(4):215-228.
81. Alexander EK, Pearce EN, Brent GA, Brown RS, Chen H, Dosiou C, Grobman WA, Laurberg P, Lazarus JH, Mandel SJ, Peeters RP, Sullivan S. 2017 Guidelines of the American Thyroid Association for the Diagnosis and Management of Thyroid Disease During Pregnancy and the Postpartum. Thyroid 2017;27(3):315-389.
82. Biondi B, Cappola AR, Cooper DS. Subclinical Hypothyroidism: A Review. JAMA 2019;322(2):153-160.
83. Simon C, Weidman-Evans E, Allen S. Subclinical hypothyroidism: To treat or not to treat? JAAPA 2020;33(5):21-26.
84. Gharib H, Papini E, Garber JR, Duick DS, Harrell RM, Hegedus L, Paschke R, Valcavi R, Vitti P, Nodules AAATFoT. American Association of Clinical Endocrinologists, American College of Endocrinology, and Associazione Medici Endocrinologi Medical Guidelines for Clinical Practice for the Diagnosis and Management of Thyroid Nodules--2016 Update. Endocr Pract 2016;22(5):622-639.
85. Pacini F, Basolo F, Bellantone R, Boni G, Cannizzaro MA, De Palma M, Durante C, Elisei R, Fadda G, Frasoldati A, Fugazzola L, Guglielmi R, Lombardi CP, Miccoli P, Papini E, Pellegriti G, Pezzullo L, Pontecorvi A, Salvatori M,

Seregni E, Vitti P. Italian consensus on diagnosis and treatment of differentiated thyroid cancer: joint statements of six Italian societies. J Endocrinol Invest 2018;41(7):849-876.
86. Baldini M, Serafino S, Zanaboni L, Cappellini MD. Thyroid cancer in beta-thalassemia. Hemoglobin 2012;36(4):407-408.
87. Origa R, Gianesin B, Longo F, Di Maggio R, Cassinerio E, Gamberini MR, Pinto VM, Quarta A, Casale M, La Nasa G, Caocci G, Piroddi A, Piolatto A, Di Mauro A, Romano C, Gigante A, Barella S, Maggio A, Graziadei G, Perrotta S, Forni GL. Incidence of cancer and related deaths in hemoglobinopathies: A follow-up of 4631 patients between 1970 and 2021. Cancer 2023;129(1):107-117.
88. Ibrahim AS, Abd El-Fatah AH, Abd El-Halim AF, Mohamed FF. Serum Ferritin Levels and Other Associated Parameters with Diabetes Mellitus in Adult Patients Suffering from Beta Thalassemia Major. J Blood Med 2023;14:67-81.
89. De Sanctis V, Soliman AT, Elsedfy H, Pepe A, Kattamis C, El Kholy M, Yassin M. Diabetes and Glucose Metabolism in Thalassemia Major: An Update. Expert Rev Hematol 2016;9(4):401-408.
90. Garber AJ, Handelsman Y, Grunberger G, Einhorn D, Abrahamson MJ, Barzilay JI, Blonde L, Bush MA, DeFronzo RA, Garber JR, Garvey WT, Hirsch IB, Jellinger PS, McGill JB, Mechanick JI, Perreault L, Rosenblit PD, Samson S, Umpierrez GE. Consensus Statement by the American Association of Clinical Endocrinologists and American College of Endocrinology on the Comprehensive Type 2 Diabetes Management Algorithm - 2020 Executive Summary. Endocr Pract 2020;26(1):107-139.
91. Pinto VM, Poggi M, Russo R, Giusti A, Forni GL. Management of the aging beta-thalassemia transfusion-dependent population - The Italian experience. Blood Rev 2019;38:100594.
92. Zanfardino A, Bruzzi P, Trada M, Rapini N, Laudani E, Tornese G, Ortolani F, Piccolo G, Matonti L, Saltarelli MA, Timpanaro T, D'Annunzio G, Predieri B, Rossi F, Mura R, Barat V, Prete A, Schiaffini R, Zucchini S, Diabetes Study Group of the Italian Society for Pediatric E, Diabetes. The real-life management of glucose homeostasis abnormalities in pediatric onco-hematological diseases: data from a national survey. Pediatr Hematol Oncol 2024;41(3):198-210.
93. Taneera J, Mahgoub E, Qannita R, Alalami A, Shehadat OA, Youssef M, Dib A, Hajji AA, Hajji AA, Al-Khaja F, Dewedar H, Hamad M. beta-Thalassemia and Diabetes Mellitus: Current State and Future Directions. Horm Metab Res 2024;56(4):272-278.
94. Desouza CV, Holcomb RG, Rosenstock J, Frias JP, Hsia SH, Klein EJ, Zhou R, Kohzuma T, Fonseca VA. Results of a Study Comparing Glycated Albumin to Other Glycemic Indices. J Clin Endocrinol Metab 2020;105(3):677-687.
95. De Sanctis V, Soliman AT, Elsedfy H, Yaarubi SA, Skordis N, Khater D, El Kholy M, Stoeva I, Fiscina B, Angastiniotis M, Daar S, Kattamis C. The ICET-A Recommendations for the Diagnosis and Management of Disturbances of Glucose Homeostasis in Thalassemia Major Patients. Mediterr J Hematol Infect Dis 2016;8(1):e2016058.
96. Buse JB, Wexler DJ, Tsapas A, Rossing P, Mingrone G, Mathieu C, D'Alessio DA, Davies MJ. 2019 Update to: Management of Hyperglycemia in Type 2 Diabetes, 2018. A Consensus Report by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). Diabetes Care 2020;43(2):487-493.
97. American Diabetes, Association. 9. Pharmacologic Approaches to Glycemic Treatment: Standards of Medical Care in Diabetes-2021. Diabetes Care 2021;44(Suppl 1):S111-S124.
98. Tzoulis P, Yavropoulou MP, Banchev A, Modeva I, Daar S, De Sanctis V. Recent advancements in glucose dysregulation and pharmacological management of osteoporosis in transfusion-dependent thalassemia (TDT): an update of ICET-A (International Network of Clinicians for Endocrinopathies in Thalassemia and Adolescence Medicine). Acta Biomed 2023;94(3):e2023178.
99. Bornstein SR, Allolio B, Arlt W, Barthel A, Don-Wauchope A, Hammer GD, Husebye ES, Merke DP, Murad MH, Stratakis CA, Torpy DJ. Diagnosis and Treatment of Primary Adrenal Insufficiency: An Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab 2016;101(2):364-389.

100. De Sanctis V, Soliman AT, Elsedfy H, Albu A, Al Jaouni S, Yaarubi SA, Anastasi S, Canatan D, Di Maio M, Di Maio S, El Kholy M, Karimi M, Khater D, Kilinc Y, Lum SH, Skordis N, Sobti P, Stoeva I, Tzoulis P, Wali Y, Kattamis C. The ICET-A Survey on Current Criteria Used by Clinicians for the Assessment of Central Adrenal Insufficiency in Thalassemia: Analysis of Results and Recommendations. Mediterr J Hematol Infect Dis 2016;8(1):e2016034.
101. Matin S, Jahromi MG, Karemizadeh Z, Haghpanah S, De Sanctis V, Soliman A, Dehbozorgian J, Majd Z, Rezaei N, Karimi M. The Frequency of Adrenal Insufficiency in Adolescents and Young Adults with Thalassemia Major versus Thalassemia Intermedia in Iran. Mediterr J Hematol Infect Dis 2015;7(1):e2015005.
102. Poggi M, Samperi I, Mattia L, Di Rocco A, Iorio C, Monti S, Pugliese G, Toscano V. New Insights and Methods in the Approach to Thalassemia Major: The Lesson From the Case of Adrenal Insufficiency. Front Mol Biosci 2020;6:162.
103. Yanase T, Tajima T, Katabami T, Iwasaki Y, Tanahashi Y, Sugawara A, Hasegawa T, Mune T, Oki Y, Nakagawa Y, Miyamura N, Shimizu C, Otsuki M, Nomura M, Akehi Y, Tanabe M, Kasayama S. Diagnosis and treatment of adrenal insufficiency including adrenal crisis: a Japan Endocrine Society clinical practice guideline [Opinion]. Endocr J 2016;63(9):765-784.
104. Cortet C, Barat P, Zenaty D, Guignat L, Chanson P. Group 5: Acute adrenal insufficiency in adults and pediatric patients. Ann Endocrinol (Paris) 2017;78(6):535-543.
105. Ospina NS, Al Nofal A, Bancos I, Javed A, Benkhadra K, Kapoor E, Lteif AN, Natt N, Murad MH. ACTH Stimulation Tests for the Diagnosis of Adrenal Insufficiency: Systematic Review and Meta-Analysis. J Clin Endocrinol Metab 2016;101(2):427-434.
106. Castinetti F, Guignat L, Bouvattier C, Samara-Boustani D, Reznik Y. Group 4: Replacement therapy for adrenal insufficiency. Ann Endocrinol (Paris) 2017;78(6):525-534.
107. Chanson P, Guignat L, Goichot B, Chabre O, Boustani DS, Reynaud R, Simon D, Tabarin A, Gruson D, Reznik Y, Raffin Sanson ML. Group 2: Adrenal insufficiency: screening methods and confirmation of diagnosis. Ann Endocrinol (Paris) 2017;78(6):495-511.
108. De Sanctis V, Soliman AT, Canatan D, Elsedfy H, Karimi M, Daar S, Rimawi H, Christou S, Skordis N, Tzoulis P, Sobti P, Kakkar S, Kilinc Y, Khater D, Alyaarubi SA, Kaleva V, Lum SH, Yassin MA, Saki F, Obiedat M, Anastasi S, Galati MC, Raiola G, Campisi S, Soliman N, Elshinawy M, Jaouni SA, Di Maio S, Wali Y, Elhakim IZ, Kattamis C. An ICET-A survey on Hypoparathyroidism in Patients with Thalassaemia Major and Intermedia: A preliminary report. Acta Biomed 2018;88(4):435-444.
109. Brandi ML, Bilezikian JP, Shoback D, Bouillon R, Clarke BL, Thakker RV, Khan AA, Potts JT, Jr. Management of Hypoparathyroidism: Summary Statement and Guidelines. J Clin Endocrinol Metab 2016;101(6):2273-2283.
110. Shoback DM, Bilezikian JP, Costa AG, Dempster D, Dralle H, Khan AA, Peacock M, Raffaelli M, Silva BC, Thakker RV, Vokes T, Bouillon R. Presentation of Hypoparathyroidism: Etiologies and Clinical Features. J Clin Endocrinol Metab 2016;101(6):2300-2312.
111. Maeda SS, Moreira CA, Borba VZC, Bandeira F, Farias MLF, Borges JLC, Paula FJA, Vanderlei FAB, Montenegro FLM, Santos RO, Ferraz-de-Souza B, Lazaretti-Castro M. Diagnosis and treatment of hypoparathyroidism: a position statement from the Brazilian Society of Endocrinology and Metabolism. Arch Endocrinol Metab 2018;62(1):106-124.
112. Tecilazich F, Formenti AM, Frara S, Giubbini R, Giustina A. Treatment of hypoparathyroidism. Best Pract Res Clin Endocrinol Metab 2018;32(6):955-964.
113. Orloff LA, Wiseman SM, Bernet VJ, Fahey TJ, 3rd, Shaha AR, Shindo ML, Snyder SK, Stack BC, Jr., Sunwoo JB, Wang MB. American Thyroid Association Statement on Postoperative Hypoparathyroidism: Diagnosis, Prevention, and Management in Adults. Thyroid 2018;28(7):830-841.
114. Khan AA, Koch CA, Van Uum S, Baillargeon JP, Bollerslev J, Brandi ML, Marcocci C, Rejnmark L, Rizzoli R, Shrayyef MZ, Thakker R, Yildiz BO, Clarke B. Standards of care for hypoparathyroidism in adults: a Canadian and International Consensus. Eur J Endocrinol 2019;180(3):P1-P22.

115. Mannstadt M, Clarke BL, Bilezikian JP, Bone H, Denham D, Levine MA, Peacock M, Rothman J, Shoback DM, Warren ML, Watts NB, Lee HM, Sherry N, Vokes TJ. Safety and Efficacy of 5 Years of Treatment With Recombinant Human Parathyroid Hormone in Adults With Hypoparathyroidism. J Clin Endocrinol Metab 2019;104(11):5136-5147.
116. Zavatta G, Clarke BL. Challenges in the management of chronic hypoparathyroidism. Endocr Connect 2020;9(10):R229-240.
117. Bollerslev J, Rejnmark L, Marcocci C, Shoback DM, Sitges-Serra A, van Biesen W, Dekkers OM, European Society of E. European Society of Endocrinology Clinical Guideline: Treatment of chronic hypoparathyroidism in adults. Eur J Endocrinol 2015;173(2):G1-20.
118. Soliman A, De Sanctis V, Elsedfy H, Yassin M, Skordis N, Karimi M, Sobti P, Raiola G, El Kholy M. Growth hormone deficiency in adults with thalassemia: an overview and the I-CET recommendations. Georgian Med News 2013;(222):79-88.
119. De Sanctis V, Soliman AT, Candini G, Kattamis C, Raiola G, Elsedfy H. Liver Iron Concentration and Liver Impairment in Relation to Serum IGF-1 Levels in Thalassaemia Major Patients: A Retrospective Study. Mediterr J Hematol Infect Dis 2015;7(1):e2015016.
120. Molitch ME, Clemmons DR, Malozowski S, Merriam GR, Vance ML, Endocrine S. Evaluation and treatment of adult growth hormone deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2011;96(6):1587-1609.
121. Chanson P, Arnoux A, Mavromati M, Brailly-Tabard S, Massart C, Young J, Piketty ML, Souberbielle JC, Investigators V. Reference Values for IGF-I Serum Concentrations: Comparison of Six Immunoassays. J Clin Endocrinol Metab 2016;101(9):3450-3458.
122. Yuen KCJ, Johannsson G, Ho KKY, Miller BS, Bergada I, Rogol AD. Diagnosis and testing for growth hormone deficiency across the ages: a global view of the accuracy, caveats, and cut-offs for diagnosis. Endocr Connect 2023;12(7).
123. US FDA. Drug Trials Snapshots: Marcrilen. 01 April 2018. Available at: https://www.fda.gov/drugs/drug-approvals-and-databases/drug-trials-snapshots-marcrilen. Accessed: 05 Dec 2024.
124. EMA. Ghryvelin (previously Macimorelin Aeterna Zentaris). Available at: https://www.ema.europa.eu/en/medicines/human/EPAR/ghryvelin. Accessed: 05 Dec 2024.
125. Garcia JM, Swerdloff R, Wang C, Kyle M, Kipnes M, Biller BM, Cook D, Yuen KC, Bonert V, Dobs A, Molitch ME, Merriam GR. Macimorelin (AEZS-130)-stimulated growth hormone (GH) test: validation of a novel oral stimulation test for the diagnosis of adult GH deficiency. J Clin Endocrinol Metab 2013;98(6):2422-2429.
126. Garcia JM, Biller BMK, Korbonits M, Popovic V, Luger A, Strasburger CJ, Chanson P, Medic-Stojanoska M, Schopohl J, Zakrzewska A, Pekic S, Bolanowski M, Swerdloff R, Wang C, Blevins T, Marcelli M, Ammer N, Sachse R, Yuen KCJ. Macimorelin as a Diagnostic Test for Adult GH Deficiency. J Clin Endocrinol Metab 2018;103(8):3083-3093.
127. Giusti A, Pinto V, Forni GL, Pilotto A. Management of beta-thalassemia-associated osteoporosis. Ann N Y Acad Sci 2016;1368(1):73-81.
128. Vogiatzi MG, Macklin EA, Fung EB, Cheung AM, Vichinsky E, Olivieri N, Kirby M, Kwiatkowski JL, Cunningham M, Holm IA, Lane J, Schneider R, Fleisher M, Grady RW, Peterson CC, Giardina PJ, Thalassemia Clinical Research N. Bone disease in thalassemia: a frequent and still unresolved problem. J Bone Miner Res 2009;24(3):543-557.
129. Charoenngam N, Rittiphairoj T, Ponvilawan B. Fracture prevalence in thalassemia: a systematic review and meta-analysis. Arch Osteoporos 2021;16(1):171.
130. Baldan A, Giusti A, Bosi C, Malaventura C, Musso M, Forni GL, Volpato S, Zuliani G, Borgna-Pignatti C. Klotho, a new marker for osteoporosis and muscle strength in beta-thalassemia major. Blood Cells Mol Dis 2015;55(4):396-401.

131. Baldini M, Marcon A, Cassin R, Ulivieri FM, Spinelli D, Cappellini MD, Graziadei G. Beta-thalassaemia intermedia: evaluation of endocrine and bone complications. Biomed Res Int 2014;2014:174581.
132. Fung EB, Harmatz PR, Milet M, Coates TD, Thompson AA, Ranalli M, Mignaca R, Scher C, Giardina P, Robertson S, Neumayr L, Vichinsky EP, Multi-Center Iron Overload Study G. Fracture prevalence and relationship to endocrinopathy in iron overloaded patients with sickle cell disease and thalassemia. Bone 2008;43(1):162-168.
133. NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis, and Therapy. Osteoporosis prevention, diagnosis, and therapy. JAMA 2001;285(6):785-795.
134. Black DM, Rosen CJ. Clinical Practice. Postmenopausal Osteoporosis. N Engl J Med 2016;374(3):254-262.
135. Johansson H, Siggeirsdottir K, Harvey NC, Oden A, Gudnason V, McCloskey E, Sigurdsson G, Kanis JA. Imminent risk of fracture after fracture. Osteoporos Int 2017;28(3):775-780.
136. Gordon CM, Leonard MB, Zemel BS, International Society for Clinical D. 2013 Pediatric Position Development Conference: executive summary and reflections. J Clin Densitom 2014;17(2):219-224.
137. Shuhart C, Cheung A, Gill R, Gani L, Goel H, Szalat A. Executive Summary of the 2023 Adult Position Development Conference of the International Society for Clinical Densitometry: ≤ Reporting, Follow-up BMD Testing and Trabecular Bone Score Application and Reporting. J Clin Densitom 2024;27(1):101435.
138. Wong P, Fuller PJ, Gillespie MT, Kartsogiannis V, Kerr PG, Doery JC, Paul E, Bowden DK, Strauss BJ, Milat F. Thalassemia bone disease: a 19-year longitudinal analysis. J Bone Miner Res 2014;29(11):2468-2473.
139. Rossini M, Adami S, Bertoldo F, Diacinti D, Gatti D, Giannini S, Giusti A, Malavolta N, Minisola S, Osella G, Pedrazzoni M, Sinigaglia L, Viapiana O, Isaia GC. Guidelines for the diagnosis, prevention and management of osteoporosis. Reumatismo 2016;68(1):1-39.
140. Bertoldo F, Cianferotti L, Di Monaco M, Falchetti A, Fassio A, Gatti D, Gennari L, Giannini S, Girasole G, Gonnelli S, Malavolta N, Minisola S, Pedrazzoni M, Rendina D, Rossini M, Chiodini I. Definition, Assessment, and Management of Vitamin D Inadequacy: Suggestions, Recommendations, and Warnings from the Italian Society for Osteoporosis, Mineral Metabolism and Bone Diseases (SIOMMMS). Nutrients 2022;14(19).
141. Bhardwaj A, Swe KMM, Sinha NK. Treatment for osteoporosis in people with beta-thalassaemia. Cochrane Database Syst Rev 2023;5(5):CD010429.
142. Voskaridou E, Ntanasis-Stathopoulos I, Papaefstathiou A, Christoulas D, Dimopoulou M, Repa K, Papatheodorou A, Peppa M, Terpos E. Denosumab in transfusion-dependent thalassemia osteoporosis: a randomized, placebo-controlled, double-blind phase 2b clinical trial. Blood Adv 2018;2(21):2837-2847.
143. Giusti A. Bisphosphonates in the management of thalassemia-associated osteoporosis: a systematic review of randomised controlled trials. J Bone Miner Metab 2014;32(6):606-615.
144. Gagliardi I, Celico M, Gamberini MR, Pontrelli M, Fortini M, Carnevale A, Napoli N, Zatelli MC, Ambrosio MR. Efficacy and Safety of Teriparatide in Beta-Thalassemia Major Associated Osteoporosis: A Real-Life Experience. Calcif Tissue Int 2022;111(1):56-65.
145. Fuggle NR, Reginster JY, Al-Daghri N, Bruyere O, Burlet N, Campusano C, Cooper C, Perez AD, Halbout P, Ghi T, Kaufman JM, Kurt A, Matijevic R, Radermecker RP, Tuzun S, Veronese N, Rizzoli R, Harvey NC, Brandi ML, Brandi ML. Radiofrequency echographic multi spectrometry (REMS) in the diagnosis and management of osteoporosis: state of the art. Aging Clin Exp Res 2024;36(1):135.

07 FERTILITY AND PREGNANCY

Authors: **Marina Baldini[1], Maddalena Casale[2], Yi Li[3], Farzana Sayani[4,5], James Smith[3], Evangelia Vlachodimitropoulou[6], Sylvia Titi Singer[7]**

[1.] Unit of Medicine and Metabolic Disease, Department of Internal Medicine, IRCCS Fondazione Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

[2.] Centre of Paediatric Haematology and Oncology, Department of Woman, Child and General and Specialised Surgery, University of Campania Luigi Vanvitelli, Naples, Italy

[3.] Department of Urology and Center for Reproductive Sciences, University of California San Francisco (UCSF), San Francisco, CA, USA

[4.] Red Blood Cell Disorders Program, Division of Medical Oncology and Haematology, Department of Medicine, University Health Network, University of Toronto, Toronto, Canada

[5.] Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

[6.] Department of Hematology/Oncology, University of California San Francisco (UCSF) Benioff Children's Hospital Oakland, Oakland, CA, USA

[7.] Department of Obstetrics and Gynaecology, King's College Hospital, London, United Kingdom

Competing interest: None to disclose.

1. INTRODUCTION

As a follow on to Chapter 6, which featured aspects related to delayed puberty, female and male hypogonadism in patients with transfusion-dependent β-thalassaemia (TDT), this Chapter takes a closer look at fertility in both men and women, and considerations for the care of women who attempt to achieve successful pregnancy. Early fertility evaluation, optimal management of iron overload and organ function, and application of advances in reproductive technology and prenatal screening are essential to achieving patients' goals. With more women with TDT seeking pregnancy, ensuring the best outcomes for both mother and baby require concerted and collaborative efforts between the haematologist, obstetrician, and various healthcare specialists [1].

2. FEMALE FERTILITY

Current care for women with TDT with regular monitoring and adequate iron chelation, along with advances in reproductive technologies, made pregnancy not only possible but also increasingly safe with marked improvements in maternal and foetal survival and outcomes. It is however evident that a high rate of fertility problems exist, estimated at 40-90%, depending on the time-period and the population studied [2, 3]. Women will present with primary or secondary amenorrhoea or failure to conceive.

Transfusion-induced haemosiderosis through damage to the pituitary gland and to the hypothalamic-pituitary axis can cause hypogonadotropic hypogonadism, the primary cause of developing subfertility. To a lesser extent, direct iron-induced ovarian damage can occur, mostly with higher iron levels and older age (mid-late 30's) impairing oocyte number and measures of ovarian reserve compared to age-matched controls [4]. However, even with iron overload compromising ovarian tissue, most women with TDT have sufficient ovarian function to allow successful ovulation induction with exogenous gonadotropin therapy. In addition, despite lower

ovarian reserve, oocyte quality is not significantly affected as shown in analysis of oocytes and embryos quality in *in vitro* fertilisation (IVF) cases performed in women with TDT [5].

2.1. Diagnosis and evaluation of subfertility

Gonadotropin levels (luteinizing hormone [LH] and follicle-stimulating hormone [FSH]) and oestradiol along with pubertal development can define hypogonadism in TDT, but they have poor predictive values of female reproductive potential.

Ovarian reserve testing determines the total number of immature oocytes (eggs) in the ovaries by a direct measure with transvaginal ultrasound obtaining ovarian antral follicle count (AFC) as well as ovarian volume, and by blood test of Anti-Mullerian hormone (AMH) level that typically correlates with AFC and can help predict fertility potential. Ovarian reserve naturally declines with age in women. In TDT, AMH and AFC decline more rapidly with higher iron load compared to age-matched women and they have an inverse relationship to ferritin levels and organ iron measures [6, 7].

The age at which a woman attempts pregnancy is therefore an important consideration and early introduction of the topic is helpful. Thalassaemia physicians should address family planning and offer evaluation and referral to a reproductive specialist for assessment and consideration of fertility preservation when appropriate. Other TDT complications such as diabetes, especially if poorly controlled, can also cause subfertility and should be addressed before planning a pregnancy. Pituitary magnetic resonance imaging (MRI) for measuring iron load and volume can detect and predict subfertility but is not routinely done. However, pituitary iron correlates with ferritin, cardiac, pancreatic and liver iron; and MRI of these organs can assist in prediction of reproductive status [8, 9].

Careful planning and identifying patients that might be 'too high' risk to undergo hormonal treatment for infertility is important. In addition, screening of the male partner to identify couples whose offspring are at risk for having an inherited thalassaemia should also be included in the pre-pregnancy evaluation. Couples in this situation might proceed with pregnancy and may choose to obtain prenatal diagnosis for determining if the embryo is affected with thalassaemia. Other options include undergoing IVF and preimplantation genetic testing for (PGT) with implantation of unaffected embryos, intrauterine insemination with donor sperm from a non-carrier, and adoption. Adoption or the use of donor sperm or egg can also be valid options for TDT women with severe organ damage avoiding a high-risk pregnancy.

Table 1 summarises key points for evaluation of subfertility in women with TDT.

Table 1. *Key points in evaluation of subfertility in women with transfusion-dependent β-thalassaemia.*

Evaluation
1. After puberty, monitor annual LH/FSH and oestradiol to assess for the developing and progression of hypogonadotropic hypogonadism.
2. Starting in early 20's, obtain AMH level to screen for ovarian reserve (if patient interested).
3. Referral for transvaginal ultrasound for AFC for ovarian reserve evaluation and consultation when considering pregnancy.

Abbreviations: LH, luteinizing hormone; FSH, follicle-stimulating hormone; AMH, Anti-Mullerian hormone; AFC, antral follicle count.

2.2. Induction of ovulation

Abnormalities in ovulation occur in 30-80% of adult women with TDT due to hypogonadotropic hypogonadism. Induction of ovulation may be indicated in women with primary amenorrhoea, secondary amenorrhoea, or those with normal menstrual function who fail to conceive, and in planned pregnancy where both partners have thalassaemia or carry the gene for it.

Although a majority of these patients no longer have pulsatile gonadotropins, induction of ovulation has been highly successful with ovulation achieved in 80% of patients [10]. A variety of ovulation induction regimens exist including gonadotropins, human menopausal gonadotropin (hMG), a mixture of FSH and LH occasionally with clomiphene citrate which stimulate development of follicles, as well as human chorionic gonadotropin (hCG) that trigger ovulation after follicle development. The dose and frequency of gonadotropin injections depend on the woman's response, which is evaluated by the number and size of the growing follicles and levels of oestradiol evaluated by blood testing and by transvaginal ultrasound scans [11]. The protocols are associated with increased risk of twin or triplet pregnancies and ovarian hyperstimulation syndrome which requires close observation and frequent hospital admission. Patients should be counselled regarding the risk of hyperstimulation syndrome, multiple pregnancies, ectopic pregnancy, and miscarriage. The use of gonadotropin-releasing hormone (GnRH) agonists for ovarian stimulation has shown to have a lower incidence of ovarian hyperstimulation; however, its use in thalassaemia might not be possible due to the compromised hypothalamic–pituitary axis in most patients with subfertility.

There are no data on harmful effects of iron chelation therapy during hormonal stimulation therapy; however, some women choose to hold iron chelation despite lack of data.

Table 2 summarises key points for induction of ovulation in women with TDT.

Table 2. *Key points in induction of ovulation in women with transfusion-dependent β-thalassaemia.*

Induction of ovulation
1. Transvaginal ultrasounds assess baseline follicle number and size and endometrium thickness. 2. Therapy with hMG or hCG preparation is given to induce ovulation. 3. Assess follicle size by ultrasound to determine if treatment should continue and/or increase the dose. 4. Check progesterone level: luteal support with progesterone may be required to assure ovulation occurs. 5. Pregnancy is confirmed by BhCG testing. 6. If unsuccessful consider another cycle or refer for further discussion and considerations for IVF if required.

Abbreviations: hMG, human menopausal gonadotropin; hCG, human chorionic gonadotropin; IVF, in vitro fertilisation.

2.3. Fertility preservation

Fertility preservation options include oocyte cryopreservation and ovarian tissue cryopreservation (OTC). Oocyte cryopreservation requires first induction of ovulation (hyperstimulation) which is feasible once the patient has reached menarche. OTC involves surgical removal of ovarian tissue capable of producing mature oocytes at a later time, which offers some advantage to oocyte cryopreservation alone [12].

Fertility preservation should be considered in some situations while oocytes and ovarian tissues are still attainable, taking into account the risk of ovarian insufficiency which increases with age. However, challenges such as special health risks, financial barriers, and access to care need to be addressed. Fertility preservation is also relevant to women with TDT planning haematopoietic stem cell transplantation (HSCT) or gene therapy, which involves haematotoxic treatment [13]. If patients elect for oocyte cryopreservation, protocols utilising GnRH agonist could be considered if patients' hypothalamic-pituitary axis is still intact, since this offers the best chance for obtaining high quality oocytes with a more favourable side effect profile [14]. This is a developing field which requires more study in TDT.

These evaluations and counselling patients about their options, going through ovulation stimulation and assisted reproductive treatment (ART) take time, are psychologically challenging, and have variable outcomes. An early referral to an experienced reproductive team especially in women that are older or those with comorbidities, is of utmost importance.

3. PREGNANCY

3.1. Pre-pregnancy planning

Pregnancy in TDT is associated with significant risks to both mother and foetus. Cardiac disease, liver disease, endocrinopathies, infections, and thrombosis risk contribute to the increased risks of pregnancy. However, early planning, before conception and optimisation of a woman's health status, can help reduce complications and result in a safe pregnancy. Pre-pregnancy planning aims to optimise a woman's overall health, including thalassaemia-specific care, and involves partner testing and genetic counselling. Assessment of fertility of both patient and partner are important to undertake early to avoid delay and to allow discussion of options for achieving a pregnancy. A multidisciplinary team involving a haematologist, a thalassaemia nurse-specialist, a specialist in reproductive medicine, an obstetrician, a cardiologist, an endocrinologist, a genetic counsellor, and a psychologist are important to optimise outcomes.

Pre-pregnancy planning involves i) evaluating and optimising the woman's current health status, ii) reviewing medications that may need adjusting, and iii) discussing the personalised risks associated with pregnancy.

3.2. Evaluating and optimising thalassaemia care

Each woman with TDT contemplating pregnancy should be evaluated by the multidisciplinary team to evaluate and optimise complications of thalassaemia with special emphasis on cardiac function and iron burden, liver iron load, endocrinopathies, and viral status (Table 3).

Table 3. *Optimisation of thalassaemia care pre-conception.*

Item	Investigation/Management
Cardiac function	• Electrocardiogram, echocardiogram
Liver function	• Serum ferritin, alanine/aspartate aminotransferase, bilirubin
Iron burden – liver, heart	• Liver MRI with iron quantification in mg/gram dry weight • Cardiac MRI with T2* in ms
Gallstones screen	• Abdominal ultrasound
Endocrinopathies	• Calcium and vitamin D levels • Bone DXA scan • Glucose tolerance test, fructosamine • Thyroid function
Infections	• Test for hepatitis B, hepatitis C, HIV status • Check rubella titres • Vaccination review and administration where due
Partner screening	• Test partner and offer genetic counselling where indicated
Fertility evaluation	• Referral to fertility expert

Abbreviations: MRI, magnetic resonance imaging; DXA, dual-energy X-ray absorptiometry; HIV, human immunodeficiency viruses.

3.2.1. Cardiac iron and function

Cardiac complications are the leading cause of death in individuals with TDT, and pre-existing cardiac dysfunction, pulmonary hypertension, arrhythmia, or iron overload significantly increase the risk of complications in pregnancy. Baseline evaluation and monitoring during pregnancy is important for best outcomes. The increased metabolic demands and blood volume in pregnancy exert significant stress on the maternal cardiovascular system [15]. Many of the changes are transient and return to normal after delivery. Complications including dysrhythmia, right ventricular dysfunction, and cardiac failure in pregnant women with TDT, have been reported in 1.1% to 15.6% of patients [16, 17]. Since iron chelation is held during pregnancy, the resulting increase in iron burden may worsen cardiac function in those with marginal function or cardiac iron overload [18, 19]. It is thus essential to optimise chelation and reduce liver and cardiac iron burden before embarking on pregnancy.

All women with TDT should have a cardiac evaluation, including echocardiogram, electrocardiogram, 24-hour Holter monitor for rhythm disorders, and a cardiac MRI T2* where available [20]. Since most non-invasive cardiac tests are insensitive for detecting early cardiac iron loading, MRI using gradient T2* measurements to quantify iron burden is key [21]. Ideally, a cardiac T2* MRI >20 ms should be achieved before pregnancy. Patients with cardiac iron overload (T2* MRI

<20 ms), left ventricular dysfunction, or arrhythmias should be discouraged from planning a pregnancy at that time, and aim to intensify iron chelation. Chelation intensification can improve cardiac function over several months, and thus repeat risk-benefit assessment should be performed regularly for a woman hoping to conceive [22].

3.2.2 Liver iron and function

Prior to pregnancy, all women should have biochemical tests including serum ferritin, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) to evaluate liver function, and a liver MRI to evaluate liver iron load. The liver iron concentration (LIC) should be less than 7 mg/g dry weight for best outcomes, especially since LIC increases during pregnancy when chelation is held [18]. If the LIC is significantly elevated, chelation should be intensified to optimise the LIC before conception. An abdominal ultrasound should be considered to evaluate for the presence of gallstones. In symptomatic patients with evidence of biliary sludge or gallstones, cholecystectomy may be considered prior to pregnancy.

3.2.3. Endocrine evaluation

Prior to embarking on pregnancy, women with TDT should be comprehensively evaluated and managed for thalassaemia-associated endocrinopathies in collaboration with an endocrinologist. Since osteopenia and osteoporosis are common in TDT, plain radiography of the spine and bone mineral density with dual-energy X-ray absorptiometry (DXA) of the hip and spine should be performed, and treatment initiated where indicated. Vitamin D levels should be checked and optimised before and during pregnancy to optimise bone health. Endocrine evaluation should also involve screening for diabetes with an oral glucose tolerance test, optimisation of glucose control and evaluation of thyroid function.

3.2.4. Infections

All patients should be screened for human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), rubella, and other pathogens that may affect pregnancy. Hepatitis B immune status should be reviewed and vaccines administered where indicated. Splenectomised patients should have their vaccinations updated. Collaboration with an infection disease specialist and therapy should be recommended for women with HIV and/or HCV.

3.2.5. Medication review

Prior to conception, medications should be reviewed since some may need to be optimised, and others may need to be discontinued (Table 4). Education around healthy eating, smoking cessation, and alcohol abstinence are also appropriate at this time. Folic acid demand increases during pregnancy and thus an increased dose of 5 mg daily is recommended at least 3 months prior to conception to reduce the risk of neural tube defects. Vitamin D and calcium should be optimised to preserve bone health. Thalassaemia disease-modifying therapies including luspatercept, pyruvate kinase activators, and hydroxyurea should be stopped at least 3 months prior to conception and ovulation/spermatogenesis induction due to concern for teratogenicity and/or limited data on effect in a developing foetus. Oral iron chelators including deferiprone and deferasirox have been shown to be teratogenic in animal models and should be switched to deferoxamine at least 3 months prior to attempting ovulation induction or conception [23]. All chelation should, however, be stopped as soon as a pregnancy is confirmed, and the need for chelation re-evaluated after the first trimester. Angiotensin-converting enzymes (ACE)

inhibitors should be discontinued prior to conception. Bisphosphonates are contraindicated during pregnancy since they have a long half-life and should be stopped 6 months prior to conception. Oral hypoglycaemic agents and thyroid hormone replacement should be evaluated in collaboration with an endocrinologist. For those on antiviral treatments, changes may be needed, and thus early planning and engagement with the infectious disease team is key.

Table 4. *Medication review prior to conception in women with transfusion-dependent β-thalassaemia.*

Medication	Action
Folic acid	• Start 5 mg daily at least 3 months before conception
Vitamin D supplementation	• Optimise dose
Disease-modifying therapies*	• Stop at least 3 months prior
Iron chelator	• Customise based on degree of liver and cardiac iron load • Goal pre-conception: LIC <7 mg/g dry weight, cardiac T2* >20 ms • If not at goal: increase chelation, delay pregnancy • If at target goal: stop oral agents and change to deferoxamine at least 3 months prior to conception
ACE inhibitors	• Stop
Diabetes medicines	• Review in collaboration with endocrinologist
Thyroid hormone replacement	• Review in collaboration with endocrinologist
Bisphosphonates	• Stop at least 6 months prior
Antiviral therapy	• Review in collaboration with infectious disease specialist

**Luspatercept, pyruvate kinase activators, hydroxyurea.*
Abbreviations: LIC, liver iron concentration; ACE, angiotensin-converting enzyme.

4. RISKS ASSOCIATED WITH PREGNANCY AND OVERALL MANAGEMENT

Collaboration with an obstetrical team with experience caring for patients with TDT is important to ensure best outcomes. It is also important to start conversations on risks of pregnancy early to equip the patient and the couple with information on family planning.

Patients should be informed that pregnancy itself does not alter the natural progression of thalassaemia. There is no strong evidence to suggest that the incidence of antepartum haemorrhage (APH) is higher in thalassaemia patients compared to the general population, where it affects 2-5% of pregnancies [24]. However, the risk of pre-eclampsia is elevated in TDT, with a prevalence of up to 11.3%, compared to approximately 4.6% in the general population, thought to be due to endothelial dysfunction and an imbalance in angiogenesis [18, 25-29].

The risk of gestational diabetes mellitus is also increased, reported at a prevalence of 18-25% in women with TDT, compared to 3-10% in the general population.

Despite increased clotting factors and decreased fibrinolysis during pregnancy [30], the incidence of venous thromboembolism (VTE) in TDT during pregnancy remains low, likely due to the mitigating effects of regular transfusion protocols [18, 31]. The incidence observed in pregnant TDT women is 0.92% which is the same as in the general pregnant population (as opposed to a higher rate of 3.7% in non-transfusion-dependent β-thalassaemia [NTDT]) [18].

Heart failure was a significant risk, particularly in earlier years before the widespread use of modern cardiac MRI and iron chelation therapies. Historical data suggest that heart failure risks were similar in TDT (1.6%) and NTDT (1.1%) patients, with maternal mortality in TDT as high as 3.7% [18]. However, with current management strategies, these risks are now rare. It is important to note that the main pregnancy risk to the mother remains cardiac complications, which can be minimised by ensuring optimal cardiac function and good control of iron overload prior to pregnancy.

The rate of miscarriage is not significantly higher than in the general population. However, the proportion of small-for-gestational-age infants is higher in TDT (18.1%) compared to the 11% observed in the general population [18]. The overall proportion of preterm birth is significantly higher in TDT (25.2%) than in NTDT (8.9%) [18], exceeding the global incidence of 5-18% [32].

There is a significant increase in transfusion requirements to maintain a safe haemoglobin range. Transfusion volumes may increase by 30-50% due to anaemia exacerbated by an increase in plasma volume during pregnancy. The cessation of chelation therapy in most TDT pregnant women leads to a notable rise in serum ferritin level, reported as a mean ferritin increase of 1005 ng/mL in TDT [18]. The high exposure to blood units also increases the risk of red blood cell alloimmunisation and proper cross-matching protocols need to be applied.

Bone deformities and cephalopelvic disproportion (CPD) pose significant challenges during pregnancy and delivery. CPD may contribute to the high rates of Caesarean delivery, observed at 83.9% in TDT and 67% in NTDT [18], which are substantially higher than the global rate of 21% [33]. Osteoporosis and scoliosis are common in TDT, despite transfusion therapy, resulting in vertebral bodies with reduced height and the segmental position of the conus may be lower than predicted. It is therefore important to attempt and reduce the severity of osteoporosis pre-pregnancy by hormone replacement and bisphosphonates, when required. This can increase bone density and reduce skeletal abnormalities, making spinal anaesthesia more feasible. Bisphosphonates should be stopped 6 months prior to pregnancy due to their long biological half-life.

4.1. Management of pregnancy

Pregnancy outcomes in thalassaemia patients have significantly improved due to advances in transfusion practices, iron chelation therapy, and adequate pre-conception planning [34-37]. However, these pregnancies are considered high-risk and require careful management by a multidisciplinary team that includes an obstetrician, midwife, haematologist, cardiologist, and endocrinologist.

4.1.1. Monitoring and medications

As mentioned earlier, folic acid supplementation (5 mg daily) should start at least 3 months before conception. Adequate vitamin D level with routine administration should also be optimised during pregnancy. Due to the increased risk of pre-eclampsia, prophylactic administration of low-dose aspirin (150-160 mg daily) is recommended, starting from 12-16 weeks of gestation, and continuing until 36 weeks [38]. Splenectomised patients require penicillin prophylaxis to prevent infections from encapsulated bacteria, such as *Neisseria meningitidis, Streptococcus pneumoniae*, and *Haemophilus influenzae* type b.

Pregnant patients with TDT should undergo monthly reviews until 28 weeks of gestation, after which biweekly assessments should be conducted until delivery. The multidisciplinary team must ensure the provision of both general and specialised antenatal care tailored to the needs of the patient.

4.1.2. Iron chelation and cardiac monitoring

Pre-conception optimisation of iron burden is critical as cardiac failure, arrythmias, and new endocrinopathies can develop when chelation is paused during pregnancy. Cardiac function monitoring is essential in pregnant patients with TDT, and echocardiography and cardiac T2* assessments by MRI can be safely conducted. If there have been no cardiac assessments in the 12 months preceding conception, imaging to evaluate iron burden and cardiac function should be performed during pregnancy. It should be noted that women who have successfully reversed myocardial iron overload may still develop arrhythmias and should be carefully monitored for palpitations during pregnancy.

Iron chelation therapy during pregnancy must be approached with caution, particularly in the first trimester, due to the lack of comprehensive safety data. Deferoxamine remains the only chelation agent with an established safety profile for use in the second and third trimesters. Over three decades, studies on more than 45 pregnancies in iron overloaded patients receiving deferoxamine have shown no adverse effects on the foetus or neonate [18].

For patients without significant myocardial iron loading (cardiac MRI T2* >20 ms) and a normal echocardiogram, cardiac assessment should be conducted early in the third trimester to develop a delivery plan, focusing on ejection fraction monitoring.

Women with significant myocardial iron loading (cardiac MRI T2* <10 ms) face a high risk of cardiac decompensation during pregnancy. These patients should begin low-dose subcutaneous deferoxamine (20 mg/kg/day), administered at least 4-5 days per week, from 20 to 24 weeks of gestation under the close supervision of haematology and cardiology specialists. Regular cardiology evaluations are critical throughout pregnancy, with a particular focus on monitoring ejection fraction. A pregnant woman with a reduction in left ventricular ejection fraction greater than 10%, evidence of heart failure by echocardiogram, or the onset of arrhythmias necessitates the initiation of chelation therapy. In cases of cardiac decompensation, an intravenous deferoxamine infusion over 24 hours should be started at an optimal dose of 40-50 mg/kg/day.

Patients presenting with symptoms such as palpitations, shortness of breath, or leg oedema require immediate cardiac assessment [39-41], as these may indicate worsening cardiac function

or new-onset arrhythmias. This is especially important for patients with a history of myocardial iron overload. Acute heart failure symptoms in the first trimester are particularly concerning and associated with adverse outcomes. Echocardiography showing a declining ejection fraction or increasing ventricular volumes should prompt immediate intervention to prevent heart failure [42]. For patients with a history of myocardial iron deposition or borderline cardiac function, deferoxamine may be considered in the final trimester or peri-delivery period, especially since prolonged labour can heighten the risk of cardiac decompensation. Decisions regarding chelation therapy during pregnancy should be made in a multidisciplinary approach, including a perinatologist, haematologists, and cardiologists, to ensure the best maternal and foetal outcomes.

4.1.3. Endocrine monitoring

Thyroid function tests (free thyroxine [FT4] and thyroid-stimulating hormone [TSH]) should be conducted periodically throughout pregnancy. In cases of hypothyroidism, the dose of thyroxine should be adjusted accordingly. All women should be screened for gestational diabetes at 16 weeks of gestation. If the initial screening is normal, it should be repeated between 24-28 weeks. For those diagnosed with diabetes, monthly monitoring of serum fructosamine levels is recommended, and patients should be reviewed in a specialist diabetic pregnancy clinic.

4.1.4. Monitoring for foetal growth restriction

An early viability ultrasound scan should be offered at 7-9 weeks of gestation to confirm pregnancy viability. Starting from 24-26 weeks of gestation, serial ultrasound scans should be conducted to monitor foetal growth closely and detect any early signs of foetal growth restriction and consider intervention. The standard scans, the 12-week for nuchal translucency assessment and a 20-week foetal anatomy scan should be performed according to standard care protocols. Maintaining a pretransfusion haemoglobin level of no less than 10 g/dL, as also typically recommended for other indications, can mitigate the higher incidence of small for gestational age (SGA) foetuses in TDT pregnancies.

4.1.5. Transfusion requirements

As mentioned earlier, maintaining pre-transfusion haemoglobin levels above 10 g/dL is critical to prevent maternal and foetal morbidity, with post-transfusion haemoglobin levels ideally reaching no less than 12 g/dL. This may result in an increase in transfusion volumes by 30-50% to meet the heightened red blood cell demand.

4.1.6. Antenatal anticoagulation management

The incidence of VTE in TDT during pregnancy is low. Still, low-molecular-weight heparin (LMWH) thromboprophylaxis is strongly recommended for all antenatal hospital admissions. Additionally, it should be considered from 28 weeks of gestation until six weeks postpartum if there are additional risk factors such as elevated platelet counts, prior splenectomy, or a history of VTE in an early time of pregnancy. For patients with platelet counts exceeding 600 $\times 10^9$/L, combining LMWH with low-dose aspirin (75-160 mg daily) is recommended to further reduce VTE risk.

A schedule for antenatal care is provided in Table 5.

Table 5. *Schedule of antenatal care in pregnancy women with transfusion-dependent β-thalassaemia.*

Gestational Age	Care and Monitoring
Early pregnancy	• MDT visit; obstetrician, haematologist, cardiologist, and endocrinologist: - Assess endocrine, hepatic, and cardiac status - Educate about pregnancy in thalassaemia - Review partner results - Review partner results and discuss prenatal diagnosis if appropriate - Conduct baseline blood tests and screen for infections - Prescribe folic acid (5 mg daily) and penicillin prophylaxis if splenectomised - VTE risk assessment and start low-dose daily aspirin - Discuss and update vaccination status
7-9 weeks	• Offer a viability scan to confirm pregnancy
11-14 weeks	• Conduct the first trimester ultrasound scan, including nuchal translucency
16 weeks	• MDT review • Routine antenatal care as per NICE guidelines (NG201) • Perform GTT
20 weeks	• MDT review • Perform the second trimester ultrasound for foetal anatomy
20-24 weeks	• Assess cardiac iron overload - If cardiac MRI T2* <10 ms, commence low-dose deferoxamine - If cardiac MRI T2* >20 ms, continue monitoring without deferoxamine • Check thyroid function
24 weeks	• MDT review • Perform ultrasound for foetal growth and amniotic fluid volume
26-28 weeks	• GTT for non-diabetic patients • If diabetic - Obtain monthly fructosamine level - Aim for fructosamine level <300 nmol/L
28 weeks	• MDT review • Perform ultrasound for foetal biometry • Specialist cardiology review to plan delivery • Consider VTE thromboprophylaxis
30 weeks	• Routine review as per NICE antenatal guidelines
32 weeks	• MDT review • Perform ultrasound for foetal growth and amniotic fluid volume

34 weeks	• Routine review
36 weeks	• MDT review • Perform ultrasound for foetal growth and amniotic fluid volume • Discuss timing and mode of delivery and analgesia • Obstetric anaesthetic review for potential complications during delivery, especially in patients with skeletal deformities that may impact epidural anaesthesia • Cross-match blood and prepare for delivery • Discontinue Aspirin
38 weeks	• Routine review • Consider induction of labour if diabetic
40 weeks	• Routine review and discussion on induction if not yet delivered

Abbreviations: MDT, multidisciplinary team; VTE, venous thromboembolism; NICE, National Institute for Health and Care Excellence; GTT, glucose tolerance test; MRI, magnetic resonance imaging.

4.2. Managing delivery and postpartum care

4.2.1. Timing and mode of delivery

The timing of delivery in pregnant women with TDT should be individualised, considering both maternal and foetal conditions. While there is no strict consensus on the optimal timing, delivery is often recommended prior to the due date of 40 weeks of gestation in cases of uncomplicated pregnancies. This approach aims to minimise the risks associated with prolonged pregnancy, such as worsening maternal anaemia, increased iron overload, and potential cardiac complications. In cases where the mother has pre-pregnancy morbidities such as diabetes or cardiac disease, earlier delivery may be considered to avoid further maternal or foetal complications. Maternal health, foetal considerations and woman's wish should all impact timing and mode of delivery; currently C-section is more commonly performed, estimated at 84% of cases in TDT [18].

4.2.2. Resumption of iron chelation therapy and breastfeeding

The use of oral iron chelators, such as deferasirox or deferiprone, during breastfeeding is not well supported by clinical data [43, 44], and generally avoided due to the potential risks to the infant. The timing of resuming chelation therapy should be individualised, balancing the need to manage iron overload with safe concentrations in breast milk. Although data are limited, deferoxamine concentration in breast milk is considered very low, and it is not absorbed orally by the infant [45], it is therefore the preferred option during lactation due to its safety profile.

Worsening iron accumulation, cardiac failure, and arrhythmias in the peripartum/postpartum period can occur requiring intense chelation after delivery with subcutaneous deferoxamine at 40-60 mg/kg/day for 5 days a week.

Breastfeeding should be encouraged in all cases except for those who are HIV and/or hepatitis C RNA-positive and/or HBV surface antigen (HBsAg) positive due to the risk of vertical transmission through breast milk. Calcium and vitamin D supplementation should continue during breast-

feeding to maintain bone health. However, bisphosphonate therapy, used to treat osteoporosis, should only be resumed after the cessation of breastfeeding to avoid potential risks to the infant [46].

Although lactation is a physiological state of hypo-oestrogenism (serum levels 50-125 pmol/L), patients with profound hypogonadism and undetectable oestrogen levels postpartum may struggle to breastfeed unless re-prescribed oestradiol-only hormone replacement therapy at very low dose, 25 mcg patches.

4.2.3. Venous thromboembolism prophylaxis

The true magnitude of postpartum VTE risk in women with TDT is difficult to ascertain, as most patients in studies received prophylactic subcutaneous LMWH for 6 weeks postpartum. It should be noted, however, that the postpartum period presents a significantly higher daily risk of VTE in the general population [47-49].

In patients with TDT who have undergone splenectomy, the risk of VTE is notably elevated [50] and thromboprophylaxis is recommended in particular if other risk factors for thrombosis exist [51]. LMWH prophylaxis should be administered while the patient is in the hospital and for at least 7 days following delivery. Prolonged anticoagulation for 6 weeks should be considered in those with additional risk factors such as a platelet count above 600×10^9/L. In the event of miscarriage or termination of pregnancy, the risk of VTE persists, and LMWH prophylaxis should be provided during and for at least 7 days following the loss.

4.2.4. Postpartum care and support

Postpartum monitoring should include the assessment of iron overload status and cardiac function. A liver and cardiac MRI at approximately 3 months postpartum, can guide the resumption and adjustment of iron chelation therapy, unless myocardial iron overload necessitates earlier evaluation.

Patients should receive counselling regarding contraception. If intrauterine devices are used, women should be warned about possible higher risk of infection. Oestrogen-containing birth control pills are generally not recommended due to the increased risk of VTE, instead, the progesterone-only pill or barrier methods are typically advised.

The postpartum period can be emotionally and physically challenging. Providing comprehensive psychosocial support, including counselling and access to support groups, is important. Coordination of care among the patient's healthcare providers is necessary to ensure a smooth transition from pregnancy to the postpartum period.

5. MALE FERTILITY

Men and adolescents with TDT commonly develop hypogonadotropic hypogonadism (see Chapter 6) resulting in decreased serum testosterone level and low sperm count thus a diminished reproductive capacity. Whereas pregnancy has been often reported in women with TDT, paternity is less common in men with TDT. Prior studies estimate that more than 50% of male TDT patients have some delayed pubertal development, sexual dysfunction, and infertility [52]. The prevalence and severity of hypogonadism varies among studies, depending on patients' age, transfusion frequency and starting age and effectiveness of iron chelation.

Pituitary iron deposition is the primary cause of hypogonadotropic hypogonadism [8]. Additionally, direct effects of iron overload on semen quality and subfertility may occur. Lower sperm concentration and lower ratio of sperm with normal morphology were shown, as well as high seminal plasma concentrations of iron and higher markers of sperm DNA damage. Some of the iron toxicity is attributed to increased levels of reactive oxygen species that ultimately lead to cytotoxicity impacting directly testicular cell populations [53-56].

5.1. Evaluation

Hypogonadism and subfertility in TDT are complex and often require a multidisciplinary approach (Table 6). In adolescents, hypogonadism can present as delayed or absent onset of pubertal maturation. Adult-onset hypogonadism can present with decreased libido, erectile dysfunction, or infertility as well as less specific symptoms suggestive of androgen deficiency such as low energy and decreased motivation. Physical exam findings will show diminished facial and body hair, muscle mass, gynaecomastia, and small testes [52, 57]. While this is a relatively common endocrinopathy in this patient population, symptoms of hypogonadotropic hypogonadism are often attributed to the overall chronic disease state of TDT patients and can cause a delay in the evaluation for hypogonadism. Early identification and confirmation of the diagnosis by measurement of testosterone level can prevent long term morbidity, infertility, and other clinical impacts of prolonged hypogonadotropic hypogonadism. Hypogonadotropic hypogonadism was defined as LH and FSH levels <2 IU/L and testosterone concentration <3 ng/mL in a study of TDT men [53] and abnormal semen characteristics are classified based on the World Health Organization reference values [58].

Table 6. *Key points for the evaluation of hypogonadism and subfertility in men with transfusion-dependent β-thalassaemia.*

Evaluation
1. At puberty, evaluate for physical changes indicating testosterone such as facial and pubic hair, muscle mass, testicular size, and genital development. 2. If hypogonadism is suspected, obtain lab tests (testosterone, LH, FSH) and refer to endocrinology for evaluation. 3. Semen analysis should be obtained in patients interested in fertility with early referral to a male fertility specialist.

Abbreviations: LH; luteinizing hormone; FSH, follicle-stimulating hormone.

5.2. Treatment

5.2.1. Hormone replacement treatment

Testosterone replacement options for adults with hypogonadotropic hypogonadism include intramuscular, transdermal patches or gels, subcutaneous implant, or oral configurations based on what is best suited for the individual patient.

Male patients with onset of hypogonadotropic hypogonadism before completion of pubertal development generally have testes smaller than 5 mL in volume. In these patients, testosterone replacement therapy can be augmented with hCG monotherapy or hCG and FSH combination therapy for improved testicular growth and fertility outcomes.

5.2.2. Induction of spermatogenesis

Men with TDT and concerns for infertility should be evaluated by a reproductive urologist and an endocrinologist. A physical exam to rule out other possible causes of infertility such as varicoceles is indicated as well as assessment of the female partner for reproductive issues. Success rates of spermatogenesis induction in males with TDT are typically lower than those for ovulation induction female counterparts. In patients with moderate to severe iron overload, success rates were reported to be in the 10-15% range in older studies [10, 59]. However, success rates are likely higher in recent years thanks to improved iron chelation regimens and earlier intervention [60].

Initial treatment for induction of spermatogenesis is typically hCG monotherapy, with a goal of stimulating intra-testicular testosterone production that will initiate generation of spermatozoa. This treatment may also benefit overall serum testosterone levels and testosterone stability as compared to direct testosterone supplementation. While hCG treatment is more onerous and costly compared to testosterone supplementation, this approach is more effective. Successful hCG treatment can have long term persistent effects on spermatogenesis and fertility even after cessation of therapy [56].

If spermatogenesis is not achieved (i.e., a semen analysis does not demonstrate sperm production) after 6-12 months of hCG treatment, adding recombinant FSH (rFSH) or menotropins (mixture of FSH and LH) can be considered. These combined therapies are often expensive. If pregnancy is successful, FSH therapy can be stopped, and spermatogenesis may be maintained with hCG therapy alone [56].

To decrease the length of time (and cost) of using FSH or hCG, men can cryopreserve semen samples in preparation for future fertility treatments. If spontaneous pregnancy does not occur after two years of either treatment, ART should then be considered [52]. These fertility treatments can also utilise cryopreserved semen samples. In general, sperm banking should be considered in all patients who have an interest in future fertility unless they are azoospermic (Table 7).

Table 7. *Key points for induction of spermatogenesis in men with transfusion-dependent β-thalassaemia.*

Induction of spermatogenesis
1. Semen analysis and labs should be obtained as initial evaluation (testosterone, LH, FSH).
2. Fertility evaluation of female partner is critical.
3. Early referral to male fertility specialist is critical.
4. First line therapy for inducing spermatogenesis is hCG therapy for 6-12 months.
5. If no spermatogenesis, consider adding recombinant FSH (rFSH) or menotropins (mixture of FSH and LH).
6. In men with continued azoospermia, consider microTESE to evaluate for intra-testicular sperm.
7. Cryopreservation of sperm is recommended if future pregnancies are desired.

Abbreviations: FSH, follicle-stimulating hormone; hCG, human chorionic gonadotropin; LH; luteinizing hormone; microTESE, microsurgical testicular sperm extraction.

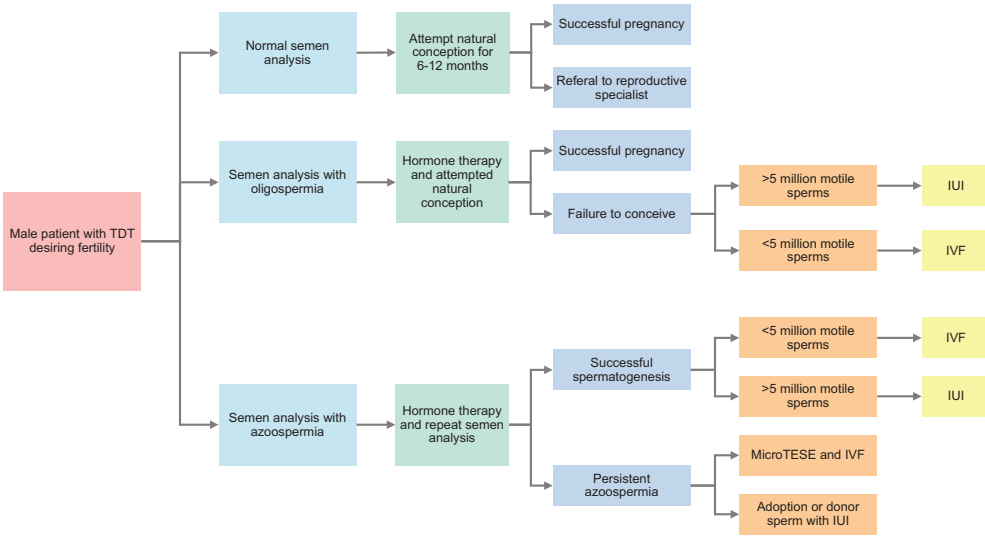
5.2.3. Assisted reproductive treatment

Recent advancements in ART have resulted in improved conception rates in TDT patients regardless of semen parameters. For patients with total motile sperm counts greater than 5 million sperms per mL, intrauterine insemination can be considered. In patients with even lower numbers of motile sperms, in IVF techniques such as intracytoplasmic sperm injection can result in a successful pregnancy.

Another option for those with persistent azoospermia after endocrine therapy is microsurgical testicular sperm extraction (MicroTESE). MicroTESE is a surgical procedure that can successfully identify sperms in approximately 50% of patients who have non-obstructive azoospermia [61]. If sperms are found during surgery, they can then be used in IVF. Men with persistent azoospermia should also be informed of other options for building their family by using donor sperms with intrauterine insemination and adoption.

Figure 1 summarises the approach to treatment of infertility in men with TDT.

Figure 1. *Infertility treatment approach in men with transfusion-dependent β-thalassaemia. Abbreviations: TDT, transfusion-dependent β-thalassaemia; microTESE, microsurgical testicular sperm extraction; IVF, in vitro fertilisation; IUI, intrauterine insemination.*



KEY POINTS AND RECOMMENDATIONS

1. Recommendations for evaluation and treatment of subfertility in women with transfusion-dependent β-thalassaemia (TDT).

- Discuss with adolescents and young adults with poor adherence to iron chelation the deleterious effect on future fertility potential (**Grade B, Class I**).
- Prior to discussion of therapy options, perform a comprehensive evaluation for fertility status in women with amenorrhoea, irregular menstrual cycle, indication of having hypogonadism, failure to conceive 6-12 months despite normal menstrual cycle (**Grade B, Class I**).
- Screen for fertility status with the following (**Grade B, Class I**):
  - Review past and current labs of hypothalamic-pituitary-ovarian axis (follicle-stimulating hormone [FSH], luteinizing hormone [LH], oestradiol, progesterone, prolactin).
  - Obtain Anti-Mullerian hormone [AMH] level and transvaginal ultrasound for antral follicle count (AFC) ovarian reserve and ovarian volume.
- Assess iron overload level (serum ferritin trend, cardiac, liver and pancreatic iron) and pituitary iron and volume if available for prediction of ovarian reserve (**Grade B, Class IIa**).
- Refer to an endocrinologist when planning pregnancy for evaluation of fertility status and other endocrinopathies (**Grade B, Class I**).
- Discuss therapy options for infertility including ovarian stimulation for timed intercourse, intrauterine insemination and *in vitro* fertilisation with embryo transfer (IVF-ET) and refer to a gynaecologist if wishing to proceed and become pregnant (**Grade B, Class I**).
- Prior to initiation of ovulation induction, discuss the potential complications and risks: chances of treatment failure, possibility of multiple pregnancy, hyperstimulation response, and need for close monitoring with blood tests and ultrasound (**Grade B, Class I**).
- Discuss considerations for fertility preservation (oocyte cryopreservation or ovarian tissue cryopreservation [OTC]) where appropriate (**Grade B, Class IIb**):
  - Younger TDT women with no partner who wish to do so.
  - Comorbidities that preclude undergoing ovulation stimulation but can permit ovarian tissue cryopreservation.
  - Older women (>30-35 years) with declining ovarian reserve markers.

2. Recommendations for pre-conception planning in women with TDT.

- Pre-pregnancy planning involves evaluating and optimising the woman's current health status including thalassaemia care, genetic counselling, and fertility evaluation (**Grade B, Class I**) (Table 3).
- Medications should be reviewed pre-conception including:
  - Start high dose folic acid at least 3 months prior to conception (**Grade A, Class 1**).
  - Optimise vitamin D supplementation (**Grade B, Class 1**).
  - Stop thalassaemia disease-modifying therapies (including luspatercept, pyruvate kinase activators, hydroxyurea) at least 3 months prior to conception (**Grade C, Class 1**).
  - Stop oral iron chelators and change to deferoxamine pre-conception (**Grade B, Class 1**).

- Stop angiotensin converting enzyme (ACE) inhibitors (**Grade A, Class 1**).
- Stop bisphosphonates at least 6 months before conception (**Grade A, Class 1**).
- Evaluate diabetes, thyroid, and antiviral medications with appropriate subspecialists (**Grade A, Class 1**).

- Early education and discussion of the personalised risks associated with pregnancy is important (**Grade C, Class I**).

3. Recommendations for management of pregnancy in women with TDT.

- Start high-dose folic acid (5 mg daily) three months prior to conception and continue throughout pregnancy to reduce neural tube defects (**Grade A, Class I**).
- Optimise vitamin D supplementation during pregnancy (**Grade B, Class 1**).
- Splenectomised patients should receive penicillin prophylaxis to prevent bacterial infections (**Grade B, Class I**).
- To prevent pre-eclampsia, administer low-dose aspirin (150-160 mg daily) from 12-16 weeks until 36 weeks of gestation (**Grade A, Class I**).
- Monthly reviews are recommended until 28 weeks, then biweekly until delivery, with both general and specialised antenatal care provided by a multidisciplinary team (**Grade C, Class I**).
- Perform cardiac imaging (echocardiography and cardiac magnetic resonance imaging [MRI] T2*) during pregnancy if no assessments were done in the 12 months before conception **(Grade B, Class II**).
- Assess cardiac function in the third trimester for patients without significant myocardial iron loading prior to pregnancy, to plan delivery and chelation therapy if needed (**Grade B, Class I**).
- All TDT patients with cardiac MRI T2* >20 ms before conception require specialist assessment in the third trimester (**Grade C, Class I**).
- Women with significant myocardial iron loading (cardiac MRI T2* <10 ms) should start low-dose deferoxamine from 20-24 weeks under specialist supervision (**Grade B, Class I**). Monitor ejection fraction regularly, and initiate or adjust chelation if cardiac function declines (**Grade B, Class I**).
- Regularly monitor thyroid function and adjust thyroxine dosage in hypothyroid patients (**Grade B, Class I**).
- Screen for gestational diabetes at 16 weeks and repeat at 24-28 weeks if initial screening is normal. Monthly serum fructosamine monitoring is recommended for those with diabetes (**Grade C, Class I**).
- Offer a viability scan at 7-9 weeks and serial growth scans from 24-26 weeks to monitor for foetal growth restriction (**Grade C, Class I**).
- In TDT, maintain pretransfusion haemoglobin levels above 10 g/dL to prevent maternal and foetal morbidity (**Grade B, Class I**).

- Recommend low molecular weight heparin (LMWH) thromboprophylaxis for all antenatal hospital admissions (**Grade B, Class I**). Consider LMWH from 28 weeks until six weeks postpartum in patients who have undergone splenectomy or have elevated platelet counts above 600 x10^9/L (**Grade B, Class II).**
- Combine LMWH with low-dose aspirin for patients with elevated platelet counts (**Grade A, Class I**).
- Offer third trimester anaesthetic assessment, especially for patients with skeletal deformities (**Grade C, Class II**).

4. Recommendations for managing delivery in women with TDT.

- Delivery could be planned prior to 40 weeks of gestation in uncomplicated pregnancies to minimise risks such as worsening maternal anaemia, increased iron overload, and potential cardiac complications (**Grade C, Class II**).
- The mode of delivery should be individualised, with vaginal delivery considered in the absence of specific obstetric or medical contraindications (**Grade C, Class I**).
- If red cell antibodies are present or if the haemoglobin is less than 10 g/L, cross-match two units of blood on admission to the labour ward (**Grade C, Class I**).
- Epidural anaesthesia is preferred during labour to avoid the risks associated with general anaesthesia, particularly in patients with maxillofacial deformities (**Grade C, Class I**).
- For patients with pre-existing cardiac conditions, prolonged pregnancy should be avoided (**Grade C, Class I**), and the use of low-dose deferoxamine during prolonged labour may be considered (**Grade C, Class II**).
- Active management of the third stage of labour should be employed to minimise bleeding (**Grade A, Class I**).

5. Recommendations for postpartum care in women with TDT.

- Administer LMWH prophylaxis during hospitalisation (**Grade B, Class I**) and for at least 7 days postpartum, extending to 6 weeks in patients with additional venous thromboembolism (VTE) risk factors, splenectomy, or platelet count >600 × 10^9/L (**Grade C, Class I**).
- Provide LMWH prophylaxis for at least 7 days following miscarriage or termination of pregnancy (**Grade C, Class I**).
- Generally, avoid oestrogen-containing birth control pills postpartum due to increased VTE risk; recommend progesterone-only pills or barrier methods instead (**Grade C, Class I**).
- Assess iron burden at 3 months postpartum, unless myocardial iron overload requires earlier evaluation (**Grade C, Class I**).
- Continue calcium and vitamin D supplementation during breastfeeding to maintain bone health (**Grade C, Class I**); resume bisphosphonate therapy only after breastfeeding cessation (**Grade C, Class II**).

6. Recommendations for evaluation and treatment of infertility in men with TDT.

- Discuss with adolescents and young adults with poor adherence to iron chelation the deleterious effect on future fertility potential (**Grade B, Class I**).
- Perform a comprehensive evaluation for hypogonadotropic hypogonadism in peripubertal boys with physical exam findings of delayed puberty (**Grade B, Class I**).
- Screen for fertility status with the following (**Grade A, Class I**):
  - Review past and current labs of hypothalamic-pituitary-testicular axis (testosterone, LH, FSH).
  - Obtain semen analysis to evaluate number of motile sperm.
- Refer to an endocrinologist and male fertility specialist when planning pregnancy for evaluation of fertility status and other endocrinopathies (**Grade B, Class I**).
- Discuss therapy options for including testicular stimulation for timed intercourse, intrauterine insemination and IVF (**Grade B, Class I**).
- Discuss considerations for fertility preservation (sperm or testicular cryopreservation) where appropriate (**Grade B, Class IIb**).

REFERENCES

1. Sayani FA, Singer ST, Carlberg KT, Vichinsky EP. Fertility and Pregnancy in Women with Transfusion-Dependent Thalassemia. Hematol Oncol Clin North Am 2023;37(2):393-411.
2. Uysal A, Alkan G, Kurtoglu A, Erol O, Kurtoglu E. Diminished ovarian reserve in women with transfusion-dependent beta-thalassemia major: Is iron gonadotoxic? Eur J Obstet Gynecol Reprod Biol 2017;216:69-73.
3. Borgna-Pignatti C, Gamberini MR. Complications of thalassemia major and their treatment. Expert Rev Hematol 2011;4(3):353-366.
4. Talaulikar VS, Bajoria R, Ehidiamhen AJ, Mujawar E, Chatterjee R. A 10-year longitudinal study of evaluation of ovarian reserve in women with transfusion-dependent beta thalassaemia major. Eur J Obstet Gynecol Reprod Biol 2019;238:38-43.
5. Mensi L, Borroni R, Reschini M, Cassinerio E, Vegetti W, Baldini M, Cappellini MD, Somigliana E. Oocyte quality in women with thalassaemia major: insights from IVF cycles. Eur J Obstet Gynecol Reprod Biol X 2019;3:100048.
6. Singer ST, Vichinsky EP, Gildengorin G, van Disseldorp J, Rosen M, Cedars MI. Reproductive capacity in iron overloaded women with thalassemia major. Blood 2011;118(10):2878-2881.
7. Chang HH, Chen MJ, Lu MY, Chern JP, Lu CY, Yang YL, Jou ST, Lin DT, Yang YS, Lin KH. Iron overload is associated with low anti-mullerian hormone in women with transfusion-dependent beta-thalassaemia. BJOG 2011;118(7):825-831.
8. Noetzli LJ, Panigrahy A, Mittelman SD, Hyderi A, Dongelyan A, Coates TD, Wood JC. Pituitary iron and volume predict hypogonadism in transfusional iron overload. Am J Hematol 2012;87(2):167-171.
9. Singer ST, Fischer R, Allen I, Lal A, Vichinsky E, Yuan Q, Wang ZJ. Pituitary iron and factors predictive of fertility status in transfusion dependent thalassemia. Haematologica 2021;106(6):1740-1744.
10. Skordis N, Petrikkos L, Toumba M, Hadjigavriel M, Sitarou M, Kolnakou A, Skordos G, Pangalou E, Christou S. Update on fertility in thalassaemia major. Pediatr Endocrinol Rev 2004;2 Suppl 2:296-302.
11. Lindheim SR, Glenn TL, Smith MC, Gagneux P. Ovulation Induction for the General Gynecologist. J Obstet Gynaecol India 2018;68(4):242-252.
12. Haering C, Coyne K, Daunov K, Anim S, Christianson MS, Flyckt R. Ovarian Tissue Cryopreservation for Fertility Preservation in Patients with Hemoglobin Disorders: A Comprehensive Review. J Clin Med 2024;13(13).
13. Bedrick BS, Kohn TP, Pecker LH, Christianson MS. Fertility preservation for pediatric patients with hemoglobinopathies: Multidisciplinary counseling needed to optimize outcomes. Front Endocrinol (Lausanne) 2022;13:985525.
14. Merkison J, Malcom C, Decherney A. Use of gonadotropin-releasing hormone (GnRH) agonist trigger in fertility preservation for patients with inherited genetic disorders. Front Endocrinol (Lausanne) 2022;13:826419.
15. Tsironi M, Karagiorga M, Aessopos A. Iron overload, cardiac and other factors affecting pregnancy in thalassemia major. Hemoglobin 2010;34(3):240-250.
16. Ansari S, Azarkeivan A, Tabaroki A. Pregnancy in patients treated for beta thalassemia major in two centers (Ali Asghar Children's Hospital and Thalassemia Clinic): outcome for mothers and newborn infants. Pediatr Hematol Oncol 2006;23(1):33-37.
17. Lao TT. Obstetric care for women with thalassemia. Best Pract Res Clin Obstet Gynaecol 2017;39:89-100.
18. Vlachodimitropoulou E, Mogharbel H, Kuo KHM, Hwang M, Ward R, Shehata N, Malinowski AK. Pregnancy outcomes and iron status in beta-thalassemia major and intermedia: a systematic review and meta-analysis. Blood Adv 2024;8(3):746-757.
19. Cassinerio E, Baldini IM, Alameddine RS, Marcon A, Borroni R, Ossola W, Taher A, Cappellini MD. Pregnancy in patients with thalassemia major: a cohort study and conclusions for an adequate care management approach. Ann Hematol 2017;96(6):1015-1021.

20. Aessopos A, Karabatsos F, Farmakis D, Katsantoni A, Hatziliami A, Youssef J, Karagiorga M. Pregnancy in patients with well-treated beta-thalassemia: outcome for mothers and newborn infants. Am J Obstet Gynecol 1999;180(2 Pt 1):360-365.
21. Anderson LJ, Holden S, Davis B, Prescott E, Charrier CC, Bunce NH, Firmin DN, Wonke B, Porter J, Walker JM, Pennell DJ. Cardiovascular T2-star (T2*) magnetic resonance for the early diagnosis of myocardial iron overload. Eur Heart J 2001;22(23):2171-2179.
22. Farmaki K, Tzoumari I, Pappa C, Chouliaras G, Berdoukas V. Normalisation of total body iron load with very intensive combined chelation reverses cardiac and endocrine complications of thalassaemia major. Br J Haematol 2010;148(3):466-475.
23. Singer ST, Vichinsky EP. Deferoxamine treatment during pregnancy: is it harmful? Am J Hematol 1999;60(1):24-26.
24. Sinha P, Kuruba N. Ante-partum haemorrhage: an update. Journal of obstetrics and gynaecology : the journal of the Institute of Obstetrics and Gynaecology 2008;28(4):377-381.
25. Abalos E, Cuesta C, Grosso AL, Chou D, Say L. Global and regional estimates of preeclampsia and eclampsia: a systematic review. Eur J Obstet Gynecol Reprod Biol 2013;170(1):1-7.
26. Tantawy AAG, Adly AAM, Ismail EAR, Youssef OI, Ali ME. Soluble fms-Like Tyrosine Kinase 1 as a Link Between Angiogenesis and Endothelial Dysfunction in Pediatric Patients With beta-Thalassemia Intermedia. Clin Appl Thromb Hemost 2017;23(8):943-950.
27. Kaitu'u-Lino TJ, Brownfoot FC, Hastie R, Chand A, Cannon P, Deo M, Tuohey L, Whitehead C, Hannan NJ, Tong S. Activating Transcription Factor 3 Is Reduced in Preeclamptic Placentas and Negatively Regulates sFlt-1 (Soluble fms-Like Tyrosine Kinase 1), Soluble Endoglin, and Proinflammatory Cytokines in Placenta. Hypertension 2017;70(5):1014-1024.
28. Ammad Ud Din M, Chowdhury M, Shahzad M, Liaqat H, Jaglal M. Increased risk of eclampsia and preeclampsia during delivery hospitalizations in women with beta-thalassemia; An analysis of the National Inpatient Sample database. Eur J Obstet Gynecol Reprod Biol X 2023;17:100175.
29. Kelaidi C, Kattamis A, Apostolakou F, Poziopoulos C, Lazaropoulou C, Delaporta P, Kanavaki I, Papassotiriou I. PlGF and sFlt-1 levels in patients with non-transfusion-dependent thalassemia: Correlations with markers of iron burden and endothelial dysfunction. Eur J Haematol 2018;100(6):630-635.
30. Bremme KA. Haemostatic changes in pregnancy. Best Pract Res Clin Haematol 2003;16(2):153-168.
31. Taher AT, Otrock ZK, Uthman I, Cappellini MD. Thalassemia and hypercoagulability. Blood Rev 2008;22(5):283-292.
32. Blencowe H, Cousens S, Oestergaard MZ, Chou D, Moller AB, Narwal R, Adler A, Vera Garcia C, Rohde S, Say L, Lawn JE. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. Lancet 2012;379(9832):2162-2172.
33. WHO. Caesarean section rates continue to rise, amid growing inequalities in access. 16 June 2021. Available at: https://www.who.int/news/item/16-06-2021-caesarean-section-rates-continue-to-rise-amid-growing-inequalities-in-access. Accessed: 18 Dec 2024.
34. Nassar AH, Naja M, Cesaretti C, Eprassi B, Cappellini MD, Taher A. Pregnancy outcome in patients with beta-thalassemia intermedia at two tertiary care centers, in Beirut and Milan. Haematologica 2008;93(10):1586-1587.
35. Toumba M, Kanaris C, Simamonian K, Skordis N. Outcome and management of pregnancy in women with thalassaemia in Cyprus. East Mediterr Health J 2008;14(3):628-635.
36. Thompson AA, Kim HY, Singer ST, Vichinsky E, Eile J, Yamashita R, Giardina PJ, Olivieri N, Parmar N, Trachtenberg F, Neufeld EJ, Kwiatkowski JL, Thalassemia Clinical Research N. Pregnancy outcomes in women with thalassemia in North America and the United Kingdom. Am J Hematol 2013;88(9):771-773.

37. Vlachodimitropoulou E, Thomas A, Shah F, Kyei-Mensah A. Pregnancy and iron status in beta-thalassaemia major and intermedia: six years' experience in a North London Hospital. J Obstet Gynaecol 2018;38(4):567-570.
38. Rolnik DL, Wright D, Poon LCY, Syngelaki A, O'Gorman N, de Paco Matallana C, Akolekar R, Cicero S, Janga D, Singh M, Molina FS, Persico N, Jani JC, Plasencia W, Papaioannou G, Tenenbaum-Gavish K, Nicolaides KH. ASPRE trial: performance of screening for preterm pre-eclampsia. Ultrasound Obstet Gynecol 2017;50(4):492-495.
39. Walker JM. Thalassaemia major and the heart: a toxic cardiomyopathy tamed? Heart 2013;99(12):827-834.
40. Kirk P, Roughton M, Porter JB, Walker JM, Tanner MA, Patel J, Wu D, Taylor J, Westwood MA, Anderson LJ, Pennell DJ. Cardiac T2* magnetic resonance for prediction of cardiac complications in thalassemia major. Circulation 2009;120(20):1961-1968.
41. Pennell DJ, Udelson JE, Arai AE, Bozkurt B, Cohen AR, Galanello R, Hoffman TM, Kiernan MS, Lerakis S, Piga A, Porter JB, Walker JM, Wood J, American Heart Association Committee on Heart F, Transplantation of the Council on Clinical C, Council on Cardiovascular R, Imaging. Cardiovascular function and treatment in beta-thalassemia major: a consensus statement from the American Heart Association. Circulation 2013;128(3):281-308.
42. Maggio A, Vitrano A, Calvaruso G, Barone R, Rigano P, Mancuso L, Cuccia L, Capra M, Pitrolo L, Prossomariti L, Filosa A, Caruso V, Gerardi C, Campisi S, Cianciulli P, Elefteriou A, Angastiniotis M, Hamza H, Telfer P, Walker JM, Phrommintikul A, Chattipakorn N. Serial echocardiographic left ventricular ejection fraction measurements: a tool for detecting thalassemia major patients at risk of cardiac death. Blood Cells Mol Dis 2013;50(4):241-246.
43. Asmari M, Waqas M, Ibrahim AE, Halim SA, Khan A, Al-Harrasi A, Watzig H, El Deeb S. Microscale Thermophoresis and Molecular Modelling to Explore the Chelating Drug Transportation in the Milk to Infant. Molecules 2022;27(14).
44. Giampreti A, Mattioli F, Faraoni L, Bedussi F, Spada C, Gallo M, Eleftherioua G, Butera R, Bacis G. Lactation in beta-thalassemiamajor: is deferasirox compatible? Thefirst case with clinical data andbreastmilk concentrations [abstract]. Clin Toxicol 2018;56(6):539.
45. Surbek DV, Glanzmann R, Nars PW, Holzgreve W. Pregnancy and lactation in homozygous beta-thalassemia major. J Perinat Med 1998;26(3):240-243.
46. Stathopoulos IP, Liakou CG, Katsalira A, Trovas G, Lyritis GG, Papaioannou NA, Tournis S. The use of bisphosphonates in women prior to or during pregnancy and lactation. Hormones (Athens) 2011;10(4):280-291.
47. Kamel H, Navi BB, Sriram N, Hovsepian DA, Devereux RB, Elkind MS. Risk of a thrombotic event after the 6-week postpartum period. N Engl J Med 2014;370(14):1307-1315.
48. Heit JA, Kobbervig CE, James AH, Petterson TM, Bailey KR, Melton LJ, 3rd. Trends in the incidence of venous thromboembolism during pregnancy or postpartum: a 30-year population-based study. Ann Intern Med 2005;143(10):697-706.
49. Ray JG, Chan WS. Deep vein thrombosis during pregnancy and the puerperium: a meta-analysis of the period of risk and the leg of presentation. Obstet Gynecol Surv 1999;54(4):265-271.
50. Panigrahi I, Agarwal S. Thromboembolic complications in beta-thalassemia: Beyond the horizon. Thromb Res 2007;120(6):783-789.
51. RCOG. Management of Beta Thalassaemia in Pregnancy. Green-top Guideline No. 66. March 2014. Available at: https://www.rcog.org.uk/guidance/browse-all-guidance/green-top-guidelines/management-of-beta-thalassaemia-in-pregnancy-green-top-guideline-no-66/. Accessed: 19 Dec 2024.
52. De Sanctis V, Soliman AT, Elsedfy H, Di Maio S, Canatan D, Soliman N, Karimi M, Kattamis C. Gonadal dysfunction in adult male patients with thalassemia major: an update for clinicians caring for thalassemia. Expert Rev Hematol 2017;10(12):1095-1106.
53. Chen MJ, Peng SS, Lu MY, Yang YL, Jou ST, Chang HH, Chen SU, Lin DT, Lin KH. Effect of iron overload on impaired fertility in male patients with transfusion-dependent beta-thalassemia. Pediatr Res 2018;83(3):655-661.

54. Singer ST, Killilea D, Suh JH, Wang ZJ, Yuan Q, Ivani K, Evans P, Vichinsky E, Fischer R, Smith JF. Fertility in transfusion-dependent thalassemia men: effects of iron burden on the reproductive axis. Am J Hematol 2015;90(9):E190-192.
55. Perera D, Pizzey A, Campbell A, Katz M, Porter J, Petrou M, Irvine DS, Chatterjee R. Sperm DNA damage in potentially fertile homozygous beta-thalassaemia patients with iron overload. Hum Reprod 2002;17(7):1820-1825.
56. De Sanctis V, Soliman AT, Yassin MA, Di Maio S, Daar S, Elsedfy H, Soliman N, Kattamis C. Hypogonadism in male thalassemia major patients: pathophysiology, diagnosis and treatment. Acta Biomed 2018;89(2-S):6-15.
57. Bhasin S, Cunningham GR, Hayes FJ, Matsumoto AM, Snyder PJ, Swerdloff RS, Montori VM, Task Force ES. Testosterone therapy in men with androgen deficiency syndromes: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2010;95(6):2536-2559.
58. Cooper TG, Noonan E, von Eckardstein S, Auger J, Baker HW, Behre HM, Haugen TB, Kruger T, Wang C, Mbizvo MT, Vogelsong KM. World Health Organization reference values for human semen characteristics. Hum Reprod Update 2010;16(3):231-245.
59. De Sanctis V, Vullo C, Katz M, Wonke B, Nannetti C, Bagni B. Induction of spermatogenesis in thalassaemia. Fertil Steril 1988;50(6):969-975.
60. Gabrielsen JS, Lamb DJ, Lipshultz LI. Iron and a Man's Reproductive Health: the Good, the Bad, and the Ugly. Curr Urol Rep 2018;19(8):60.
61. Achermann APP, Pereira TA, Esteves SC. Microdissection testicular sperm extraction (micro-TESE) in men with infertility due to nonobstructive azoospermia: summary of current literature. Int Urol Nephrol 2021;53(11):2193-2210.

08 OTHER COMPLICATIONS

Authors: Yesim Aydinok[1], Antonis Kattamis[2], Polyxeni Delaporta[2], Rayan Bou-Fakhredin[3], Ali T. Taher[4]

[1.] Department of Pediatric Hematology, Ege University Medical School, Izmir, Turkey

[2.] Thalassemia Unit, First Department of Pediatrics, National and Kapodistrian University of Athens, 'Aghia Sophia' Children's Hospital, ERN-EuroBloodNet Center, Athens, Greece

[3.] SC Medicina ad Indirizzo Metabolico, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico; Department of Clinical Sciences and Community Health, University of Milan, Milan, Italy

[4.] Department of Internal Medicine, American University of Beirut Medical Center, Beirut, Lebanon

Competing interest: YA reports consultancy fees from Bristol Myers Squibb (Celgene Corp), CRISPR/Vertex and Silence Therapeutics; research funding from Agios, Bristol Myers Squibb (Celgene Corp), Cerus, Sobi Inc. and Novartis; membership on an advisory committee for Cerus, Bristol Myers Squibb (Celgene Corp), Agios and Chiesi. AK reports receiving consulting fees/honoraria from Agios Pharmaceuticals, Bristol Myers Squibb (Celgene Corp), Chiesi Farmaceutici, CRISPR Therapeutics/Vertex, CSL Vifor, Novartis and Novo Nordisk; and research support from Bristol Myers Squibb (Celgene Corp) and Novartis. ATT reports consultancy fees from Novo Nordisk, Bristol Myers Squibb (Celgene Corp), Agios Pharmaceuticals, Pharmacosmos and Roche; and research funding from Novo Nordisk, Bristol Myers Squibb (Celgene Corp), Agios Pharmaceuticals, Pharmacosmos and Roche. The remaining authors have no competing interests to disclose. All competing interests are outside the present work.

1. INTRODUCTION

Several emerging complications continue to be observed in patients with transfusion-dependent β-thalassaemia (TDT), which underscore the complex nature of the disease and its long-term impact on various bodily systems beyond the heart, liver, and endocrine organs. These are primarily attributed to suboptimal anaemia and/or iron overload management or side effects of conventional therapies [1]. Improved survival in patients with TDT has also allowed several previously unrecognised complications to manifest, including those associated with aging in the general population [2, 3]. This Chapter will provide an overview of these additional complications, highlighting their clinical significance, potential impact on patient health, and the need for comprehensive, multidisciplinary care to address them effectively.

2. CHOLELITHIASIS

Cholelithiasis (gall stones) is one complication that may be seen in suboptimally transfused patients with TDT [4-7]. The pathogenesis of cholelithiasis is influenced by several factors, including the precipitation of bilirubin in bile due to increased peripheral haemolysis. Iron deposition in the gallbladder has also been implicated in the development of cholelithiasis and the subsequent formation of gallstones. Unrelated genetic factors such as mutations or inherited variability in the function of the *UGT1A1* gene (responsible for Gilbert syndrome) can also substantially increase gallstone risk [7-10].

Diagnosis usually follows what is commonly practiced in the general population, with signs and symptoms of gall stones and typical findings on laboratory studies and abdominal ultrasonography. The removal of the gallbladder during splenectomy is common practice, especially when stones are symptomatic. This is particularly important, as acute cholecystitis can lead to serious complications in splenectomised patients.

3. MALIGNANCY

Beyond documentation in case reports and case series, several epidemiological and survival studies have reported incidence and prevalence rates for malignancies in patients with TDT, especially in older adults [11-18]. In a recent large follow up of 4631 patients with haemoglobinopathies between 1970 and 2021 in Italy, the incidence rate of cancer was 442 cases per 100,000 person-years. The liver (hepatocellular carcinoma [HCC]) was the most frequent site of tumours in both sexes, with a higher incidence (9-fold) in comparison with the general population for all haemoglobinopathy types including TDT [19]. In recent years, liver disease, especially HCC, has become the second cause of death in patients with TDT which is primarily attributed to decreased deaths from other causes like iron-related heart disease [15, 20]. HCC in TDT patients is primarily attributed to viral hepatitis and/or iron overload or any other risk factor that promoted hepatic necro-inflammation [18, 21] (see Chapter 5 for further details on HCC screening, diagnosis, and management).

Haematologic malignancies, particularly leukaemia and lymphoma, have also been reported to be more frequent in patients with haemoglobinopathies compared to the general population [12]. However, these findings were not observed by other studies [16]. Several factors have been identified as contributing to the increased risk of both solid and haematologic malignancies in TDT patients compared to the general population. These include oxidative damage caused by iron overload, which can lead to DNA mutations and cellular dysfunction; immunological abnormalities and chronic inflammation that impair the body's ability to fight cancerous cells; and transfusion-induced immunomodulation, which can alter immune response [16, 18]. Furthermore, the transmission of oncogenic viruses through blood transfusions may increase the risk of malignancy, while hydroxyurea therapy, which affects DNA synthesis and repair mechanisms, may also play a role. Chronic anaemia in TDT patients could stimulate bone marrow activity, leading to erythropoietin-driven erythroid hyperplasia (e.g., JAK2/STAT5 pathway activation), which can increase the likelihood of malignant transformation [16, 18].

Given these potentially increased risks of cancer development, and beyond HCC, patients with TDT should be regular screened for common cancers per local guidelines to allow for timely diagnosis and intervention.

4. SKIN MANIFESTATIONS

Dermatological manifestations have also been observed in TDT patients. A study by Zulfiqar and colleagues found that 89.4% of TDT patients had cutaneous manifestations, with xerosis (44.2%), freckles (39.8%), and pruritus (44.2%) being the most frequent [22]. Similarly, other studies reported high incidences of xerosis and pruritus in TDT patient cohorts [23, 24]. Iron deposition in the skin may trigger histamine release from mast cells, potentially explaining the frequent pruritus in these patients [25]. Zulfiqar and colleagues also identified 13 cases of pityriasis alba, a condition

characterised by hypopigmented patches, which has been linked to reduced copper levels in TDT patients [22, 24, 26]. Few rare cases of colloid milium, linked to iron overload and the formation of hydroxyl radicals that disrupt elastin metabolism and cause structural abnormalities in elastic fibres, have also been reported in the literature [27, 28].

Iron chelators have been associated with a range of dermatological side effects [29]. These can include hyperpigmentation, skin rash, itching, and photosensitivity. Strategies to manage these skin complications include topical antioxidants and corticosteroids to reduce inflammation, antihistamines for pruritus, and sun protection to alleviate photosensitivity [29]. Adjusting chelation dosages or using combination therapies can help minimise dermatological side effects. General measures, such as proper hydration and a balanced diet, also support skin health and overall well-being [29]. Additionally, long-term use of hydroxyurea has been linked to dermatological side effects, such as actinic keratosis, leg ulcers, and skin carcinomas [30, 31].

Regular examination for various skin disorders through skin inspection is essential in managing TDT patients, as it can significantly improve their quality of life. To ensure comprehensive care, once suspected, a dermatologist should be consulted as part of the multidisciplinary management team. This collaborative approach allows for early detection and tailored treatments, addressing skin-related concerns that may otherwise be overlooked.

5. LEG ULCERS

Leg ulcers may be seen in suboptimally transfused and/or chelated TDT patients, but they are generally more common in non-transfusion-dependent β-thalassaemia (NTDT) [32, 33]. Risk factors include advanced age, severe anaemia and tissue hypoxia, splenectomy and hypercoagulability, high venous pressure with right-sided heart failure, and (local) iron overload [34]. Leg ulcers are often very painful and indolent. Observational studies in NTDT patients indicate that optimisation of blood transfusion or hydroxyurea therapy with or without erythropoietin may have a role in prevention and management [32, 33, 35, 36]. Pentoxifylline, the use of an oxygen chamber, the vasodilator dilazep, skin grafts, platelet-derived wound healing factors and granulocyte macrophage colony-stimulating factor, anticoagulation, sodium nitrite cream, and the erythroid maturation agent luspatercept have all shown some benefit in healing chronic leg ulcers in small studies or case reports/series of patients with β-thalassaemia [37-43].

6. RENAL DISEASE

Renal disease is a rare cause of death in TDT patients. On the other hand, many studies have shown that a considerable percentage of patients exhibit signs of renal disease. There is evidence, mainly from studies in the paediatric population, of tubular dysfunction and glomerular filtration rate (GFR) abnormalities leading reflected by findings in different biochemical markers of renal function. Of these, the most common are proteinuria and hypercalciuria [52-56].

The pathogenesis of renal dysfunction in TDT is multifactorial with chronic anaemia, hypoxia, and oxidative stress playing a significant role. Iron toxicity further contributes to nephron damage, while iron chelation therapy may be nephrotoxic by different mechanisms like

aggressive iron removal and local iron depletion [56]. These changes seem to be nonprogressive, resolve spontaneously in most part, or may require iron chelator dose modifications [56] (see Chapter 3 for further details on the effects of different iron chelators on renal function).

Other factors like renal stones and urinary tract infections may further contribute to the pathogenesis. Renal damage can affect the glomeruli, the tubules or/and the interstitial space, while it usually affects a combination of these. As damage progresses, it can lead to fibrosis and decrease GFR. Many other causes like viral infections (including hepatitis B [HBV] and C [HCV]), hepatopathy, cardiomyopathy, endocrinopathies, drug toxicity, and immunological disorders contribute to the development of renal dysfunction.

Renal dysfunction may progress from non-clinically significant biochemical abnormalities to decreased GFR and, finally, to end-stage renal disease (ESRD) requiring dialysis [54, 57, 58].

Beyond requirements for renal monitoring associated with specific iron chelation therapies (see Chapter 3), periodic evaluation should include:

- Serum creatinine: it should be noted that patients with TDT may have lower creatinine levels than reference values, because of the chronic hyperfiltration state and decreased muscle mass. Thus, a steady worsening of creatinine levels, even if they remained within normal ranges, should be promptly evaluated.
- Serum calcium, phosphorus, magnesium, and uric acid: to assess for respective increased urinary losses.
- Urine analysis: to evaluate for protein, haemoglobin, blood cells and casts.
- Urine ratio of protein/creatinine: to evaluate for proteinuria, either in a spot sample or 24 hours collection.
- 24 hours urine calcium excretion or urine calcium/creatinine ratio: to evaluate for hypercalciuria.
- Specific markers of renal function like β2-microglobulin, cystatin, neutrophil gelatinase-associated lipocalin can be analysed, but their usefulness in routine practice has not been established.
- Renal ultrasound is advised especially in the presence of renal abnormalities and/or signs of nephrocalcinosis.

Timely diagnosis is important to avoid further deterioration of renal function. Respective measures should be instituted upon documentation of abnormal markers. Thorough evaluation for possible causes of renal dysfunction, like drug toxicities, infections and autoimmune processes should be performed. Special attention should be given to nephrotoxicity induced by iron chelators, which, even though it has been described with all available chelators, is more frequent with the use of deferasirox (see Chapter 3).

Worsening of creatinine levels is a common feature observed with the use of deferasirox especially at the initiation of therapy and upon dose increases. It has been, at least partially, attributed to haemodynamic changes and not to direct renal toxicity. The latter is further supported by the fact that there is no progression with long-term use. On the other hand, deferasirox has been shown to induce acute tubular toxicity / renal tubular acidosis (Fanconi syndrome) with electrolyte imbalance, and metabolic acidosis, especially in young children.

Renal toxicity of deferasirox has been shown to be related to high deferasirox trough levels and disproportionate ratio of deferasirox dose to the level of iron overload. In the event of renal abnormalities, prompt adjustment of dosage and even temporary interruption of therapy is essential to avoid further deterioration. If serious renal complications occur, rechallenge with close monitoring and upon resolution of the adverse reaction can be attempted [59-63].

Renal dysfunction may lead to loss of electrolytes and minerals, which need to be replenished. Calcium and vitamin D supplementation are covered in Chapter 10. The efficacy of thiazide diuretics in altering the course of renal dysfunction is debatable [52, 64]. Management of patients with ESRD, including renal transplantation, should be according to local guidelines and similar to non-thalassaemic patients [65]. Chelation therapy for patients with ESRD should be adjusted according to the chelators' kinetics and characteristics according to local prescribing information [66].

7. SPLENOMEGALY

Splenic enlargement (splenomegaly) and hyperactivity (hypersplenism) are common in β-thalassaemia patients in view of chronic, premature haemolysis of circulating red cells as well as splenic extramedullary haematopoiesis (EMH, see EXTRAMEDULLARY HAEMATOPOIESIS section below). About 80% of damaged red blood cells (RBC) are removed extravascularly by macrophages present mainly in the spleen [67]. Splenectomy was previously a common practice to increase haemoglobin level and/or reduce transfusion requirement. However, adverse events following splenectomy including increased rates of infections and sepsis (see INFECTIOUS DISEASE section below) and higher long-term risks of thromboembolism and pulmonary hypertension due to an aggravated hypercoagulable state, especially in older adults with NTDT [68-70], led to a decline in its use over the years in most Western countries [71]. It is currently reserved for cases of severe/clinically significant splenomegaly or hypersplenism. When indicated, laparoscopic splenectomy has become the gold standard for the removal of the spleen, considering lower rates of intraoperative blood loss, postoperative morbidity and mortality, a shorter length of hospital stay, as well as a more favourable body image and cosmesis than open splenectomy, even in patients with massive or supra-massive spleens [72-82].

8. EXTRAMEDULLARY HAEMATOPOIESIS

The soft tissues producing blood elements outside the bone marrow are called extramedullary haemopoietic tissues, and their process is termed EMH. β-thalassaemia is among the most common causes of EMH worldwide. EMH is a common complication in patients with NTDT, affecting up to 15-20% of cases, while it occurs in only 1-5% of patients with TDT [83, 84], with higher rates noted in suboptimally transfused patients [32]. These prevalence rates are also mostly based on symptomatic presentations or incidental findings on imaging, and higher rates may be observed if patients are actively screened for EMH. They also include varying definitions of EMH based on pseudotumor presentation and/or overt hepatosplenomegaly. In a prospective study of patients with NTDT, levels of the soluble form of transferrin receptor (sTfR) showed a high predictive power for EMH development particularly in patients with intact spleen, indirectly indicating a correlation with the severity of underlying disease and ineffective erythropoiesis [85].

During foetal development, haematopoiesis first occurs in the yolk sac, then develops in the aorta–gonad–mesonephros region before moving to the foetal liver, spleen, and thymus. The spleen is colonised but contributes to haematopoiesis to a lesser extent than the liver. Later in gestation, haematopoietic stem and progenitor cells (HSPCs) colonise the bone marrow, which remains the primary site of haematopoiesis throughout adulthood, and blood elements production in other tissues declines. However, HSPCs and their differentiation have been observed outside the bone marrow in various conditions that are characterised by increased erythropoietic demand, as occurs in severe chronic haemolytic anaemias, and the process is hence termed EMH [86].

This redirected differentiation and EMH can occur in virtually every organ of the body with extramedullary haemopoietic vascular connective tissues that are thought to be involved in active haematopoiesis during foetal development, but also retain the ability to produce RBC after birth in cases of chronic ineffective erythropoiesis. The reactivation of foetal sites for haematopoietic production primarily occurs in the spleen and liver. Other sites most involved in EMH are lymph nodes and the paravertebral regions. The thymus, heart, breasts, prostate, broad ligaments, kidneys, adrenal glands, pleura, retroperitoneal tissue, skin, peripheral nerves, cranial nerves, and spinal canal may also be involved.

In another theory, the occurrence of para-osseous masses is explained by the extrusion of hypercellular marrow through the thinned cortex of certain bony structures, such as the vertebrae, proximal ribs, and sacrum, which results in the development of EMH in the paravertebral and presacral spaces [87].

8.1. Clinical presentation

Most patients' symptoms are related to the site of EMH involvement but are typically discovered incidentally in many patients. Symptomatic EMH is due to the mass effect upon the adjacent organs, with these masses commonly called pseudotumours.

8.1.1. Hepatosplenic

The most common manifestation of EMH is hepatosplenomegaly with or without focal soft-tissue masses. Hepatosplenomegaly is a non-specific feature of EMH in most cases since it often occurs without an associated focal mass. Pain can be the presenting symptom of splenic EMH if splenic infarction occurs [88]. In addition to focal hepatic masses, periportal/peribiliary hepatic involvement has been observed [89].

8.1.2. Paraspinal

After hepatosplenomegaly, the most frequent occurrence of EMH is the development of paraspinal masses, which are significantly more prevalent in the thorax than in the abdomen or pelvis. Paraspinal EMH occurs in 11-15% of cases with EMH and mainly presents as a pseudotumor, which remains asymptomatic in more than 80% of cases. Nevertheless, the size and location of lesions and the extent of spinal cord involvement determine the severity, acuteness, and multiplicity of neurological symptoms resulting from spinal compression, most frequently being reported during the third and fourth decades of life. The male-to-female ratio reaches 5:1 [90].

8.1.3. Thoracic

Other thoracic manifestations of EMH include rib expansion and, less frequently, pulmonary nodules, diffuse interstitial changes, or lung masses which might be present with pleural

effusion and spontaneous haemothorax (from pleural involvement), dyspnoea (from diffuse infiltration or pulmonary hypertension), respiratory failure, or haemoptysis (from alveolar haemorrhage). The thymus is a rare location for EMH [87, 91-94].

8.1.4. Abdominal

Perirenal involvement is a common abdominal site for EMH and the most common retroperitoneal manifestation. EMH can occur in the pre-sacral area and remains largely asymptomatic [95].

8.1.5. Craniospinal

EMH can manifest as multiple epidural soft tissue masses in the brain or spine. Given the narrow and rigid spinal canal, masses in the extra-axial tissues within the spine are generally symptomatic. The clinical manifestations of central nervous system (CNS) involvement may include seizures, hydrocephalus, or cord compression with various clinical presentations including back pain, abnormal sensation, proprioception, weakness, hyperreflexia, urgency of urination, or bowel incontinence. Early diagnosis of intra-spinal EMH is essential to reduce the incidence of irreversible neurological damage [96].

EMH can also occur in the nasopharynx causing obstructive sleep apnoea, and in the paranasal sinuses which may be confused with chronic sinusitis or mucoceles [97, 98].

8.2. Diagnosis

Depending on the location of EMH, radiographic findings can confirm the diagnosis. Paraspinal or rib involvement can be readily seen on chest X-rays as well-demarcated, smooth, or lobulated masses. EMH can present as a distinct solid mass with internal vascularity on ultrasound. Active lesions are usually hypoechoic, and inactive and long-standing lesions have variable echogenicity and may be echogenic.

On computed tomography, paraspinal masses typically appear as multiple, smooth-surfaced, lobulated shapes, bilaterally multifocal located lesions. Although intralesional fat may be present, calcification is uncommon. EMH masses are usually soft and do not erode adjacent bone from the outside. Haemopoietically active masses demonstrate mild homogeneous enhancement after contrast (high vascularity), whereas old burnt-out lesions have inhomogeneous contrast enhancement owing to iron deposition and fat infiltration.

Magnetic resonance imaging is the modality of choice for diagnosing EMH, although findings are nonspecific. It is also the preferred diagnostic tool for intraspinal and intracranial masses.

Active lesions typically show intermediate signal intensity on T1-weighted images and hyperintensity on T2-weighted images compared to the nearest skeletal muscle. Older lesions usually show hypointensity on both T1- and T2-weighted images because of the signal loss associated with the intralesional haemosiderin accumulation. The typical imaging appearance of perirenal EMH is the soft tissue surrounding the kidneys, which does not affect the kidney's contour or function. Focal and diffuse peritoneal nodularity or masses, multiple enlarged retroperitoneal lymph nodes, and presacral irregular nodular thickening are also observed [88, 89, 97].

8.3. Differential diagnosis

EMH is usually seen as an incidental finding when patients with haematological disorders are imaged for other purposes. Clinical history is crucial in suggesting EMH when a soft tissue mass

is present, in addition to the typical imaging characteristics of EMH described earlier. Although the imaging findings of older lesions often demonstrate characteristic features such as haemosiderin and fat deposition, the newer, actively haematopoietic lesions often mimic neoplasms requiring differential diagnosis.

Since the marrow contains haematopoietic cells, radionuclide tracers which localise in these cells, have been used to identify EMH and distinguish them from neoplastic lesions, which may eliminate the need for biopsy. ^{52}Fe (a positron emitter) and 111Inchloride (a gamma emitter) bind to transferrin, localise in the erythroid precursors, and are the most specific radiotracers for haematopoietic cells. These have been used for positron-emission tomography (PET) and scintigraphy/single-photon-emission computed tomography (SPECT). 99mTc-colloid scintigraphy imaging can confirm the presence of bone marrow elements in a mass suspicious of EMH [87].

Histological examination of the fine-needle aspiration, biopsy, or surgical specimen can provide a definitive diagnosis. However, sampling of vascular EMH masses, even with an imaging-guided procedure, is not without risk, especially in the thorax and spleen where massive haemorrhage can occur. Fine-needle aspiration tends to be safer than biopsy, with a lower risk of fatal haemorrhage. This stresses the importance of correctly diagnosing EMH on imaging to avoid potentially catastrophic sampling. Histopathological diagnostic confirmation should be reserved for older patients with a high probability of malignant disease and for cases where the clinical and radiological picture is equivocal [99].

8.4. Management

A wait-and-see strategy may be indicated if the lesion is asymptomatic and located in an anatomic site that poses no danger. If this condition is not met, several treatment options are available, such as blood transfusion, hydroxyurea, low-dose radiotherapy, or surgery, individually or in combination.

8.4.1. Transfusion

Early diagnosis is crucial for controlling EMH masses and preventing severe neurologic complications (in paraspinal locations). Blood transfusions with a target pretransfusion haemoglobin level above 10 g/dL are the principal treatment modality for stopping or decreasing the compensatory medullary tissue expansion in patients with β-thalassaemia. Although the initial response results primarily from a decrease in blood flow to the EMH tissues, masses regress slowly. This modality cannot be considered alone for patients with paraspinal masses with severe neurological or rapidly progressive symptoms because urgent relief is required [90]. However, some case reports described rapid reversal of neurological symptoms with a hypertransfusion regimen (i.e., weekly transfusions) to maintain haemoglobin levels around 15 g/dL [100-102]. This can be considered in patients when other treatment modalities like radiotherapy, hydroxyurea, or surgical decompression are contraindicated, like pregnancy.

8.4.2. Hydroxyurea

Pharmacological agents stimulating foetal haemoglobin synthesis, reduce the imbalance between α and non-α globin chains thereby enhancing erythropoiesis efficacy and decreasing overall erythropoietic activity. An additional cytoreductive effect of these agents could reduce the size of EMH masses and be suitable as a first-choice therapy for EMH masses before radiotherapy or surgery in patients with milder neurological symptoms.

Relatively lower doses of hydroxyurea (7.5-12 mg/kg/day) have been associated with regression of EMH masses while increasing total and foetal haemoglobin in case reports of NTDT patients [103]. In observational studies, NTDT patients maintained on hydroxyurea also have lower rates of EMH [32]. Higher doses of hydroxyurea (15-20 mg/kg/day) may also exert cytostatic effects, contributing directly to tumour regression. Although hydroxyurea doses of 20 mg/kg per day may be well-tolerated without any side effects, higher doses could potentially be hepatotoxic and myelotoxic which needs careful monitoring [104-106].

8.4.3. Radiotherapy

Since EMH tissue is highly radiosensitive, relatively low doses (10-30 Gy) of radiotherapy usually result in rapid therapeutic response and alleviation of severe neurological symptoms in paraspinal EMH cases. Although the dose-response relationship for radiotherapy has not been clearly defined, doses between the given ranges are decided based on the extent of the radiotherapy field. The total dose is delivered in fractions of usually 1-2.5 Gy daily doses. Radiotherapy can produce an initial worsening of symptoms secondary to tissue oedema during the first days of treatment, which can be prevented with the administration of steroids. The risks of late side effects after irradiation of the spinal cord and adjacent organs, as well as EMH relapse rates of 19-37%, cannot be dismissed. Adding a combination of hydroxyurea and optimising transfusions to a target haemoglobin >10 g/dL may further suppress and prevent the recurrence of EMH [93, 107-111].

8.4.4. Surgery

Decompressive surgery for paraspinal masses, including laminectomy and excision of the EMH tissue, is effective in spinal cord compression with immediate resolution of neurological symptoms. However, the high vascularity of the EMH mass poses the risk of excessive bleeding, requiring intensive transfusion. Surgery should be reserved for cases with acute, progressive, and severe neurologic deterioration that do not respond to adequate transfusion and radiotherapy. If the excision of EMH mass is incomplete due to the diffuse nature of the mass, radiotherapy can be introduced as an adjunct to surgery to prevent recurrence when the wound is healed, in addition to adequate transfusions [112-116].

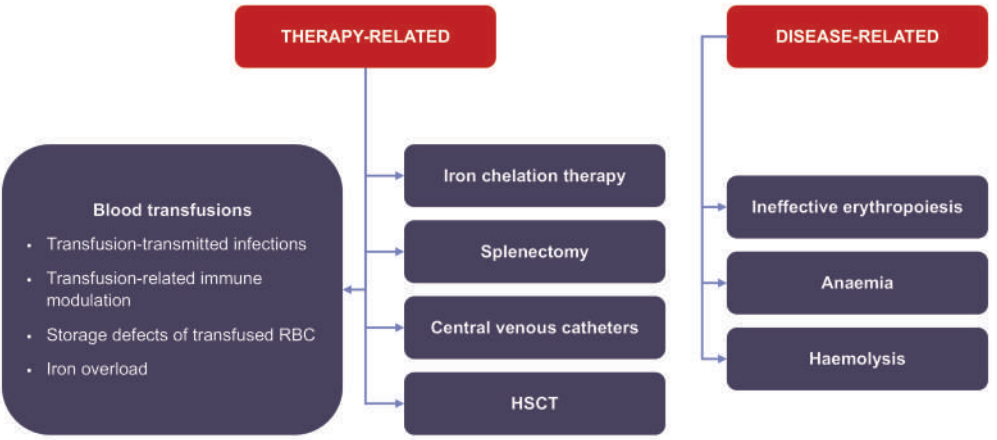
9. INFECTIOUS DISEASE

Infections and their complications, mainly due to HCV and associated liver disease, have become among the leading causes of death in TDT patients in Western countries due, in part, to a significant reduction in the number of deaths from iron-induced cardiac disease [15, 17, 117, 118]. Infections were already reported as the primary cause of mortality among severe haemoglobin E/β-thalassaemia patients in Thailand years ago [119], and recent studies confirmed that infections are still the most common cause of death in TDT patients in North Thailand [121] and South India [122].

Differences in the epidemiology of infections, socioeconomic status, preventative strategies, and accessibility to healthcare in each country explain the variability in infection-related morbidity and mortality rates for TDT patients worldwide. In TDT, allogeneic packed RBC (pRBC) transfusions come with a significant burden, including direct exposure to the risks of transfusion-transmitted infections (TTI) [123], indirect risks of transfusion-related immunomodulation (TRIM) [124], and increased risk of infection due to iron overload [125]. Pathophysiological mechanisms in TDT such as ineffective erythropoiesis, haemolysis, and anaemia can also negatively impact the immune system and

increase susceptibility to infections [120]. Furthermore, some other therapeutic interventions, such as iron chelation therapy, splenectomy, central venous catheters, and haematopoietic stem cell transplantation (HSCT) may contribute to infectious complications and subsequent morbidity and mortality (Figure 1).

Figure 1. *Factors contributing to infection risk in transfusion-dependent β-thalassaemia. Abbreviations: RBC, red blood cells; HCT, haematopoietic stem cell transplantation.*



9.1. Transfusion-transmitted infections

Transfusion is associated with the risk of TTI due to undetected known pathogens [126], emerging pathogens [127], and unrecognised bacterial contamination [128]. Despite improved donor testing, long-term transfusion support has a substantial cumulative lifetime residual risk of TTI [129] and splenectomised TDT patients may have increased TTI morbidity [130]. Further, the blood safety chain (donor selection, TTI testing, and haemovigilance, including post-transfusion surveillance, see Chapter 2) is suboptimal in low-income countries [131].

HCV, HBV, human immunodeficiency virus (HIV), and syphilis are the most common infectious agents that may be transmitted via pRBC transfusions in TDT. There has also been a steadily increasing number of reported cases of transfusion-transmitted hepatitis E virus in blood donation recipients [132]. Transfusion-transmitted malaria remains one of the most common TTI on a global scale [133]. Assessments of viraemia prevalence in blood donations for arthropod-borne viruses (arboviruses), including West Nile virus, Dengue virus, Chikungunya virus, and Zika virus indicate substantial transfusion transmission in epidemic areas [132, 134, 135]. Fundamental principles for providing safe blood include:

- The deferral of high-risk prospective donors is the first level of defence against TTI.
- Strategies regarding donor recruitment through voluntary, non-remunerated blood donation should be implemented because such donors are less likely to have a risk for TTIs.
- The routine testing of donor blood for HBV, HCV, HIV, and syphilis by validated technology should be implemented in blood banks.

- Many of these pathogens are geographically distributed, allowing the exploration of alternative strategies to reduce TTI beyond universal screening.

Testing of donations for infectious agents is a key factor in ensuring that the risk of disease transmission is minimised and that blood components are suitable for their intended purpose. Current tests are based on detecting relevant antigens and/or antibody and gene sequences. The minimum mandatory serological blood donor screening tests are:

- Antibody to HIV-1 and HIV-2 including outlying types (e.g., HIV-1 type O).
- Antibody to HCV.
- Hepatitis B surface antigen (HBsAg) assay.
- Treponema pallidum haemagglutination assay (TPHA) for syphilis.

Nucleic acid screening or nucleic acid amplification techniques (NAT) for HCV-RNA, HIV-RNA, and HBV-DNA in mini pools or single donations represent the state of the art in many countries. It was developed to remedy limitations in serological testing, like the window period which is the time lapse from the appearance of the virus in blood until the detectability of a given marker (antibody, antigen, or nucleic acid) [136]. However, policy debates continue over the cost-effectiveness of NAT-testing strategies in different settings, particularly in resource-constrained countries [137].

Apart from seronegative donors during the infectious window period, the greatest threat to the safety of the blood supply using serological markers is posed by the silent/occult period of HBV. This period is characterised by very low viral load and undetectable HBsAg at the tail end of chronic carriage or the occurrence of escape mutants interfering with HBsAg synthesis and the interference of other viruses in HBV replication [138].

Vaccination against HBV should protect all patients with TDT who are seronegative for HBV markers. Since the protection offered by vaccination is not absolute, vaccinated patients should be tested annually for anti-HBs. A booster dose of the HBV vaccine is considered if the anti-HBs titre decreases. Patients should also be tested annually for other TTI such as HIV and HCV (anti-HCV), which if positive, should be followed by HCV RNA.

The diversity of blood-borne infectious agents transmitted through transfusion of infected blood donated by apparently healthy and asymptomatic blood donors also includes human T-cell lymphotropic viruses (HTLV-1/2), cytomegalovirus (CMV), human parvovirus B19 (HPVB19), West Nile virus, Dengue virus, *Babesia* spp., *Plasmodium* spp., *Trypanosoma cruzi*, and the prions that cause variant Creutzfeldt-Jakob disease (CJD) [139]. Additional serological screening tests for blood donors may thus be required by the national authorities for specific components or epidemiological conditions [140], for example:

- Anti-HTLV-1 and HTLV-2.
- Antibody to hepatitis B core antigen (anti-HBc).
- Antibody to CMV for the transfusion of highly susceptible patients and in cases of candidates for HSCT.
- Chagas testing for *T. cruzi* antibodies in endemic areas and elsewhere for travellers returning from an endemic area.
- NAT screening for detection of West Nile virus, Zika virus, Chikungunya virus, *Babesia microti*.

When it comes to prevention, the cell-associated nature of CMV infections coupled with the adoption of universal leucocyte reduction in most developed countries has led to the reconsideration of the need for serological screening for CMV in blood donors, since pre-storage leucodepletion of pRBC units is considered equivalent to CMV antibody-negative blood products with reduced transmission of CMV by some authorities [141]. Pre-storage leucodepletion may also be effective in reducing the risk of several additional TTI including infections due to herpes viruses (e.g., Epstein-Barr virus [EBV] and human herpesvirus-8 [HHV-8]), retroviruses (e.g., HTLV-1 and HIV), bacteria (e.g., *Yersinia enterocolitica*), protozoa (e.g., *Leishmania* spp. and *T. cruzi*), and infectious prions. It should be noted that leucodepletion does not provide 100% risk prevention from these infections, but it may offer an additional and justified measure of caution [142].

Pathogen reduction treatment (PRT) of pRBC, which refers to the inactivation of all viruses, bacteria, parasites, and any replicating structures, could be a method to achieve the goal of almost absolute blood safety, albeit there are some limitations of this technology. Some pathogens (HPVB19, hepatitis A virus, and hepatitis E virus) show partially intrinsic resistance to the inactivation process [129]. PRT is undergoing clinical trials but is not yet commercially available. Photochemical inactivation of whole blood using riboflavin (vitamin B2) and ultraviolet light energy (Mirasol Pathogen Reduction System®) has been investigated in a phase 3 clinical trial for whole blood use [139], but has not yet been evaluated for RBC use. Chemical inactivation of RBC using amustaline-glutathione (Intercept Blood System®) has been assessed in phase 3 clinical trials for supporting chronic transfusion programmes in patients with TDT. PRT of pRBC appeared well-tolerated and logistically feasible for chronic transfusion therapy without a significant increase in pRBC utilisation in TDT patients [143]. The universal utilisation of an approved PRT of pRBC could significantly revise donor testing and may ultimately be less complex and less expensive than continued assay development. However, cost will be essential in implementing PRT technology, particularly in low-income countries.

Haemovigilance is another crucial pillar for safeguarding blood safety through epidemiological surveillance of adverse reactions and adverse events in donors and recipients (European Union Commission Directive 2005/61/EC; available at: https://eur-lex.europa.eu/eli/dir/). Its goal is to prevent the recurrence of adverse events and reactions. To accomplish this task, haemovigilance must be a shared responsibility of professionals in the field and the competent authorities for blood safety [144].

Bacterial contamination of pRBC units and associated septic transfusion reactions present with high fever, rigors, and hypotension beginning during or shortly after the transfusion. The pRBC unit is presumably contaminated by transient donor bacteraemia due to a recent infection. Causative bacteria are often gram-negative bacilli, mainly *Y. enterocolitica* and *Serratia marcescens* [145]. Leucodepletion can eliminate or markedly reduce the growth of the bacterium in processed blood. However, it cannot provide 100% protection from the risk of these infections. It may offer an additional and justified measure of caution [146].

9.2. Transfusion-related immune modulation

TRIM may contribute to all immunological alterations observed in TDT patients, and it is assumed that either allogeneic mononuclear cells in the pRBC unit or the soluble substances released during storage play a central role in the pathogenesis of TRIM. Pre-storage leucodepletion of pRBC units has no protective effect on immune alterations observed in patients with TDT [147]. In a

large study performed Chinese children with TDT, increased T-lymphocyte activation and decreased natural killer cell numbers were found to be prominent features associated with repeated transfusions [148].

9.3. Storage defects of transfused red blood cells

It is suggested that free haem compounds released from the lysis of transfused RBC can readily provide iron for bacteria and promote infection [149]. This hypothesis could be augmented by evidence suggesting that low molecular mass iron complexes occur in pRBC units stored for more than 10 days [150]. Marked increases in non-transferrin-bound iron and decreased antioxidant capacity have been observed in pRBCs stored for more than 14 days [151]. An extensive comparative study is required to reveal whether prolonged storage of pRBC is associated with an increased risk of nosocomial infection. Transfusions with pRBC units stored for less than 14 days may provide benefits to avoid deleterious effects of storage defects. Transfusion of pRBC units stored less than 2 weeks also reduces the risk of transfusion-associated *Yersinia* septicaemia, since *Yersinia* only grows in the contaminated RBC unit after a lag time of 2 weeks.

9.4. Transfusional iron overload-related risks of infection

Iron overload is suggested to be a risk factor predisposing to infections since all groups of protozoa, fungi, and gram-positive and negative bacteria require iron for survival and replication, with the only exception being pathogenic *Borrelia burgdorferi*, which uses manganese instead of iron. Some pathogens such as *Y. enterocolitica, Klebsiella* spp., *Escherichia coli*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa, Listeria monocytogenes,* and *Legionella pneumophila* increase their virulence and pathogenicity in the presence of excess iron [152]. A gram-negative bacterium, *Vibrio vulnificus,* which can be transmitted by ingestion of uncooked warm seawater fish, crustaceans, and molluscs and can cause a lethal infection in 20-50% of iron-overloaded patients [153]. Although viruses do not require iron, studies have reported that iron also increases the risk of viral infections [154] and impairs the clinical response to antiviral therapy in HCV infection [155]. Further, iron overload is associated with faster HIV-1 disease progression and poor outcomes in TDT patients [156]. Iron availability is also linked to the pathogenicity of *Candida albicans* and *Aspergillum fumigates.*

Iron has subtle effects on cell-mediated immune effector pathways which may further explain why systemic iron overload is associated with unfavourable outcomes in many types of infections [157]. In fact, numerous immune abnormalities have been described in patients with TDT, although factors behind these immune alterations are poorly understood but iron overload may have a prominent role [158]:

- Increased number and activity of CD8 suppressor cells.
- Decreased CD4/CD8 ratio.
- Decreased T-cell proliferation.
- Increased number and activation of B lymphocytes.
- Increased immunoglobulin (Ig)G, IgM, and IgA levels.
- Decreased C3 and C4 levels.
- Defective chemotaxis and phagocytosis of neutrophils and macrophages.
- Defective natural killer cell function.

Despite the lack of well-controlled studies, control of iron overload may have preventive/therapeutic benefits against infections.

9.5. Infections and iron chelation therapy

Controlling systemic iron and preventing iron from invading microbes are essential to host defence strategies. As a siderophore, some benefits of deferoxamine have been demonstrated in particular infections; for example, deferoxamine was able to promote recovery from coma in children with cerebral malaria [159], and experimental studies indicate beneficial effects of deferoxamine in infections with *Histoplasma capsulatum* and *T. cruzi* [160]. This is partly attributable to the immunomodulatory role of iron chelation via increased nitric oxide and decreased interleukin-4 (IL-4) production in deferoxamine-treated patients. However, a certain amount of iron is vital for the formation of oxygen radicals by the Fenton reaction and via the catalytic action of phagocyte oxidase (PHOX), while iron overload has immune-debilitating effects. In fact, treating *Salmonella*-infected mice with deferoxamine impairs pathogen clearance due to reduced reactive oxygen species (ROS) generation [161]. Furthermore, certain pathogens, including *Y. enterocolitica, V. vulnificus,* and *Mucorales,* can utilise deferoxamine as a siderophore to increase their pathogenicity. As a precautionary measure, temporary discontinuation of deferoxamine during a febrile illness until establishing whether the episode is caused by a pathogen that can use deferoxamine as a siderophore or taken under control is strongly advised.

Non-siderophoric iron chelators such as deferasirox have been studied for possible anti-infective properties. In an *in vitro* study, *V. vulnificus* was stimulated by deferoxamine; in contrast, orally bioavailable iron chelators such as deferasirox and deferiprone had an inhibitory effect on the growth of *V. vulnificus* [162]. Further, deferasirox and deferiprone limit the growth of *Chlamydia psittaci, Chlamydia trachomatis,* and *L. pneumophila* and may be suitable as add-on therapies in mucormycosis [163, 164]. However, the latter could not be supported by a subsequent double-blind, placebo-controlled, phase 2 trial that aimed to define the safety and efficacy of short-term therapy with deferasirox for patients with acute mucormycosis [165]. Deferiprone did not have the virulence-enhancing effect observed with deferoxamine during experimental *Y. enterocolitica* infection in mice [166]. Deferasirox and deferiprone can be continued during febrile episodes in TDT patients.

9.6. Splenectomy-related risks of infection

Splenectomy plays a significant role in susceptibility to infections in β-thalassaemia, since the spleen has a crucial function in immune defence as a phagocytic filter for blood-borne microorganisms, particularly encapsulated bacteria, and also produces antibodies [167]. The polysaccharide capsules of *S. pneumoniae, Haemophilus influenzae* type B, and *Neisseria meningitidis* protect them from phagocytosis and are a significant factor in their pathogenicity. Therefore, recognition and clearance of these bacteria depend on opsonising anti-polysaccharide antibodies produced by the IgM memory B cells generated from the marginal zone of the spleen. IgM memory B cells produce opsonising antibodies stimulating phagocytosis of encapsulated bacteria by resident macrophages and secrete natural antibodies that can neutralise a larger variety of bacterial and viral pathogens, thereby limiting the initial infections. They further enhance the adaptive immune response by localising the antigens in the spleen. IgM memory B cells differentiate into IgA plasma cells in the intestinal mucosa, playing a role in mucosal defence [168].

Overwhelming post-splenectomy infection (OPSI) is defined as fulminating sepsis, meningitis, or pneumonia triggered mainly by *S. pneumoniae*, followed by *H. influenzae* type B and *N. meningitidis.*

The risk of OPSI is more than 50 times higher than in the general population. Although the risk is highest in the first two years following splenectomy, it is a permanent, life-long condition [169]. Preventive strategies for OPSI include pre/post-splenectomy vaccinations, post-splenectomy antibiotic prophylaxis, and patient education.

Other pathogens responsible for post-splenectomy infections include *E. coli, P. aeruginosa,* group B streptococci, *Enterococcus* spp., and *V. vulnificus* [170].

9.6.1. Pre/post-splenectomy vaccinations

Patients should be evaluated whether they are vaccinated according to the national immunisation schedule, including the vaccines listed below, and any missing vaccinations must be completed before splenectomy. Pneumococcal, meningococcal, and *H. influenzae* type B vaccines are recommended for patients undergoing splenectomy (Table 1) [171]. Furthermore, annual seasonal influenza vaccination (inactivated vaccines) is equally advised to prevent secondary bacterial coinfections, especially *S. pneumoniae* and *Staphylococcus aureus.* Live influenza vaccines are contraindicated. Adhering to local health authorities' recommendations for COVID-19 is also vital.

Immunisation should be completed 14 days before surgery to ensure adequate antibody responses. A recent study involving children who have not received vaccines has suggested that the peak of IgG serum concentration following pneumococcal polysaccharide vaccine (PPSV) administration is recorded between days 9 and 11 [172]. The optimal timing of post-splenectomy immunisation has yet to be identified. However, it appears more than reasonable that patients who are unlikely to receive immunisation post-discharge should be immunised just before discharge. Theoretically, this strategy should guarantee an initial 100% vaccination coverage rate.

Two anti-pneumococcal formulations (pneumococcal conjugate vaccine [PCV] 13 and PPSV 23) are currently recommended. PPSV23 can provide an efficient antibody response after an initial dose of PCV13 but not when PPSV23 is given initially. Theoretically, PCV13 should act as a primer for immunological memory, while PPSV23 should widen the protection, including a broader serotype spectrum. The inhibitory association of PPSV23 with subsequent PCV13 administrations was confirmed [173]. However, the PCV20 vaccine, which has been used in recent years, has eliminated the need to use the PPSV23 vaccine due to its broad spectrum. In cases where PCV20 is applied, there is no need to apply PPSV23 [171].

The quadrivalent meningococcal ACWY conjugate vaccine (MenACWY) allows an immune response against four *N. meningitis* serogroups (A, C, W, Y). A recent phase 3 non-randomised trial demonstrated that in splenectomised subjects, a 1 dose and 2 dose 8 weeks apart schedule of MenACWY-TT was as immunogenic as in age-matched, healthy control participants, with an acceptable safety profile [174].

To target serogroup B, which is most frequently responsible for invasive meningococcal disease, two recombinant vaccines were licensed (namely, meningococcal serogroup B [MenB]-FHbp and MenB-4C). Repeated administration of MenB-4C with 2 dose 8 weeks apart or MenB-FHbp with 2 dose 8 weeks apart schedules and 6 months later are advised [175]. A new MenABCWY vaccine targeting all 5 serogroups has also recently become available.

Because of the high immunogenic activity, booster doses are not recommended for the *H. influenzae* type B (Hib) vaccine (Table 1) [171].

Table 1. *Suggested perioperative vaccination schedules for transfusion-dependent β-thalassaemia children and adults undergoing splenectomy [171].*

Age	PCV13, PCV20	PPSV23
2 mo to <2 yr	4 doses 8 wk apart (4^{th} dose at ≥12 mo)	Postpone until the age of 2 yr
2 to 6 yr*	2 doses 8 wk apart	1^{st} dose: 8 wk after the last PCV13, PCV20 2^{nd} dose: 5 yr after the last PPSV23
>6 yr*	1 dose	

Age	Hib vaccine
0 to <1 yr	4 doses 8 wk apart (routinely scheduled for infants)
1 to 5 yr	2 doses 8 wk apart, in total; at least 1 dose before splenectomy
>5 yr	If immunised, 1 dose

Age	Conjugated MenACWY			MenB
	MenACWY-CRM	MenACWY-D	MenACWY-TT**	MenB-4C**
0 to <2 yr	8 wk to 7 mo: 4 doses 8 wk apart (4^{th} dose at ≥12 mo) >7 mo: 2 doses 12 wk apart (2^{nd} dose at ≥12 mo)	Do not administer, because of immune interference with PCV13, PCV20	6 wk to 6 mo: 3 doses 8 weeks apart (3^{rd} dose at ≥12 mo) >6 mo to 12 mo: 2 doses at least 12 wk apart (2^{nd} dose at ≥12 mo) >12 mo: 2 doses 8-12 wk apart	2 to 11 mo: 3 doses 4 wk apart (3^{rd} dose at ≥12 mo) 12-23 mo: 3 doses 8 wk apart (3^{rd} dose at 12 months after the 2^{nd} dose)
≥2 yr	2 doses 8 wk apart	2 doses 8 wk apart	2 doses 8 wk apart	2 doses 8 wk apart
≥10 yr	2 doses 8 wk apart	2 doses 8 wk apart	2 doses 8 wk apart	2 doses 8 wk apart
Revaccination	Revaccination 3 yr after primary series and afterward a booster dose is needed every 5 yr if the risk continues			Revaccinate 1 yr after primary series and afterward a booster dose is needed every 2-3 yr if the risk continues

**In patients who completed recommended PCV series but have not received PPSV23, if they previously received at least 1 dose of PCV20 then no further PCV or PPSV23 doses needed, if they did not previously receive PCV20 then administer 1 dose of PCV20 or 1 dose of PPSV23 at least 8 weeks after the most recent PCV dose. If PPSV23 is used, administer 1 dose of PCV20 or dose 2 PPSV23 at least 5 years after dose 1 PPSV23.*

***Age groups and use of recommendations for these vaccines may differ in each country. MenB-FHbp vaccine is used only in children ≥10 years of age (2 doses 8 weeks apart, and revaccination 1 year after primary series and afterward a booster dose is needed every 2-3 years if the risk continues).*

Abbreviations: PCV, pneumococcal conjugate vaccine; PPSV, pneumococcal polysaccharide vaccine; Hib, Haemophilus influenzae type B; MenACWY, quadrivalent meningococcal ACWY conjugate vaccine; MenB, meningococcal serogroup B vaccine; yr, year(s); mo, month(s); wk, week(s).

9.6.2. Post-splenectomy antibiotic prophylaxis

Chemoprophylaxis depends on the age of the individual and the treating physician's opinion (Table 2). Although there is little consensus on the duration of use, antibiotic prophylaxis should be provided for a minimum of 1 to 3 years after splenectomy. It is absolutely recommended for children aged <16 years and adults aged >50 years. Lifelong prophylactic antibiotics could be offered to patients who previously experienced an episode of sepsis or who remain immunocompromised due to underlying disease or treatment, as well as patients with an inadequate serologic response to pneumococcal vaccination.

Given the epidemiologic shifts in colonisation and infection due to newer vaccines, additional investigation is needed to better clarify the optimal type and duration of antibiotic therapy [176, 177].

Table 2. *Suggested antibiotic prophylaxis recommendations in splenectomised patients.*

Routine prophylaxis	Primary regimen	Regimen for penicillin allergy
0-5 years of age	Penicillin VK 125 mg bid OR Amoxicillin 20 mg/kg od or bid (max 500 mg)	Azithromycin 5 mg/kg od (max 250 mg)
>5 years of age	Penicillin VK 250 mg bid OR Amoxicillin 20 mg/kg od or bid (max 500 mg)	Azithromycin 5 mg/kg od (max 250 mg)
Febrile illness before ED arrival	**Primary regimen**	**Regimen for penicillin allergy**
Children	Amoxicillin-clavulanate 45 mg/kg bid (max 875 mg)	Cefuroxime 15 mg/kg bid (max 500 mg) OR Cefdinir 7 mg/kg bid (max 300 mg)
Adults	Amoxicillin-clavulanate 875/125 mg bid	Cefuroxime 500 mg bid OR Cefdinir 300 mg bid OR Levofloxacin 750 mg od

Abbreviations: ED, emergency department; od, once per day; bid, twice per day.

9.6.3. Patient education

Splenectomised patients should be educated about the increased risk of infections, how to avoid them, and what to do in case of illness. Patients should be recommended to carry their antibiotics supply in case of emergency. In case of any seemingly minor signs of infection, patients should initiate early empiric treatment with the oral antibiotics supply on hand (Table 2) and seek urgent medical attention [178, 179].

9.6.4. Management of infections in splenectomised patients

Because the individual risk of sepsis cannot be predicted, all infections in splenectomised patients must be considered potentially life-threatening.

OPSI is a medical emergency. Following brief prodromal symptoms such as fever, shivering, myalgia, vomiting, diarrhoea, and headache, septic shock develops in just a few hours, with anuria, hypotension, hypoglycaemia, and, commonly, disseminated intravascular coagulation, and massive adrenal gland haemorrhage (Waterhouse-Friderichsen syndrome), progressing to multiorgan failure and death [180]. The mortality rate is around 50% to 70%, and most deaths occur within the first 24 hours; thus, only prompt diagnosis and immediate treatment can reduce mortality [167, 181].

9.7. Disease-related risks of infection

Ineffective erythropoiesis and haemolysis result in hyperplasia of monocyte/macrophages, which phagocytose defective erythroid precursors and erythrocytes. The increased phagocytic activity resulting from the clearance of defective erythrocytes may reduce the capacity of the phagocytic system to defend against microorganisms [182] and consequently overwhelms pattern recognition receptors, including Toll-like receptors [183]. In the clinical setting, severe anaemia has also been reported as a risk factor for bacterial infections in thalassaemia [119].

The deleterious effects of ineffective erythropoiesis, anaemia, and haemolysis on the host defence mechanisms may be mitigated by maintaining pretransfusion haemoglobin levels between 9.5 and 10.5 g/dL, which corrects anaemia while suppressing ineffective erythropoiesis.

9.8. Further details on specific bacterial infections

9.8.1. Yersinia enterocolitica

Y. enterocolitica is of low pathogenicity and restricted to the gastrointestinal tract in an immune-competent host. The availability of large amounts of iron in those with iron overload or undergoing deferoxamine chelation increases the virulence of *Y. enterocolitica*. Fulminant *Y. enterocolitica* septicaemia has been reported as a common infectious risk in deferoxamine-treated TDT patients from Western countries [184] rather than in Eastern countries.

Fever is the most common presenting feature, often associated with abdominal pain and enterocolitis. Pharyngitis-tonsillitis, acute respiratory distress syndrome, and polyarthritis are other clinical manifestations of infection. The mortality rate can reach 50% in septicaemia with complications including hepatic and splenic abscesses, osteomyelitis, intussusception, nephritis, meningitis, and endocarditis.

Blood and stool samples must be cultured under specific conditions (at 22°C for 48 hours). The microbiology laboratory should be informed to enable correct culture conditions. Serological tests may display cross-reactivity. However, fourfold rises in IgG titres in samples obtained 15 days apart may suggest a recent infection.

Intravenous trimethoprim-sulfamethoxazole (400 mg sulfamethoxazole every 12 hours) for 7 days (14 days in the case of septicaemia) plus gentamicin (5-7 mg/kg every 24 hours) should be used for treatment. Intramuscular ceftriaxone (2 g every 12 hours) is an alternative in focal infections

(e.g., enteritis, pharyngitis, tonsillitis). Ciprofloxacin (400 mg every 12 hours) is also an effective antibiotic.

9.8.2. Klebsiella spp.

Klebsiella spp. have been reported as the major cause of severe bacterial infections in patients with thalassaemia from the Far East [119].

Infections present with sinusitis, intracranial infections, septicaemia, and pyogenic abscesses in the liver, lung, kidney, and parathyroid gland, which are associated with high rates of morbidity and mortality.

Ceftazidime (2 g every 8 hours) plus gentamicin (5-7 mg/kg every 24 hours) should be used for treatment. Meropenem, imipenem, and fluoroquinolones are alternative antibiotics for resistant species. Early surgical intervention should be considered.

9.9. Further details on specific viral infections

9.9.1. Human parvovirus B19

HPVB19 typically causes erythaema infectiosum or fifth disease in children with the clinical course of a flu-like syndrome. HPVB19 DNA is present in the circulation for almost one week and disappears during the production of neutralising antibodies (IgM for 6-8 weeks and IgG afterwards). This protective mechanism would not be present in immunocompromised subjects, leading to the persistence of viral DNA.

HPVB19 mainly infects erythroid progenitors and is complicated by transient red cell aplasia. Because of high erythroid turnover, patients with thalassaemia may develop severe anaemia with low reticulocyte counts during the course of HPVB19 infection [185]. Patients require intensification of the transfusion regimen during acute infection. HPVB19 infection should be suspected in patients with increased blood consumption once other responsible factors (e.g., poor haemoglobin content of RBC units, alloimmunisation, or hypersplenism) are excluded.

Although the main transmission route is respiratory, transfusions of pRBC collected from persistently infected blood donors play a secondary role [186].

9.9.2. Human immunodeficiency virus

HIV leads to CD4+ lymphocyte depletion that renders the individual at risk for many types of opportunistic infections. Due to continuous implementation and improvement of more sensitive serological methods and NAT, the residual risk of viral transmission through blood transfusions decreased to less than 1:1.3 million for HIV in the European Union and the USA [187, 188]. No case has been recorded since the implementation of NAT screening (August 2008-2015). However, in Africa, higher prevalence and less comprehensive donor testing still result in an estimated 10% to 15% of cases of HIV linked to unsafe blood transfusion (https://www.safeblood4africa.org/).

In a large multicentre study comprising 79 HIV-positive thalassaemia patients from different countries, the progression to overt-acquired immune deficiency syndrome (AIDS) after seroconversion was 1.4% after 3 years and 9% after five years. There was no statistically significant relationship between disease progression and age, sex, acute infection, or splenectomy [189]. However, a significant inverse relationship between disease progression and the dose of

deferoxamine administered was reported; the rate of progression decreases as the mean daily deferoxamine dose increases [190].

A more recent 30-year multicentre study of about 3000 TDT patients from Greece (3-65 years old, 45% splenectomised) examined data on the survival of HIV-infected patients along with national epidemiological data on donor blood. In the period 1982-2015, 43 patients tested positive for HIV infection [191]. Forty-two who had received 819,000 units of RBCs (1:19,000) were infected by the end of 1987. Transfusion-transmitted HIV infection risk was reduced significantly by 2005 with seroprevalence in only three patients transfused with seronegative blood donated from three different donors during the serologically silent window period. Therefore, the residual risk of transfusion-transmitted HIV in these patients in 1988-2015 was estimated at 1:290,666 serologically tested blood units. The corresponding figure in the blood donor population was 1:1,833,333 units. Of the 32 patients (74%) who died up to 2015, 26 (81%) progressed to AIDS at a mean age of 16.5 ± 9.2 years. One deceased patient was co-infected with HBV and HCV. HBV and HCV were higher in HIV seropositive patients than in seronegative ones. Of the 13 survivors (mean age 43 ± 7.1 years), two had a history of splenectomy, and two were anti-HCV positive and HCV RNA negative. Most patients had negative viral load and were free of HIV symptoms. A multivariate analysis demonstrated that serum ferritin levels were statistically associated with the duration of survival after diagnosis of HIV infection in this group. This 30-year study confirms Costagliola colleagues' earlier findings (1992,1994) about the inverse relationship between the rate of disease progression and iron chelation therapy.

A large spectrum of therapeutic options is currently available for HIV-infected patients, which have also been used in patients with TDT. With early diagnosis and early initiation of antiretroviral therapy (ART), the life expectancy of people with HIV has reached the same as the general population [192].

Since iron overload can have an adverse effect on HIV-1 disease progression, such as faster progression of HIV in patients with low doses of deferoxamine and high serum ferritin [156], optimal control of body iron burden with iron-chelation regimens is recommended in HIV-1-positive TDT patients. Although there is no evidence that splenectomy facilitates the progression of HIV infection, a splenectomy treatment strategy should be decided with caution in an HIV-1-positive patient.

9.9.3. Cytomegalovirus infection

CMV can be transmitted by fresh blood components containing leucocytes. It is estimated that approximately 2-12% of CMV-positive healthy donors can transmit the virus by blood donation to the recipients. The consequences of CMV infection are serious in immunocompromised patients, such as thalassaemia patients who have had HSCT.

The use of blood products from CMV-seronegative donors has been shown to be effective in preventing transmission. However, it does not fully eliminate the risk of transmission. Moreover, CMV seroprevalence reaches 50 to 100% in various geographical regions, and the availability of CMV-seronegative products is limited.

Pre-deposit leucodepletion of cellular blood products achieving a residual leucocyte count <5 $x10^6$ per unit allows the reduction of CMV transmission to a level at least equivalent to the

transfusion of seronegative blood components for those patients at significant risk of severe CMV transfusion-associated disease [193].

9.9.4. West Nile virus

West Nile virus is a mosquito-borne flavivirus that primarily causes an asymptomatic or mild disease. However, in <1%, it causes neurological disease such as encephalitis, meningitis, or more rarely acute flaccid paralysis. Elderly and immunocompromised persons are at higher risk of developing severe disease and having a fatal outcome. The risk of transmission through blood transfusion has been recognised, and preventive blood safety measures have been implemented in affected areas. The paucity of reported transfusion-transmitted infections and the blood screening results in the USA and Europe suggest that blood safety interventions are effective [132, 140].

Investigation of West Nile virus infection in 369 TDT patients in affected areas of Greece showed that 1.9% of the patients were positive for antibodies against the virus with a mild clinical course. Transmission before the implementation of blood screening with NAT was confirmed in two thalassaemia patients [194].

9.9.5. Viral hepatitis

Details of HBV and HCV infections along with associated hepatic morbidity in TDT patients are summarised in Chapter 5.

Hepatitis E is caused by infection with a non-enveloped, single-stranded RNA virus. Although underdiagnosed worldwide, it is responsible for 20 million infections yearly. Four major genotypes infect humans. Genotypes 1 and 2, endemic in many developing countries, are responsible for water-borne epidemics. Genotypes 3 and 4 are associated with zoonotic HEV infections causing sporadic infections in industrialised countries; they are transmitted to humans through uncooked infectious pork consumption and game products or by contact with infected animals. Transmissions through transfusion and transplantation have also been reported [195, 196].

Hepatitis E virus mainly causes acute self-limiting infections, but chronic infections may occur in immunocompromised patients and can lead to fulminant hepatitis and death. Acquired hepatitis E virus cases have been observed across Europe, where genotype 3 infections have raised questions for public health and blood safety. Hepatitis E virus genotype 3 infection is commonly asymptomatic or mild and self-limiting without chronic sequelae. As acute phase viraemia persists for 6-8 weeks and because most cases are asymptomatic, infected blood donors can donate blood while viraemic. A multicentre study in blood donors and thalassaemic patients in Greece found an overall prevalence of 2.9% for HEV IgG antibodies (significantly greater in males and older donors) and a prevalence of 3.6% in thalassaemic patients [197]. The increasing incidence of transfusion-transmitted hepatitis E virus has led several European countries to include blood screening in preventive strategies for this infection. The risk of transmission in groups such as thalassaemia patients with heavy exposure to donor RBCs has raised new issues in their clinical management.

Studies in donor blood in several European countries using NAT testing have shown a high frequency of viraemic donations of up to 1:726 [198]. However, hepatitis E virus testing of blood products has still not been generally implemented. The number of transfusion-transmitted hepatitis E virus has, until now, been very low, probably due to under-reporting and under-

recognition, mainly because of asymptomatic infections in transfused patients, and reports are limited to the relevant risk of in immunosuppressed patients [199].

9.9.6. COVID-19

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV 2) is a strain of coronavirus that causes COVID-19 (Coronavirus Disease 2019), the respiratory illness responsible for the COVID-19 pandemic. Since the first cases of COVID-19 caused by SARS-CoV-2 were reported in China in December 2019, as of April 13, 2024, 704,753,890 people have been affected by the infection, with 7,010,681 reported deaths. Multiple COVID-19 vaccines, such as protein subunit vaccines containing spike proteins of the SARS-CoV-2 and mRNA vaccines, have received emergency use authorisation (EUA) to prevent severe COVID-19 illness and have been instrumental in controlling the severity of this pandemic and improving clinical outcomes.

In June 2020, the Thalassaemia International Federation (TIF) published recommendations on managing thalassaemia patients during the COVID-19 pandemic. A general classification in 3 groups (low to moderate risk, high risk, and very high risk) was proposed according to the level of COVID-19 severity risks based on adequacy of transfusions, receiving optimal chelation, having intact spleen, and no co-morbidities. TIF pointed out that patients with severe forms of thalassaemia should be considered vulnerable to COVID-19 [200].

Three additional risks of severe infection should also be systematically evaluated and/or prevented for patients with severe COVID-19:

- The risk of latent or existing adrenal insufficiency decompensation.
- The increased risk of thromboembolic events with recommendation of prophylactic anticoagulation.
- The risk of secondary bacterial infection which is exceptionally high in splenectomised patients [201].

Despite immunological and cardiometabolic disturbances, oxidative stress, and prolonged low-grade inflammatory status that might explain the susceptibility of TDT patients to SARS-CoV-2 infection, most develop mild to moderate COVID-19 [202]. Vaccination should follow national policies adopted in the general population.

9.10. Further details on specific fungal infections

9.10.1. Mucor spp.

Mucormycosis or zygomycoses are opportunistic infections that may affect thalassaemia patients undergoing HSCT. Iron is a key nutrient for fungi as well as bacteria. The notion that iron chelation may serve as an effective antifungal modality was proposed more than 30 years ago. However, administration of deferoxamine resulted in worsening of mucormycosis. This was attributed to the fact that deferoxamine itself may act as a siderophore for the fungi. As mentioned earlier, observations that deferasirox chelation may be a useful adjunct to antifungal treatment [163] led to a trial of deferasirox combined with liposomal amphotericin B (AmBisome) as short-term therapy for mucormycosis. The results were disappointing as patients treated with deferasirox had a higher mortality rate at 90 days, leading the authors to conclude that the data did not support a role for initial, adjunctive deferasirox therapy for mucormycosis.

9.10.2. Phytiosum insidiosi

Pythiosis is an infrequent human infection caused by *Phytiosum insidiosum*, a fungus-like organism. Three forms of human pythiosis are recognised: 1) cutaneous form affecting the periorbital area, face, and limbs as a granulomatous, ulcerating abscess-like cellulitis; 2) ophthalmic pythiosis affecting the eyes as corneal ulcers and keratitis; 3) systemic pythiosis affecting vascular tissue and resulting in arterial occlusions leading to gangrene and amputation [158]. Pythiosis has been reported in Thailand, Australia, Haiti, India, New Zealand and the USA. The systemic form was common in patients with thalassaemia and associated with high morbidity and mortality (most patients die within 6 months) [203].

Serological tests and polymerase chain reaction (PCR) methods are being developed for diagnosis. Antifungal drugs are ineffective for disease control, and medical treatment alone is insufficient to salvage patients with systemic infections.

Two vaccines for pythiosis have been developed. One vaccine is prepared from soluble concentrated *P. insidiosum* antigen. It is administered intradermally in the first injection, subcutaneously in the following three injections, and at two weekly intervals in patients with life-threatening systemic infections. The vaccine was curative in a substantial number of cases [204].

9.11. Further details on specific parasitic infections

9.11.1. Malaria

When it comes to transfusion-transmitted diseases, parasitic infections play a significant role. Malaria is believed to be the most important parasitic disease currently facing humans. Malaria is a life-threatening disease caused by parasites transmitted to humans through the bites of infected female *Anopheles* mosquitoes (*Plasmodium falciparum, P. vivax, P. malariae, P. ovale*). The World Health Organization (WHO) estimates that 3.2 billion people live in areas at risk of malaria transmission in 106 countries and territories. Globally in 2022, there were an estimated 249 million malaria cases in 85 malaria endemic countries and areas, and Africa was home to 94% of cases [205].

Transfusion-transmitted malaria is also reported sporadically in non-endemic areas, where the risk of transmission arises from travellers to endemic areas and permanent residents with origin in endemic regions. However, autochthonous transmissions are possible due to the presence of competent vectors.

An outbreak of locally acquired *P. vivax* malaria in Greece in 2009, which peaked in 2011, raised the question of how to define spatial boundaries of an affected area and when to trigger specific blood safety measures targeted to affected areas with ongoing local transmission. In this context, the European Centre for Disease Prevention and Control advice the following expert opinion that could help the European Union's national blood safety authorities in developing a preventive strategy during malaria outbreaks [206]:

- Blood sessions will be suspended in the affected areas and in surrounding locations up to a radius of 6 km. The distance criterion is based on the mosquito vector's dispersal range.
- Temporary deferral for 6 months from blood donation of asymptomatic persons residing or working in the above areas and visitors from the beginning of an outbreak up to 4 months after the end of the mosquito activity season.

- Blood screening for malarial antibodies or DNA might be considered instead of temporary deferral, which might jeopardise the blood supply.
- Pathogen reduction could be considered if a suitable methodology is available [207, 208].

There is evidence that carriers of certain haemoglobinopathies have a reduced risk of severe and fatal *P. falciparum* malaria. However, the same is not true for the homozygous state, including β-thalassaemia major and intermedia [158]. Physicians who advise chemoprophylaxis to patients before and during periods of travel into endemic areas should take into account the evolving patterns of drug resistance in malaria parasites and changes in recommendations for malaria prevention [209].

KEY POINTS AND RECOMMENDATIONS

1. Recommendations for cholelithiasis.

- The gall bladder should always be examined during abdominal ultrasonography in transfusion-dependent β-thalassaemia (TDT), such as annual screening conducted for liver disease. Patients with symptoms or signs of cholelithiasis on laboratory studies or imaging should be managed per local standards of care (**Grade C, Class I**).
- Cholecystectomy should be considered during splenectomy when stones are symptomatic to prevent serious complications such as acute cholecystitis in splenectomised patients (**Grade C, Class IIa**).

2. Recommendations for malignancy.

- Recommendations for screening, diagnosis, and management of hepatocellular carcinoma (HCC) in TDT patients are provided in Chapter 5.
- Considering the potential increased risk of malignancy in patients with TDT, especially those with iron overload, patients should be closely screened for common cancers according to local standards and guidelines (**Grade C, Class I**).

3. Recommendations for skin manifestations and leg ulcers.

- The skin of TDT patients should always be inspected on routine physical examination for dermatological manifestations or leg ulcers, especially in patients who are suboptimally transfused and/or chelated, on long-term iron chelation, or splenectomised (**Grade C, Class I**).
- Optimisation of blood transfusion or hydroxyurea therapy may help in wound healing in leg ulcers (**Grade C, Class IIb**).
- Patients with evidence of dermatological manifestations or leg ulcers should be treated with local or systemic therapy based on recommendations from a dermatologist and/or a plastic surgeon (**Grade C, Class I**).

4. Recommendations for renal disease.

- Patients with TDT should be monitored for iron chelator-related renal toxicity with dose adjustments and/or interruption per local prescribing information (see Chapter 3) (**Grade A, Class I**).
- Patients with TDT should be screened for renal disease with the following (**Grade C, Class IIa**):
  - Serum creatinine, urine analysis with spot ratio of protein/creatinine: every 6 months.
  - Serum calcium, phosphorus, magnesium, and uric acid; 24-hour urine collection for protein/creatinine and calcium/creatinine ratio: every 12 months starting the age of 10 years or earlier if needed.
  - Renal ultrasound: every 2 years after adolescence and in case of laboratory abnormalities.
- Patients with renal dysfunction should be referred to a nephrologist for management per local standards and guidelines (**Grade C, Class I**).

5. Recommendations for splenomegaly.

- The spleen size should be examined in clinical visits and splenectomy should generally be avoided in patients younger than 5 years of age, and otherwise reserved for cases of (**Grade C, Class IIa**):
  - When other interventions to manage anaemia are contraindicated.
  - Hypersplenism leading to worsening anaemia, leukopaenia, or thrombocytopaenia and causing clinical problems such as recurrent bacterial infections or bleeding.
  - Splenomegaly accompanied by symptoms such as left upper quadrant pain or early satiety.
  - Massive splenomegaly (largest dimension >20 cm) with concern about possible splenic rupture.
- Laparoscopic splenectomy is preferred to the open procedure unless otherwise indicated by the responsible surgeon (**Grade B, Class IIa**).
- Splenectomised patients should be considered at higher risk of thromboembolic events during risk assessment for consideration of thromboprophylaxis in medical and surgical settings (**Grade C, Class IIa**).

6. Recommendations for extramedullary haematopoiesis (EMH).

- The diagnosis of EMH in TDT should be made based on clinical suspicion and imaging findings (ideally with magnetic resonance imaging), with confirmatory biopsy (fine-needle aspiration could be safer) reserved for cases of a complex differential (e.g., in older patients at risk of malignancy) and in the absence of bleeding risk (e.g., vascular lesions) (**Grade C, Class IIa**).
- In cases of asymptomatic EMH (incidental finding) in a safe anatomical location, a wait-and-see strategy should be considered (**Grade C, Class IIb**).
- EMH masses that could lead to future morbidity can be managed by increasing blood transfusions to further suppress ineffective erythropoiesis (**Grade C, Class IIa**).
- Management of symptomatic EMH masses should be tailored to each individual to prevent permanent disability. Table 3 illustrates the approach to management of paravertebral EMH masses (**Grade C, Class I**).

Table 3. *Management of paraspinal extramedullary haematopoiesis masses.*

Neurologic symptoms	Intervention
Mild	Optimise transfusions with pretransfusion haemoglobin target >10 g/dL ± hydroxyurea
Moderate	Optimise transfusions with pretransfusion haemoglobin target >10 g/dL + low-dose radiotherapy (+ steroids) ± hydroxyurea
Severe	Optimise transfusions with pretransfusion haemoglobin target >10 g/dL + preoperative transfusions + laminectomy and excision of mass + low-dose radiotherapy (if excision is incomplete)

7. Recommendations for infectious disease.

- Quality assurance guidelines, strict regulatory standards, and haemovigilance procedures should be established to safeguard the quality of donor blood and enhance the safety of the transfusion process (**Grade C, Class I**).
- The minimum mandatory serological blood donor screening tests should include (**Grade A, Class I**):
  - Antibody to human immunodeficiency virus (HIV)-1 and HIV-2 including outlying types (e.g., HIV-1 type O).
  - Antibody to hepatitis C virus (HCV).
  - Hepatitis B surface antigen (HBsAg) assay.
  - Treponema pallidum haemagglutination assay (TPHA) for syphilis.
- Additional serological screening tests for blood donors may be required by national authorities based on local infectious agents' epidemiology (**Grade C, Class IIa**).
- Vaccination against hepatitis B virus (HBV) should protect all patients with TDT who are seronegative for HBV markers. Since the protection offered by vaccination is not absolute, vaccinated patients should be tested annually for anti-HBs. A booster dose of the HBV vaccine is considered if the anti-HBs titre decreases. Patients should also be tested annually for other transfusion-transmitted infections (TTI) such as HIV and HCV (anti-HCV), which if positive, should be followed by HCV RNA (**Grade A, Class I**).
- The suspicion and approach to transfusion-related bacterial sepsis should include the following (**Grade A, Class I**):
  - If bacterial contamination is suspected, the transfusion should be halted immediately.
  - Intravenous infusion of a third-generation cephalosporin (cefotaxime 2 g every 8 hours or ceftriaxone 2 g every 12 hours) or carbapenem (meropenem or imipenem 2 g every 8 hours) combined with vancomycin (1-1.5 g every 12 hours).
  - Gram stain and blood culture should be obtained from the blood bag and the recipient.
- Preventative measures for bacterial sepsis should include enhanced phlebotomy site disinfection and diversion pouches integral to the blood collection set (to remove the first aliquot of donor blood, which may have higher concentrations of skin flora) (**Grade C, Class IIa**).
- Transfusion of pre-storage leucodepleted red blood cells stored <14 days may have preventive benefits against infections (**Grade C, Class IIa**).
- Control of iron overload may have preventive/therapeutic benefits against infections (**Grade C, Class IIa**).
- In patients with febrile illness, temporary discontinuation of deferoxamine is strongly advised until establishing whether the episode is caused by a pathogen that can use deferoxamine as a siderophore, unless the patient has a severe cardiac risk or acute heart failure (**Grade B, Class IIa**). Patients can continue to use the oral iron chelators deferiprone and deferasirox (**Grade C, Class IIa**).
- Patients undergoing splenectomy should receive appropriate pre/post-splenectomy vaccinations to be completed 14 days before surgery including pneumococcal, meningococcal, and *Haemophilus influenzae* type B (Table 1), inactivated influenza vaccine

(annually), COVID-10 vaccine per local health authorities' recommendations. Patients who underwent splenectomy without being given the vaccine may still benefit from vaccination post-splenectomy (**Grade A, Class I**).

- Patients undergoing splenectomy should receive prophylactic antibiotic therapy for at least 1-3 years following splenectomy (Table 2) (**Grade A, Class I**).
- The suspicion and approach to overwhelming post-splenectomy infection (OPSI) should include the following (**Grade A, Class I**):
  - Physicians must be aware of the potential life-threatening infections in TDT patients who underwent splenectomy, and patients should be educated on use of early empiric treatment with oral antibiotics with signs of infection and to seek early medical care when fever develops.
  - In patients at risk and with indicative symptoms, prompt initiation of empirical antibiotics is essential with intravenous infusion of third-generation cephalosporin (cefotaxime 2 g every 8 hours or ceftriaxone 2 g every 12 hours), combined with gentamicin (5-7 mg/kg every 24 hour) or ciprofloxacin (400 mg every 12 hours) or vancomycin (1-1.5 g every 12 hours).
  - While awaiting the results of the blood culture, bacteria can be visualised by Gram staining.
  - A reverse transcriptase-polymerase chain reaction (RT-PCR) test for simultaneous identification of three main encapsulated bacteria (*Streptococcus pneumonia, H. influenzae* type B, and *Nisseria meningitidis*) is available.

REFERENCES

1. El-Beshlawy A, Dewedar H, Hindawi S, Alkindi S, Tantawy AA, Yassin MA, Taher AT. Management of transfusion-dependent beta-thalassemia (TDT): Expert insights and practical overview from the Middle East. Blood Rev 2024;63:101138.
2. Taher AT, Cappellini MD. How I manage medical complications of beta-thalassemia in adults. Blood 2018;132(17):1781-1791.
3. Pinto VM, Poggi M, Russo R, Giusti A, Forni GL. Management of the aging beta-thalassemia transfusion-dependent population - The Italian experience. Blood Rev 2019;38:100594.
4. Shahramian I, Behzadmehr R, Afshari M, Allahdadi A, Delaramnasab M, Bazi A. Cholelithiasis in Thalassemia Major Patients: A Report from the South-East of Iran. Int J Hematol Oncol Stem Cell Res 2018;12(2):117-122.
5. Maulana MB, Fuadi MR. Clinical pathology aspect on diagnosis cholelithiasis in beta-Thalassemia patient: A case report. Ann Med Surg (Lond) 2022;81:104454.
6. Lotfi M, Keramati P, Assdsangabi R, Nabavizadeh SA, Karimi M. Ultrasonographic assessment of the prevalence of cholelithiasis and biliary sludge in beta-thalassemia patients in Iran. Med Sci Monit 2009;15(8):CR398-402.
7. Galanello R, Piras S, Barella S, Leoni GB, Cipollina MD, Perseu L, Cao A. Cholelithiasis and Gilbert's syndrome in homozygous beta-thalassaemia. Br J Haematol 2001;115(4):926-928.
8. Olivieri NF, Muraca GM, O'Donnell A, Premawardhena A, Fisher C, Weatherall DJ. Studies in haemoglobin E beta-thalassaemia. Br J Haematol 2008;141(3):388-397.
9. Winichagoon P, Thonglairoam V, Fucharoen S, Wilairat P, Fukumaki Y, Wasi P. Severity differences in beta-thalassaemia/haemoglobin E syndromes: implication of genetic factors. Br J Haematol 1993;83(4):633-639.
10. Premawardhena A, Fisher CA, Fathiu F, de Silva S, Perera W, Peto TE, Olivieri NF, Weatherall DJ. Genetic determinants of jaundice and gallstones in haemoglobin E beta thalassaemia. Lancet 2001;357(9272): 1945-1946.
11. Karimi M, Giti R, Haghpanah S, Azarkeivan A, Hoofar H, Eslami M. Malignancies in patients with beta-thalassemia major and beta-thalassemia intermedia: a multicenter study in Iran. Pediatr Blood Cancer 2009;53(6):1064-1067.
12. Chung WS, Lin CL, Lin CL, Kao CH. Thalassaemia and risk of cancer: a population-based cohort study. J Epidemiol Community Health 2015;69(11):1066-1070.
13. Borgna-Pignatti C, Rugolotto S, De Stefano P, Zhao H, Cappellini MD, Del Vecchio GC, Romeo MA, Forni GL, Gamberini MR, Ghilardi R, Piga A, Cnaan A. Survival and complications in patients with thalassemia major treated with transfusion and deferoxamine. Haematologica 2004;89(10):1187-1193.
14. Modell B, Khan M, Darlison M, Westwood MA, Ingram D, Pennell DJ. Improved survival of thalassaemia major in the UK and relation to T2* cardiovascular magnetic resonance. J Cardiovasc Magn Reson 2008;10:42.
15. Voskaridou E, Kattamis A, Fragodimitri C, Kourakli A, Chalkia P, Diamantidis M, Vlachaki E, Drosou M, Lafioniatis S, Maragkos K, Petropoulou F, Eftihiadis E, Economou M, Klironomos E, Koutsouka F, Nestora K, Tzoumari I, Papageorgiou O, Basileiadi A, Lafiatis I, Dimitriadou E, Kalpaka A, Kalkana C, Xanthopoulidis G, Adamopoulos I, Kaiafas P, Mpitzioni A, Goula A, Kontonis I, Alepi C, Anastasiadis A, Papadopoulou M, Maili P, Dionisopoulou D, Tsirka A, Makis A, Kostaridou S, Politou M, Papassotiriou I, Greek Haemoglobinopathies Study G. National registry of hemoglobinopathies in Greece: updated demographics, current trends in affected births, and causes of mortality. Ann Hematol 2019;98(1):55-66.
16. Origa R, Gianesin B, Longo F, Di Maggio R, Cassinerio E, Gamberini MR, Pinto VM, Quarta A, Casale M, La Nasa G. Incidence of cancer and related deaths in hemoglobinopathies: A follow-up of 4631 patients between 1970 and 2021. Cancer 2023;129(1):107-117.
17. Forni GL, Gianesin B, Musallam KM, Longo F, Rosso R, Lisi R, Gamberini MR, Pinto VM, Graziadei G, Vitucci A, Bonetti F, Musto P, Piga A, Cappellini MD, Borgna-Pignatti C, Webthal p. Overall and complication-free survival in a large cohort of patients with beta-thalassemia major followed over 50 years. Am J Hematol 2023;98(3):381-387.

18. Hodroj MH, Bou-Fakhredin R, Nour-Eldine W, Noureldine HA, Noureldine MHA, Taher AT. Thalassemia and malignancy: An emerging concern? Blood Rev 2019;37:100585.
19. Origa R, Gianesin B, Longo F, Di Maggio R, Cassinerio E, Gamberini MR, Pinto VM, Quarta A, Casale M, La Nasa G, Caocci G, Piroddi A, Piolatto A, Di Mauro A, Romano C, Gigante A, Barella S, Maggio A, Graziadei G, Perrotta S, Forni GL. Incidence of cancer and related deaths in hemoglobinopathies: A follow-up of 4631 patients between 1970 and 2021. Cancer 2023;129(1):107-117.
20. Voskaridou E, Ladis V, Kattamis A, Hassapopoulou E, Economou M, Kourakli A, Maragkos K, Kontogianni K, Lafioniatis S, Vrettou E, Koutsouka F, Papadakis A, Mihos A, Eftihiadis E, Farmaki K, Papageorgiou O, Tapaki G, Maili P, Theohari M, Drosou M, Kartasis Z, Aggelaki M, Basileiadi A, Adamopoulos I, Lafiatis I, Galanopoulos A, Xanthopoulidis G, Dimitriadou E, Mprimi A, Stamatopoulou M, Haile ED, Tsironi M, Anastasiadis A, Kalmanti M, Papadopoulou M, Panori E, Dimoxenou P, Tsirka A, Georgakopoulos D, Drandrakis P, Dionisopoulou D, Ntalamaga A, Davros I, Karagiorga M, Greek Haemoglobinopathies Study G. A national registry of haemoglobinopathies in Greece: deducted demographics, trends in mortality and affected births. Ann Hematol 2012;91(9):1451-1458.
21. Moukhadder HM, Halawi R, Cappellini MD, Taher AT. Hepatocellular carcinoma as an emerging morbidity in the thalassemia syndromes: A comprehensive review. Cancer 2017;123(5):751-758.
22. Zulfiqar Z, Kanwal A, Chaudhry M, Aadil M, Qaiser S, Malik A, Abdullah M. Cutaneous Manifestations in Patients with Beta-Thalassemia Major. J Mother Child 2023;27(1):142-146.
23. El-Dash H, Adel S. Cutaneous manifestations in Egyptian children with beta-thalassemia major: Relationship with serum ferritin, thyroid profile, and treatment modalities. Pediatr Dermatol 2018;35(5):639-643.
24. Dogramaci AC, Savas N, Ozer B, Duran N. Skin diseases in patients with beta-thalassemia major. Int J Dermatol 2009;48(10):1057-1061.
25. Brigant F, Hautefeuille V, Dadban A, Lok C, Nguyen-Khac E, Chaby G. Generalized pruritus in dysmetabolic hyperferritinemia treated by phlebotomy. Dermatol Online J 2015;21(9).
26. Fahmey SS, Taha G, El-Refaey A, Adly S. Skin Disorders in Egyptian Children with beta-Thalassemia Major. J Trop Pediatr 2018;64(2):104-109.
27. Giordano G, Ricci R, Froio E, Izzi G, Barone A, Boccaletti V. A case of colloid milium in patient with beta thalassaemia major. J Cutan Pathol 2008;35(6):566-569.
28. Azzazi Y, Khedr H, Abdel-Halim MRE. Pigmented colloid milium in a beta-thalassemia major patient: a case report. Int J Dermatol 2023;62(12):e626-e628.
29. Saeidnia M, Shadfar F, Sharifi S, Babashahi M, Ghaderi A, Shokri M. Skin complications during iron chelation therapy for beta-thalassemia: overview and treatment approach. Int J Hematol 2024;120(3):271-277.
30. Antar A, Ishak RS, Otrock ZK, El-Majzoub N, Ghosn S, Mahfouz R, Taher AT. Successful treatment of hydroxyurea-associated chronic leg ulcers associated with squamous cell carcinoma. Hematol Oncol Stem Cell Ther 2014;7(4):166-169.
31. Guillot B, Bessis D, Dereure O. Mucocutaneous side effects of antineoplastic chemotherapy. Expert Opin Drug Saf 2004;3(6):579-587.
32. Taher AT, Musallam KM, Karimi M, El-Beshlawy A, Belhoul K, Daar S, Saned MS, El-Chafic AH, Fasulo MR, Cappellini MD. Overview on practices in thalassemia intermedia management aiming for lowering complication rates across a region of endemicity: the OPTIMAL CARE study. Blood 2010;115(10):1886-1892.
33. Matta BN, Abbas O, Maakaron JE, Koussa S, Daderian RH, Taher AT. Leg ulcers in patients with beta-thalassaemia intermedia: a single centre's experience. J Eur Acad Dermatol Venereol 2014;28(9):1245-1250.
34. Taher A, Musallam K, Cappellini MD. Guidelines for the Management of Non-Transfusion-Dependent β-Thalassaemia. 3rd ed. Nicosia, Cyprus: Thalassaemia International Federation, 2023.
35. al-Momen AK. Recombinant human erythropoietin induced rapid healing of a chronic leg ulcer in a patient with sickle cell disease. Acta Haematol 1991;86(1):46-48.

36. Gamberini MR, Fortini M, De Sanctis V. Healing of leg ulcers with hydroxyurea in thalassaemia intermedia patients with associated endocrine complications. Pediatr Endocrinol Rev 2004;2 Suppl 2:319-322.
37. Dettelbach HR, Aviado DM. Clinical pharmacology of pentoxifylline with special reference to its hemorrheologic effect for the treatment of intermittent claudication. J Clin Pharmacol 1985;25(1):8-26.
38. Gimmon Z, Wexler MR, Rachmilewitz EA. Juvenile leg ulceration in beta-thalassemia major and intermedia. Plast Reconstr Surg 1982;69(2):320-325.
39. Opartkiattikul N, Sukpanichnant S, Wanachiwanawin W, Fucharoen S, Funahara Y, Sumiyoshi A, Imai K, Sangtawesin W, Thientadakul P. A double-blind placebo control trial of dilazep in beta-thalassemia/hemoglobin E patients. Southeast Asian J Trop Med Public Health 1997;28 Suppl 3:167-171.
40. Josifova D, Gatt G, Aquilina A, Serafimov V, Vella A, Felice A. Treatment of leg ulcers with platelet-derived wound healing factor (PDWHFS) in a patient with beta thalassaemia intermedia. Br J Haematol 2001;112(2):527-529.
41. Levin C, Koren A. Healing of refractory leg ulcer in a patient with thalassemia intermedia and hypercoagulability after 14 years of unresponsive therapy. Isr Med Assoc J 2011;13(5):316-318.
42. Minniti CP, Gorbach AM, Xu D, Hon YY, Delaney KM, Seidel M, Malik N, Peters-Lawrence M, Cantilena C, Nichols JS, Mendelsohn L, Conrey A, Grimes G, Kato GJ. Topical sodium nitrite for chronic leg ulcers in patients with sickle cell anaemia: a phase 1 dose-finding safety and tolerability trial. Lancet Haematol 2014;1(3):e95-e103.
43. Piga A, Perrotta S, Gamberini MR, Voskaridou E, Melpignano A, Filosa A, Caruso V, Pietrangelo A, Longo F, Tartaglione I, Borgna-Pignatti C, Zhang X, Laadem A, Sherman ML, Attie KM. Luspatercept improves hemoglobin levels and blood transfusion requirements in a study of patients with beta-thalassemia. Blood 2019;133(12):1279-1289.
44. Zafar S, Saleem K, Rashid A. An Unusual Presentation of a Patient with Leg Ulcers: A Case Report. Cureus 2019;11(12):e6293.
45. Derin S, Erdogan S, Sahan M, Azik MF, Derin H, Topal Y, Topal H. Olfactory Dysfunction in beta Thalassemia Major Patients Treated With Iron-Chelating Agents. Ear Nose Throat J 2019;98(8):NP125-NP130.
46. Osma U, Kurtoglu E, Eyigor H, Yilmaz MD, Aygener N. Sensorineural hearing loss in beta-thalassemia patients treated with iron chelation. Ear Nose Throat J 2015;94(12):481-485.
47. Khan MA, Khan MA, Seedat AM, Khan M, Khuwaja SF, Kumar R, Usama SM, Fareed S. Sensorineural Hearing Loss and Its Relationship with Duration of Chelation Among Major beta-Thalassemia Patients. Cureus 2019;11(8):e5465.
48. Manara R, Brotto D, Barillari MR, Costa G, Villani AV, Perna C, Ziello B, di Salle F, Cantone E, Pasanisi A, De Michele E, Ciancio A, D'Urzo G, Valentino P, Perrotta S, Ricchi P, Tartaglione I. Hearing loss in beta-thalassaemia: An Italian multicentre case-control study. Br J Haematol 2024;204(5):2016-2024.
49. Haghpanah S, Zekavat OR, Safaei S, Ashraf MA, Parand S, Ashraf H. Optical coherence tomography findings in patients with transfusion-dependent beta-thalassemia. BMC Ophthalmol 2022;22(1):279.
50. Baig R, Zahid N, Kausar F, Qazi HIA, Moiz B, Hussain S, Khan AH, Arain F, Ahmad K. Burden of ocular abnormalities in patients with beta thalassaemia: a cross-sectional study. BMJ Open Ophthalmol 2023;8(1).
51. Uddin MJ, Hossain MI, Sonia SF, Nazia S, Islam MK, Nahar L, Moon LY, Noshin N. Ocular Involvement in Transfusion Dependent Thalassemia Children. Mymensingh Med J 2023;32(3):807-811.
52. Aliberti L, Gagliardi I, Gamberini MR, Ziggiotto A, Verrienti M, Carnevale A, Bondanelli M, Zatelli MC, Ambrosio MR. Beta-thalassaemia major: Prevalence, risk factors and clinical consequences of hypercalciuria. Br J Haematol 2022;198(5):903-911.
53. Wong P, Milat F, Fuller PJ, Kerr PG, Doery JCG, Oh DH, Jackson D, Gillespie MT, Bowden DK, Pasricha SR, Lau KK. Urolithiasis is prevalent and associated with reduced bone mineral density in beta-thalassaemia major. Intern Med J 2017;47(9):1064-1067.
54. Demosthenous C, Vlachaki E, Apostolou C, Eleftheriou P, Kotsiafti A, Vetsiou E, Mandala E, Perifanis V, Sarafidis P. Beta-thalassemia: renal complications and mechanisms: a narrative review. Hematology 2019;24(1):426-438.

55. Lai ME, Spiga A, Vacquer S, Carta MP, Corrias C, Ponticelli C. Renal function in patients with beta-thalassaemia major: a long-term follow-up study. Nephrol Dial Transplant 2012;27(9):3547-3551.
56. Ponticelli C, Musallam KM, Cianciulli P, Cappellini MD. Renal complications in transfusion-dependent beta thalassaemia. Blood Rev 2010;24(6):239-244.
57. Quinn CT, Johnson VL, Kim HY, Trachtenberg F, Vogiatzi MG, Kwiatkowski JL, Neufeld EJ, Fung E, Oliveri N, Kirby M, Giardina PJ, Thalassemia Clinical Research N. Renal dysfunction in patients with thalassaemia. Br J Haematol 2011;153(1):111-117.
58. Musallam KM, Taher AT. Mechanisms of renal disease in beta-thalassemia. J Am Soc Nephrol 2012;23(8):1299-1302.
59. Piga A, Fracchia S, Lai ME, Cappellini MD, Hirschberg R, Habr D, Wegener A, Bouillaud E, Forni GL. Deferasirox effect on renal haemodynamic parameters in patients with transfusion-dependent beta thalassaemia. Br J Haematol 2015;168(6):882-890.
60. Origa R, Piga A, Tartaglione I, Della Corte G, Forni GL, Bruederle A, Castiglioni C, Han J. Renal safety under long-course deferasirox therapy in iron overloaded transfusion-dependent beta-thalassemia and other anemias. Am J Hematol 2018;93(7):E172-E175.
61. Yacobovich J, Stark P, Barzilai-Birenbaum S, Krause I, Pazgal I, Yaniv I, Tamary H. Acquired proximal renal tubular dysfunction in beta-thalassemia patients treated with deferasirox. J Pediatr Hematol Oncol 2010;32(7):564-567.
62. Dee CM, Cheuk DK, Ha SY, Chiang AK, Chan GC. Incidence of deferasirox-associated renal tubular dysfunction in children and young adults with beta-thalassaemia. Br J Haematol 2014;167(3):434-436.
63. Scoglio M, Cappellini MD, D'Angelo E, Bianchetti MG, Lava SAG, Agostoni C, Milani GP. Kidney Tubular Damage Secondary to Deferasirox: Systematic Literature Review. Children (Basel) 2021;8(12).
64. Morton A. A role for thiazide diuretic therapy in preventing bone loss, fracture, and nephrolithiasis in individuals with thalassemia and hypercalciuria? Osteoporos Int 2017;28(5):1759.
65. Kalamara TN, Zarkada EG, Kasimatis ED, Kofinas AG, Klonizakis PI, Vlachaki EC. Kidney transplantation in an adult with transfusion-dependent beta thalassemia: A challenging case report and literature review. Arch Clin Cases 2023;10(2):97-101.
66. Beydoun HG, Saliba AN, Taher AT. Deferasirox in thalassemia patients with end-stage renal disease. Am J Hematol 2016;91(10):E456-457.
67. Mannu F, Arese P, Cappellini MD, Fiorelli G, Cappadoro M, Giribaldi G, Turrini F. Role of hemichrome binding to erythrocyte membrane in the generation of band-3 alterations in beta-thalassemia intermedia erythrocytes. Blood 1995;86(5):2014-2020.
68. Derchi G, Galanello R, Bina P, Cappellini MD, Piga A, Lai ME, Quarta A, Casu G, Perrotta S, Pinto V, Musallam KM, Forni GL, Webthal Pulmonary Arterial Hypertension G. Prevalence and risk factors for pulmonary arterial hypertension in a large group of beta-thalassemia patients using right heart catheterization: a Webthal study. Circulation 2014;129(3):338-345.
69. Taher AT, Musallam KM, Karimi M, El-Beshlawy A, Belhoul K, Daar S, Saned M, Cesaretti C, Cappellini MD. Splenectomy and thrombosis: the case of thalassemia intermedia. J Thromb Haemost 2010;8(10):2152-2158.
70. Taher AT, Cappellini MD, Bou-Fakhredin R, Coriu D, Musallam KM. Hypercoagulability and Vascular Disease. Hematol Oncol Clin North Am 2018;32(2):237-245.
71. Piga A, Serra M, Longo F, Forni G, Quarta G, Cappellini MD, Galanello R. Changing patterns of splenectomy in transfusion-dependent thalassemia patients. Am J Hematol 2011;86(9):808-810.
72. Canda AE, Sucullu I, Ozsoy Y, Filiz AI, Kurt Y, Demirbas S, Inan I. Hospital experience, body image, and cosmesis after laparoscopic or open splenectomy. Surg Laparosc Endosc Percutan Tech 2009;19(6):479-483.
73. Winslow ER, Brunt LM. Perioperative outcomes of laparoscopic versus open splenectomy: a meta-analysis with an emphasis on complications. Surgery 2003;134(4):647-653; discussion 654-645.

74. Konstadoulakis MM, Lagoudianakis E, Antonakis PT, Albanopoulos K, Gomatos I, Stamou KM, Leandros E, Manouras A. Laparoscopic versus open splenectomy in patients with beta thalassemia major. J Laparoendosc Adv Surg Tech A 2006;16(1):5-8.
75. Musallam KM, Khalife M, Sfeir PM, Faraj W, Safadi B, Abi Saad GS, Abiad F, Hallal A, Alwan MB, Peyvandi F, Jamali FR. Postoperative outcomes after laparoscopic splenectomy compared with open splenectomy. Ann Surg 2013;257(6):1116-1123.
76. Patle NM, Tantia O, Sasmal PK, Khanna S, Sen B. Laparoscopic splenectomy in patients of beta thalassemia: Our experience. J Minim Access Surg 2010;6(3):70-75.
77. Feldman LS, Demyttenaere SV, Polyhronopoulos GN, Fried GM. Refining the selection criteria for laparoscopic versus open splenectomy for splenomegaly. J Laparoendosc Adv Surg Tech A 2008;18(1):13-19.
78. Koshenkov VP, Nemeth ZH, Carter MS. Laparoscopic splenectomy: outcome and efficacy for massive and supramassive spleens. Am J Surg 2012;203(4):517-522.
79. Zhou J, Wu Z, Cai Y, Wang Y, Peng B. The feasibility and safety of laparoscopic splenectomy for massive splenomegaly: a comparative study. J Surg Res 2011;171(1):e55-60.
80. Swanson TW, Meneghetti AT, Sampath S, Connors JM, Panton ON. Hand-assisted laparoscopic splenectomy versus open splenectomy for massive splenomegaly: 20-year experience at a Canadian centre. Can J Surg 2011;54(3):189-193.
81. Owera A, Hamade AM, Bani Hani OI, Ammori BJ. Laparoscopic versus open splenectomy for massive splenomegaly: a comparative study. J Laparoendosc Adv Surg Tech A 2006;16(3):241-246.
82. Zhu J, Ye H, Wang Y, Zhao T, Zhu Y, Xie Z, Liu J, Wang K, Zhan X, Ye Z. Laparoscopic versus open pediatric splenectomy for massive splenomegaly. Surg Innov 2011;18(4):349-353.
83. Taher A, Isma'eel H, Cappellini MD. Thalassemia intermedia: revisited. Blood Cells Mol Dis 2006;37(1):12-20.
84. Ricchi P, Meloni A, Grigoratos C, Toia P, Fina P, Pistoia L, Costantini S, Borsellino Z, Lisi R, Rocca M, Preziosi P, Midiri M, Pepe A. Prevalence of extramedullary hematopoiesis, renal cysts, splenic and hepatic lesions, and vertebral hemangiomas among thalassemic patients: a retrospective study from the Myocardial Iron Overload in Thalassemia (MIOT) network. Ann Hematol 2019;98(6):1333-1339.
85. Ricchi P, Ammirabile M, Costantini S, Di Matola T, Verna R, Diano A, Foglia MC, Spasiano A, Cinque P, Prossomariti L. A useful relationship between the presence of extramedullary erythropoeisis and the level of the soluble form of the transferrin receptor in a large cohort of adult patients with thalassemia intermedia: a prospective study. Ann Hematol 2012;91(6):905-909.
86. Mende N, Laurenti E. Hematopoietic stem and progenitor cells outside the bone marrow: where, when, and why. Exp Hematol 2021;104:9-16.
87. Malla S, Razik A, Das CJ, Naranje P, Kandasamy D, Kumar R. Marrow outside marrow: imaging of extramedullary haematopoiesis. Clin Radiol 2020;75(8):565-578.
88. Fouladi DF, Fishman EK, Kawamoto S. Extramedullary Hematopoiesis: A Forgotten Diagnosis and a Great Mimicker of Malignancy. J Comput Assist Tomogr 2023;47(3):445-452.
89. Roberts AS, Shetty AS, Mellnick VM, Pickhardt PJ, Bhalla S, Menias CO. Extramedullary haematopoiesis: radiological imaging features. Clin Radiol 2016;71(9):807-814.
90. Haidar R, Mhaidli H, Taher AT. Paraspinal extramedullary hematopoiesis in patients with thalassemia intermedia. Eur Spine J 2010;19(6):871-878.
91. Bowling MR, Cauthen CG, Perry CD, Patel NP, Bergman S, Link KM, Sane AC, Conforti JF. Pulmonary extramedullary hematopoiesis. J Thorac Imaging 2008;23(2):138-141.
92. Abdulla MAJ, Yassin MA, Abdelrazek M, Mudawi D, Ibrahim F, Soliman DS, ElOmri H, Nashwan AJ, Fernyhough LJ, De Sanctis V, Soliman AT. A persistent cough as atypical clinical presentation of intrathoracic extramedullary hematopoiesis (EMH) in a female with thalassemia intermedia. Acta Biomed 2018;89(2-S):41-46.
93. Chu KA, Lai RS, Lee CH, Lu JY, Chang HC, Chiang HT. Intrathoracic extramedullary haematopoiesis complicated by massive haemothorax in alpha-thalassaemia. Thorax 1999;54(5):466-468.

94. Taher A, Skouri H, Jaber W, Kanj N. Extramedullary hematopoiesis in a patient with beta-thalassemia intermedia manifesting as symptomatic pleural effusion. Hemoglobin 2001;25(4):363-368.
95. Youngster I, Weiss M, Drobot A, Eitan A. An unusual presacral mass: extramedullary hematopoiesis. J Gastrointest Surg 2006;10(6):927-929.
96. Zherebitskiy V, Morales C, Del Bigio MR. Extramedullary hematopoiesis involving the central nervous system and surrounding structures. Hum Pathol 2011;42(10):1524-1530.
97. Orphanidou-Vlachou E, Tziakouri-Shiakalli C, Georgiades CS. Extramedullary hemopoiesis. Semin Ultrasound CT MR 2014;35(3):255-262.
98. Bizzoni A, Lombardi D, Maroldi R, Incardona P, Nicolai P. Extramedullary hematopoiesis: a rare occurrence in the sinonasal tract. Auris Nasus Larynx 2010;37(2):233-237.
99. Georgakopoulou VE, Damaskos C, Mantzouranis K, Melemeni D, Gkoufa A, Chlapoutakis S, Garmpis N, Sklapani P, Aravantinou A, Garmpi A, Trakas N, Tsiafaki X. Invasive methods for the diagnosis and management of intrathoracic extramedullary hematopoiesis: A literature review. Respir Med Res 2021;79:100815.
100. Chehal A, Aoun E, Koussa S, Skoury H, Koussa S, Taher A. Hypertransfusion: a successful method of treatment in thalassemia intermedia patients with spinal cord compression secondary to extramedullary hematopoiesis. Spine (Phila Pa 1976) 2003;28(13):E245-249.
101. Aliberti B, Patrikiou A, Terentiou A, Frangatou S, Papadimitriou A. Spinal cord compression due to extramedullary haematopoiesis in two patients with thalassaemia: complete regression with blood transfusion therapy. J Neurol 2001;248(1):18-22.
102. Singounas EG, Sakas DE, Hadley DM, Chalevelakis G, Sfakianos G, Raptis S, Karvounis PC. Paraplegia in a pregnant thalassemic woman due to extramedullary hematopoiesis: successful management with transfusions. Surg Neurol 1991;36(3):210-215.
103. Cario H, Wegener M, Debatin KM, Kohne E. Treatment with hydroxyurea in thalassemia intermedia with paravertebral pseudotumors of extramedullary hematopoiesis. Ann Hematol 2002;81(8):478-482.
104. Meo A, Cassinerio E, Castelli R, Bignamini D, Perego L, Cappellini MD. Effect of hydroxyurea on extramedullary haematopoiesis in thalassaemia intermedia: case reports and literature review. Int J Lab Hematol 2008;30(5):425-431.
105. Konstantopoulos K, Vagiopoulos G, Kantouni R, Lymperi S, Patriarcheas G, Georgakopoulos D, Fessas P. A case of spinal cord compression by extramedullary haemopoiesis in a thalassaemic patient: a putative role for hydroxyurea? Haematologica 1992;77(4):352-354.
106. Cianciulli P, di Toritto TC, Sorrentino F, Sergiacomi L, Massa A, Amadori S. Hydroxyurea therapy in paraparesis and cauda equina syndrome due to extramedullary haematopoiesis in thalassaemia: improvement of clinical and haematological parameters. Eur J Haematol 2000;64(6):426-429.
107. Malik M, Pillai LS, Gogia N, Puri T, Mahapatra M, Sharma DN, Kumar R. Paraplegia due to extramedullary hematopoiesis in thalassemia treated successfully with radiation therapy. Haematologica 2007;92(3):e28-30.
108. Goerner M, Gerull S, Schaefer E, Just M, Sure M, Hirnle P. Painful spinal cord compression as a complication of extramedullary hematopoiesis associated with beta-thalassemia intermedia. Strahlenther Onkol 2008;184(4):224-226.
109. Ruo Redda MG, Allis S, Reali A, Bartoncini S, Roggero S, Anglesio SM, Piga A. Complete recovery from paraparesis in spinal cord compression due to extramedullary haemopoiesis in beta-thalassaemia by emergency radiation therapy. Intern Med J 2014;44(4):409-412.
110. Ileri T, Azik F, Ertem M, Uysal Z, Gozdasoglu S. Extramedullary hematopoiesis with spinal cord compression in a child with thalassemia intermedia. J Pediatr Hematol Oncol 2009;31(9):681-683.
111. Kaufmann T, Coleman M, Giardina P, Nisce LZ. The role of radiation therapy in the management of hematopoietic neurologic complications in thalassemia. Acta Haematol 1991;85(3):156-159.
112. Mann KS, Yue CP, Chan KH, Ma LT, Ngan H. Paraplegia due to extramedullary hematopoiesis in thalassemia. Case report. J Neurosurg 1987;66(6):938-940.

113. Ghieda U, Elshimy M, El Beltagi AH. Progressive spinal cord compression due to epidural extramedullary hematopoiesis in thalassemia intermedia. A case report and literature review. Neuroradiol J 2013;26(1): 111-117.
114. Tan TC, Tsao J, Cheung FC. Extramedullary haemopoiesis in thalassemia intermedia presenting as paraplegia. J Clin Neurosci 2002;9(6):721-725.
115. Amirjamshidi A, Abbassioun K, Ketabchi SE. Spinal extradural hematopoiesis in adolescents with thalassemia. Report of two cases and a review of the literature. Childs Nerv Syst 1991;7(4):223-225.
116. Niggemann P, Krings T, Hans F, Thron A. Fifteen-year follow-up of a patient with beta thalassaemia and extramedullary haematopoietic tissue compressing the spinal cord. Neuroradiology 2005;47(4):263-266.
117. Modell B, Khan M, Darlison M, Westwood MA, Ingram D, Pennell DJ. Improved survival of thalassaemia major in the UK and relation to T2* cardiovascular magnetic resonance. J Cardiovasc Magn Reson 2008;10(1):42.
118. Dessi C, Leoni G, Moi P, Danjou F, Follesa I, Foschini ML, Morittu M, Zappu A, Defraia E, Bina P, Cunico A, Civolani A, Podda RA, Origa R. Thalassemia major between liver and heart: Where we are now. Blood Cells Mol Dis 2015;55(1):82-88.
119. Wanachiwanawin W. Infections in E-beta thalassemia. J Pediatr Hematol Oncol 2000;22(6):581-587.
120. Wanachiwanawin W, Siripanyaphinyo U, Fucharoen S, Wasi P, Mawas F, Wiener E, Wickramasinghe SN. Activation of monocytes for the immune clearance of red cells in beta zero-thalassaemia/HbE. Br J Haematol 1993;85(4):773-777.
121. Tantiworawit A, Kamolsripat T, Piriyakhuntorn P, Rattanathammethee T, Hantrakool S, Chai-Adisaksopha C, Rattarittamrong E, Norasetthada L, Fanhchaksai K, Charoenkwan P. Survival and causes of death in patients with alpha and beta-thalassemia in Northern Thailand. Ann Med 2024;56(1):2338246.
122. Dhanya R, Sedai A, Ankita K, Parmar L, Agarwal RK, Hegde S, Ramaswami G, Gowda A, Girija S, Gujjal P, Pushpa H, Ramaiah JD, Karri C, Jali S, Tallur NR, Shenoy UV, Pinto D, Ramprakash S, Raghuram CP, Trivedi D, Cao X, Faulkner L. Life expectancy and risk factors for early death in patients with severe thalassemia syndromes in South India. Blood Adv 2020;4(7):1448-1457.
123. Vamvakas EC, Blajchman MA. Transfusion-related mortality: the ongoing risks of allogeneic blood transfusion and the available strategies for their prevention. Blood 2009;113(15):3406-3417.
124. Blajchman MA. Transfusion immunomodulation or TRIM: what does it mean clinically? Hematology 2005;10 Suppl 1:208-214.
125. Marx JJ. Iron and infection: competition between host and microbes for a precious element. Best Pract Res Clin Haematol 2002;15(2):411-426.
126. Candotti D, Assennato SM, Laperche S, Allain JP, Levicnik-Stezinar S. Multiple HBV transfusion transmissions from undetected occult infections: revising the minimal infectious dose. Gut 2019;68(2):313-321.
127. Stramer SL, Dodd RY, Subgroup AT-TDEID. Transfusion-transmitted emerging infectious diseases: 30 years of challenges and progress. Transfusion 2013;53(10 Pt 2):2375-2383.
128. Damgaard C, Magnussen K, Enevold C, Nilsson M, Tolker-Nielsen T, Holmstrup P, Nielsen CH. Viable bacteria associated with red blood cells and plasma in freshly drawn blood donations. PLoS One 2015;10(3):e0120826.
129. Kleinman S, Stassinopoulos A. Risks associated with red blood cell transfusions: potential benefits from application of pathogen inactivation. Transfusion 2015;55(12):2983-3000.
130. Teawtrakul N, Jetsrisuparb A, Sirijerachai C, Chansung K, Wanitpongpun C. Severe bacterial infections in patients with non-transfusion-dependent thalassemia: prevalence and clinical risk factors. Int J Infect Dis 2015;39:53-56.
131. Shyamala V. Transfusion transmitted infections in thalassaemics: need for reappraisal of blood screening strategy in India. Transfus Med 2014;24(2):79-88.
132. Domanovic D, Gossner CM, Lieshout-Krikke R, Mayr W, Baroti-Toth K, Dobrota AM, Escoval MA, Henseler O, Jungbauer C, Liumbruno G, Oyonarte S, Politis C, Sandid I, Vidovic MS, Young JJ, Ushiro-Lumb I, Nowotny N.

West Nile and Usutu Virus Infections and Challenges to Blood Safety in the European Union. Emerg Infect Dis 2019;25(6):1050-1057.
133. Kitchen AD, Chiodini PL. Malaria and blood transfusion. Vox Sang 2006;90(2):77-84.
134. Gimenez-Richarte A, Ortiz de Salazar MI, Gimenez-Richarte MP, Collado M, Fernandez PL, Clavijo C, Navarro L, Arbona C, Marco P, Ramos-Rincon JM. Transfusion-transmitted arboviruses: Update and systematic review. PLoS Negl Trop Dis 2022;16(10):e0010843.
135. Petersen LR, Busch MP. Transfusion-transmitted arboviruses. Vox Sang 2010;98(4):495-503.
136. Candotti D, Allain JP. Molecular virology in transfusion medicine laboratory. Blood Transfus 2013;11(2):203-216.
137. Janssen MP, van Hulst M, Custer B, Economics ARH, Outcomes Working G, Collaborators. An assessment of differences in costs and health benefits of serology and NAT screening of donations for blood transfusion in different Western countries. Vox Sang 2017;112(6):518-525.
138. Paraskevis D, Magiorkinis G, Magiorkinis E, Ho SY, Belshaw R, Allain JP, Hatzakis A. Dating the origin and dispersal of hepatitis B virus infection in humans and primates. Hepatology 2013;57(3):908-916.
139. Allain JP, Owusu-Ofori AK, Assennato SM, Marschner S, Goodrich RP, Owusu-Ofori S. Effect of Plasmodium inactivation in whole blood on the incidence of blood transfusion-transmitted malaria in endemic regions: the African Investigation of the Mirasol System (AIMS) randomised controlled trial. Lancet 2016;387(10029):1753-1761.
140. Busch MP, Bloch EM, Kleinman S. Prevention of transfusion-transmitted infections. Blood 2019;133(17):1854-1864.
141. Smith D, Lu Q, Yuan S, Goldfinger D, Fernando LP, Ziman A. Survey of current practice for prevention of transfusion-transmitted cytomegalovirus in the United States: leucoreduction vs. cytomegalovirus-seronegative. Vox Sang 2010;98(1):29-36.
142. Cervia JS, Wenz B, Ortolano GA. Leukocyte reduction's role in the attenuation of infection risks among transfusion recipients. Clin Infect Dis 2007;45(8):1008-1013.
143. Aydinok Y, Piga A, Origa R, Mufti N, Erickson A, North A, Waldhaus K, Ernst C, Lin JS, Huang N, Benjamin RJ, Corash L. Amustaline-glutathione pathogen-reduced red blood cell concentrates for transfusion-dependent thalassaemia. Br J Haematol 2019;186(4):625-636.
144. Politis C, Wiersum JC, Richardson C, Robillard P, Jorgensen J, Renaudier P, Faber JC, Wood EM. The International Haemovigilance Network Database for the Surveillance of Adverse Reactions and Events in Donors and Recipients of Blood Components: technical issues and results. Vox Sang 2016;111(4):409-417.
145. Lindholm PF, Annen K, Ramsey G. Approaches to minimize infection risk in blood banking and transfusion practice. Infect Disord Drug Targets 2011;11(1):45-56.
146. Kim DM, Brecher ME, Bland LA, Estes TJ, McAllister SK, Aguero SM, Carmen RA, Nelson EJ. Prestorage removal of Yersinia enterocolitica from red cells with white cell-reduction filters. Transfusion 1992;32(7):658-662.
147. Sirchia G, Rebulla P, Mascaretti L, Greppi N, Andreis C, Rivolta S, Parravicini A. The clinical importance of leukocyte depletion in regular erythrocyte transfusions. Vox Sang 1986;51 Suppl 1:2-8.
148. Li K, Li CK, Wong RP, Wong A, Shing MM, Chik KW, Yuen PM. Transfusion-related immunomodulation in Chinese children with thalassaemia. Vox Sang 1997;73(3):167-173.
149. Griffiths E, Williams P. The iron-uptake systems of pathogenic bacteria, fungi, and protozoa. In: Bullen JJ, Griffiths E, eds. Iron and Infection Molecular, Physiological, and Clinical aspects Chichester: Wiley, 1999.
150. Marwah SS, Blann A, Harrison P, Lumley MA, Wright J, McDowell J, Phillips JD, Rea C, Bareford D. Increased non-transferrin bound iron in plasma-depleted SAG-M red blood cell units. Vox Sang 2002;82(3):122-126.
151. Ozment CP, Turi JL. Iron overload following red blood cell transfusion and its impact on disease severity. Biochim Biophys Acta 2009;1790(7):694-701.
152. Weinberg ED. Microbial pathogens with impaired ability to acquire host iron. Biometals 2000;13(1):85-89.

153. Kuo CH, Dai ZK, Wu JR, Hsieh TJ, Hung CH, Hsu JH. Septic arthritis as the initial manifestation of fatal Vibrio vulnificus septicemia in a patient with thalassemia and iron overload. Pediatr Blood Cancer 2009;53(6) :1156-1158.
154. Weinberg ED. Iron availability and infection. Biochim Biophys Acta 2009;1790(7):600-605.
155. Pietrangelo A. Hemochromatosis gene modifies course of hepatitis C viral infection. Gastroenterology 2003;124(5):1509-1523.
156. Gordeuk VR, Delanghe JR, Langlois MR, Boelaert JR. Iron status and the outcome of HIV infection: an overview. J Clin Virol 2001;20(3):111-115.
157. Nairz M, Schroll A, Sonnweber T, Weiss G. The struggle for iron - a metal at the host-pathogen interface. Cell Microbiol 2010;12(12):1691-1702.
158. Vento S, Cainelli F, Cesario F. Infections and thalassaemia. The Lancet Infectious diseases 2006;6(4):226-233.
159. Gordeuk V, Mukiibi J, Hasstedt SJ, Samowitz W, Edwards CQ, West G, Ndambire S, Emmanual J, Nkanza N, Chapanduka Z, et al. Iron overload in Africa. Interaction between a gene and dietary iron content. N Engl J Med 1992;326(2):95-100.
160. Arantes JM, Francisco AF, de Abreu Vieira PM, Silva M, Araujo MS, de Carvalho AT, Pedrosa ML, Carneiro CM, Tafuri WL, Martins-Filho OA, Eloi-Santos SM. Trypanosoma cruzi: desferrioxamine decreases mortality and parasitemia in infected mice through a trypanostatic effect. Exp Parasitol 2011;128(4):401-408.
161. Collins HL, Kaufmann SH, Schaible UE. Iron chelation via deferoxamine exacerbates experimental salmonellosis via inhibition of the nicotinamide adenine dinucleotide phosphate oxidase-dependent respiratory burst. J Immunol 2002;168(7):3458-3463.
162. Neupane GP, Kim DM. Comparison of the effects of deferasirox, deferiprone, and deferoxamine on the growth and virulence of Vibrio vulnificus. Transfusion 2009;49(8):1762-1769.
163. Ibrahim AS, Gebermariam T, Fu Y, Lin L, Husseiny MI, French SW, Schwartz J, Skory CD, Edwards JE, Jr., Spellberg BJ. The iron chelator deferasirox protects mice from mucormycosis through iron starvation. J Clin Invest 2007;117(9):2649-2657.
164. Paradkar PN, De Domenico I, Durchfort N, Zohn I, Kaplan J, Ward DM. Iron depletion limits intracellular bacterial growth in macrophages. Blood 2008;112(3):866-874.
165. Spellberg B, Ibrahim AS, Chin-Hong PV, Kontoyiannis DP, Morris MI, Perfect JR, Fredricks D, Brass EP. The Deferasirox-AmBisome Therapy for Mucormycosis (DEFEAT Mucor) study: a randomized, double-blinded, placebo-controlled trial. J Antimicrob Chemother 2012;67(3):715-722.
166. Lesic B, Foulon J, Carniel E. Comparison of the effects of deferiprone versus deferoxamine on growth and virulence of Yersinia enterocolitica. Antimicrob Agents Chemother 2002;46(6):1741-1745.
167. Di Sabatino A, Carsetti R, Corazza GR. Post-splenectomy and hyposplenic states. Lancet 2011;378(9785):86-97.
168. Brendolan A, Rosado MM, Carsetti R, Selleri L, Dear TN. Development and function of the mammalian spleen. Bioessays 2007;29(2):166-177.
169. Hansen K, Singer DB. Asplenic-hyposplenic overwhelming sepsis: postsplenectomy sepsis revisited. Pediatr Dev Pathol 2001;4(2):105-121.
170. Cullingford GL, Watkins DN, Watts AD, Mallon DF. Severe late postsplenectomy infection. Br J Surg 1991;78(6):716-721.
171. US CDC. Recommended Child and Adolescent Immunization Schedule for ages 18 years or younger. 2025. Available at: https://www.cdc.gov/vaccines/hcp/imz-schedules/downloads/child/0-18yrs-child-combined-schedule.pdf. Accessed: 04 Jan 2025.
172. Kimaro Mlacha SZ, Warira A, Gatakaa H, Goldblatt D, Scott JAG. Outpacing the pneumococcus: Antibody dynamics in the first few days following pneumococcal capsular antigen stimulation. Sci Rep 2018;8(1):15376.
173. Casciani F, Trudeau MT, Vollmer CM, Jr. Perioperative Immunization for Splenectomy and the Surgeon's Responsibility: A Review. JAMA Surg 2020;155(11):1068-1077.

174. Klein NP, Habanec T, Kosina P, Shah NR, Kolhe D, Miller JM, Hezareh M, Van der Wielen M. Immunogenicity and safety of the quadrivalent meningococcal ACWY-tetanus toxoid conjugate vaccine (MenACWY-TT) in splenectomized or hyposplenic children and adolescents: Results of a phase III, open, non-randomized study. Vaccine 2018;36(17):2356-2363.
175. Martinon-Torres F, Bernatowska E, Shcherbina A, Esposito S, Szenborn L, Marti MC, Hughes S, Faust SN, Gonzalez-Granado LI, Yu LM, D'Agostino D, Calabresi M, Toneatto D, Snape MD. Meningococcal B Vaccine Immunogenicity in Children With Defects in Complement and Splenic Function. Pediatrics 2018;142(3).
176. Guri A, Ben-Ami T. Updated Recommendations on the Prevention and Treatment of Infections in Children With Asplenia/Hyposplenism. J Pediatr Hematol Oncol 2024;46(5):225-232.
177. Lee GM. Preventing infections in children and adults with asplenia. Hematology Am Soc Hematol Educ Program 2020;2020(1):328-335.
178. Camp J, Bayrhuber M, Anka N, Heine V, Glattacker M, Farin-Glattacker E, Rieg S. Efficacy of a novel patient-focused intervention aimed at increasing adherence to guideline-based preventive measures in asplenic patients: the PrePSS trial. Infection 2023;51(6):1787-1795.
179. Hale AJ, Depo B, Khan S, Whitman TJ, Bullis S, Singh D, Peterson K, Hyson P, Catoe L, Tompkins BJ, Alston WK, Dejace J. The Impact of Standardized Infectious Diseases Consultation on Postsplenectomy Care and Outcomes. Open Forum Infect Dis 2022;9(8):ofac380.
180. Brigden ML, Pattullo AL. Prevention and management of overwhelming postsplenectomy infection--an update. Crit Care Med 1999;27(4):836-842.
181. Holdsworth RJ, Irving AD, Cuschieri A. Postsplenectomy sepsis and its mortality rate: actual versus perceived risks. Br J Surg 1991;78(9):1031-1038.
182. Wiener E, Wanachiwanawin W, Chinprasertsuk S, Siripanyaphinyo U, Mawas F, Fucharoen S, Wickramasinghe SN. Increased serum levels of macrophage colony-stimulating factor (M-CSF) in alpha- and beta-thalassaemia syndromes: correlation with anaemia and monocyte activation. Eur J Haematol 1996;57(5):364-369.
183. Ozinsky A, Underhill DM, Fontenot JD, Hajjar AM, Smith KD, Wilson CB, Schroeder L, Aderem A. The repertoire for pattern recognition of pathogens by the innate immune system is defined by cooperation between toll-like receptors. Proc Natl Acad Sci U S A 2000;97(25):13766-13771.
184. Adamkiewicz TV, Berkovitch M, Krishnan C, Polsinelli C, Kermack D, Olivieri NF. Infection due to Yersinia enterocolitica in a series of patients with beta-thalassemia: incidence and predisposing factors. Clin Infect Dis 1998;27(6):1362-1366.
185. Ricerca BM, Di Girolamo A, Rund D. Infections in thalassemia and hemoglobinopathies: focus on therapy-related complications. Mediterr J Hematol Infect Dis 2009;1(1):e2009028.
186. Lefrere JJ, Servant-Delmas A, Candotti D, Mariotti M, Thomas I, Brossard Y, Lefrere F, Girot R, Allain JP, Laperche S. Persistent B19 infection in immunocompetent individuals: implications for transfusion safety. Blood 2005;106(8):2890-2895.
187. Velati C, Romano L, Fomiatti L, Baruffi L, Zanetti AR, Group SR. Impact of nucleic acid testing for hepatitis B virus, hepatitis C virus, and human immunodeficiency virus on the safety of blood supply in Italy: a 6-year survey. Transfusion 2008;48(10):2205-2213.
188. Allain JP, Thomas I, Sauleda S. Nucleic acid testing for emerging viral infections. Transfus Med 2002;12(4):275-283.
189. Costagliola DG, Girot R, Rebulla P, Lefrere JJ. Incidence of AIDS in HIV-1 infected thalassaemia patients. European and Mediterranean W.H.O. Working Group on Haemoglobinopathies and Cooleycare. Br J Haematol 1992;81(1):109-112.
190. Costagliola DG, de Montalembert M, Lefrere JJ, Briand C, Rebulla P, Baruchel S, Dessi C, Fondu P, Karagiorga M, Perrimond H, et al. Dose of desferrioxamine and evolution of HIV-1 infection in thalassaemic patients. Br J Haematol 1994;87(4):849-852.

191. Politis C, Ladis V, Karagiorga M, Fragodimitri C, Kattamis A, Chalkia P, Vlahaki E, Kouraklis A, Vgontza N, Maili X, Voskaridou E, Diamantidis M, Athanasiou K, Richardson C. What happened to the HIV infected patients with thalassaemia syndromes in Greece? A 30 year multicenter study [abstract]. Vox Sang 2017;112(Suppl 1):280.
192. Mody A, Sohn AH, Iwuji C, Tan RKJ, Venter F, Geng EH. HIV epidemiology, prevention, treatment, and implementation strategies for public health. Lancet 2024;403(10425):471-492.
193. Bowden RA, Slichter SJ, Sayers M, Weisdorf D, Cays M, Schoch G, Banaji M, Haake R, Welk K, Fisher L, McCullough J, Miller W. A comparison of filtered leukocyte-reduced and cytomegalovirus (CMV) seronegative blood products for the prevention of transfusion-associated CMV infection after marrow transplant. Blood 1995;86(9):3598-3603.
194. Politis C, Pappa A, Hassapopoulou H, Pantelidou D, Perifanis V, Teli A. Transfusion transmitted West Nile virus (WNV) infection in thalassaemic patients in Greece [abstract]. Vox Sang 2011;101(Suppl 2):236.
195. Perez-Gracia MT, Garcia M, Suay B, Mateos-Lindemann ML. Current Knowledge on Hepatitis E. J Clin Transl Hepatol 2015;3(2):117-126.
196. Schlosser B, Stein A, Neuhaus R, Pahl S, Ramez B, Kruger DH, Berg T, Hofmann J. Liver transplant from a donor with occult HEV infection induced chronic hepatitis and cirrhosis in the recipient. J Hepatol 2012;56(2):500-502.
197. Zervou EZ, Politis CP, Hassapopoulou EH, Vini MV, Parara MP, Kavallierou LK, Fountouli K, Zaxarioudaki A, Hatzitaki M, Martinis G, Katopi DK, Megalou AM, Avrami DA, Halkia PH, Aggelou EA, Tsironi ET, Lafiatis IL, Richardson CR. Prevalence of hepatitis E virus (HEV) infection in blood donors and multi-transfused patients in Greece [abstract]. Vox Sang 2015;109(Suppl 1):242-243.
198. Hogema BM, Molier M, Sjerps M, de Waal M, van Swieten P, van de Laar T, Molenaar-de Backer M, Zaaijer HL. Incidence and duration of hepatitis E virus infection in Dutch blood donors. Transfusion 2016;56(3):722-728.
199. Pischke S, Hiller J, Lutgehetmann M, Polywka S, Rybczynski M, Ayuk F, Lohse AW. Blood-borne Hepatitis E Virus Transmission: A Relevant Risk for Immunosuppressed Patients. Clin Infect Dis 2016;63(4):569-570.
200. Farmakis D, Giakoumis A, Cannon L, Angastiniotis M, Eleftheriou A. COVID-19 and thalassaemia: A position statement of the Thalassaemia International Federation. Eur J Haematol 2020;105(4):378-386.
201. Jean-Mignard E, De Luna G, Pascal L, Agouti I, Thuret I. SARS-CoV-2 infection in patients with beta-thalassemia: The French experience. Transfus Clin Biol 2022;29(1):70-74.
202. Al-Kuraishy HM, Mazhar Ashour MH, Saad HM, Batiha GE. COVID-19 and beta-thalassemia: in lieu of evidence and vague nexus. Ann Hematol 2024;103(5):1423-1433.
203. Prasertwitayakij N, Louthrenoo W, Kasitanon N, Thamprasert K, Vanittanakom N. Human pythiosis, a rare cause of arteritis: case report and literature review. Semin Arthritis Rheum 2003;33(3):204-214.
204. Wanachiwanawin W, Mendoza L, Visuthisakchai S, Mutsikapan P, Sathapatayavongs B, Chaiprasert A, Suwanagool P, Manuskiatti W, Ruangsetakit C, Ajello L. Efficacy of immunotherapy using antigens of Pythium insidiosum in the treatment of vascular pythiosis in humans. Vaccine 2004;22(27-28):3613-3621.
205. World Health Organization. World malaria report 2023. Geneva, Switzerland: World Health Organization, 2023.
206. Domanovic D, Kitchen A, Politis C, Panagiotopoulos T, Bluemel J, Van Bortel W, Overbosch D, Lieshout-Krikke R, Fabra C, Facco G, Zeller H. Targeting of blood safety measures to affected areas with ongoing local transmission of malaria. Transfus Med 2016;26(3):161-165.
207. El Chaar M, Atwal S, Freimanis GL, Dinko B, Sutherland CJ, Allain JP. Inactivation of Plasmodium falciparum in whole blood by riboflavin plus irradiation. Transfusion 2013;53(12):3174-3183.
208. Henschler R, Seifried E, Mufti N. Development of the S-303 Pathogen Inactivation Technology for Red Blood Cell Concentrates. Transfus Med Hemother 2011;38(1):33-42.
209. Chen LH, Keystone JS. New strategies for the prevention of malaria in travelers. Infect Dis Clin North Am 2005;19(1):185-210.

09 ORAL AND DENTAL CARE

Authors: **Navdeep Kumar**[1], **Faiez N. Hattab**[2]

[1.] Royal National ENT & Eastman Dental Hospitals, University College London Hospitals NHS Foundation Trust, London, United Kingdom

[2.] Oskar-Maria-Graf-Ring 1a, Munich, Germany

Competing interest: None to disclose.

1. INTRODUCTION

Thalassaemia is one of the most common genetic disorders worldwide, presenting significant public health and social challenges in areas where incidence is high. It is a group of hereditary haemoglobinopathies, with its manifestations, including facial/oral changes, modulated by several genetic, racial, and environmental factors. As described elsewhere in these guidelines, the different subtypes of thalassaemia almost exclusively affect people of particular ethnic origins and hence the distribution is characteristic. For example, β-thalassaemia major, the most serious variant, is predominant in the Middle East and North African (MENA) region, impacted by the tradition of a close relative marriage [1]. However, it is important to note that migration has increased the prevalence of the disease in regions traditionally believed to have a low prevalence, while, at the same time, prevention and screening programmes in endemic regions have reduced the number of affected individuals [2].

Despite this widening global distribution of β-thalassaemia, dental awareness of this condition is variable with many dentists lacking experience in either identifying potential oral manifestations and/or providing appropriate dental care [3, 4]. As a consequence, patients may experience difficulties in accessing appropriate dental care or may be reluctant to attend if they feel the dentist does not understand their condition. Financial and time constraints may also contribute as the patients may have multiple other medical appointments, including blood transfusions.

Care may only be sought at a late stage when individuals experience pain and try to access emergency dental services. In this situation, the dental decay is often advanced, with the risk of infection and abscess spreading into the tissues of the face and neck. Unfortunately, as a consequence of late presentation, dental extractions may be more likely to be provided than fillings, leading to individuals losing more and more teeth.

When dental treatment is provided, the dentist may not be fully aware of the implications of β-thalassaemia on dental management and hence, fail to liaise with haematology colleagues appropriately. Conversely, fear of the unknown may be associated with a reluctance to provide anything beyond basic dental care. Indeed, many general dentists may prefer to refer these patients to either specialist dental services, or to hospital-based dental units, especially when dental extractions are required.

Hence, this Chapter reviews the key considerations in the dental care of patients with β-thalassaemia, with a focus on the most serious subtype, β-thalassaemia major or transfusion-dependent β-thalassaemia (TDT). It provides an overview of the potential oro-facial manifestations,

guidance on medical risk assessment, appropriate dental treatment modifications, in addition to an outline of dental care pathways, so that effective, timely care can be provided.

2. ORO-FACIAL FEATURES

Many oro-facial features and dental changes have been described in β-thalassaemia (Tables 1-3), with the reported prevalence of these changes variably described in different population groups (Table 4) [4, 5]. Although these changes are generally not life-threatening, they can closely link to overall health and wellbeing [6, 7].

Table 1. *Changes to the facial/jaw bones.*

Feature	Reason
Enlargement of the upper jaw (chipmunk face)	Bone marrow expansion: extent depends on anaemia severity, age, timing of blood transfusion
Maxillary sinus reduction/nasal obstruction	Bone marrow expansion
'Chicken wire-like' appearance of tooth bearing bone on radiographs	Bone marrow expansion
Medication-related osteonecrosis of the jaw	Medications including anti-resorptive medication

Table 2. *Changes to the oro-facial soft tissues.*

Feature	Reason
Periodontal disease	Multifactorial including poor oral hygiene, reduced salivary flow and buffer capacity, reduced salivary IgA levels, impaired salivary gland function, mouth breathing
Pale gingivae and oral mucosa	Anaemia
Discoloured gingivae	Iron deposits, bilirubin, anaemia
Sore or burning tongue	Folate deficiency
Painful swelling of salivary glands and dry mouth	Iron deposits
Reduced salivary protection	Reduced IgA in saliva
Oral ulceration	Very rare; related to anaemia
Necrotising gingivostomatitis	Very rare; possibly linked to agranulocytosis due to deferiprone

Table 3. *Changes to the teeth.*

Feature	Reason
Change in dental morphology e.g., teeth with enlarged pulp chambers, short crowns/roots	Disrupted dental development
Delayed dental development/delayed eruption of teeth	Physical growth delay
Dental caries	Multifactorial including, ack of regular dental care; dry mouth, change in quantity and quality of saliva, fatigue/poor motivation
Discoloured teeth	Iron deposits/bilirubin in the dentinal tubules
Varying degrees of malocclusion (overbite, open bite)	Changes to facial bones
Migration and spacing of upper anterior teeth	Changes to facial bones
Developing tightness, discomfort and ulceration from dentures	Changes to facial bones

Table 4. *Prevalence of orofacial β-thalassaemia major features in different population groups.*

β-Thalassaemia major groups	Jordan [4]	India [5]
Sample size	**54**	**72**
Frontal bone bossing, % (n)	61.1 (33)	--
Saddle nose, % (n)	59.2 (32)	56.9 (41)
Lip incompetence, % (n)	51.8 (28)	80.6 (58)
Discoloured teeth, % (n)	44.4 (24)	--
Dental and jaw pain, % (n)	40.7 (22)	--
Pallor oral mucosa, % (n)	38.9 (21)	22.2 (16)
Increased overjet, % (n)	25.9 (14)	--
Maxillary protrusion, % (n)	24.9 (13)	60.5 (72)
Chipmunk faces, % (n)	16.7 (9)	--
Nasal airway problem, % (n)	16.7 (9)	--
Lower lip paraesthesia, % (n)	13.0 (7)	--
Parotid gland enlargement, % (n)	5.6 (3)	--

2.1. Changes to the facial/jaw bones

It is known that β-thalassaemia may result in changes in the facial/jaw bones, the extent of which depends on the severity of the anaemia, the patient's age, duration of the clinical symptoms, and the timing/frequency of both therapeutic blood transfusion and splenectomy, and initiation of iron chelation. When bone changes are present, the main change to the facial bones is malformation due to bone marrow hyperplasia caused by rapid red cell turnover, particularly in β-thalassaemia major. This hypertrophy and expansion of the erythroid marrow cavity, results in impaired bone growth, thinning of the mandibular cortex, increased bone resorption, bone deformation and increased bone fragility [8-10]. Radiographic changes described in the facial/jaw bones include thickened frontal bone, thinned cortex of the mandible, maxillary sinus hypoplasia, faint/indiscernible inferior dental canal boundaries, altered trabecular pattern, and enlarged marrow spaces (Figures 1 and 2) [1, 4, 11-14].

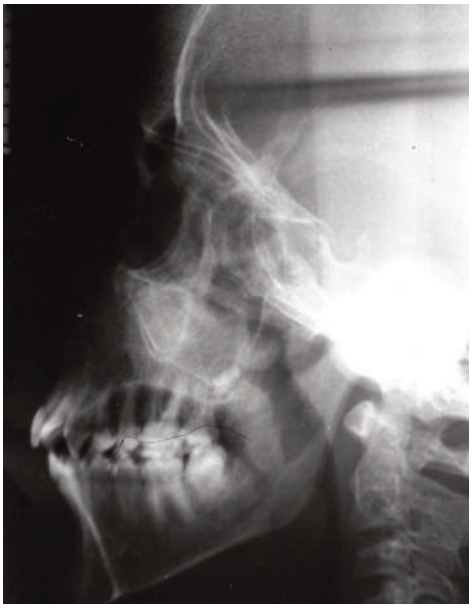


Figure 1. *Cephalometric radiograph of a 15-year-old boy with β-thalassaemia major disclosing thickened frontal bone, thinned inferior border of the mandible, premaxilla protrusion, flaring of the maxillary anterior teeth, small maxillary sinus, and widened diploic spaces in the frontal bone. Reproduced with permission from [4].*

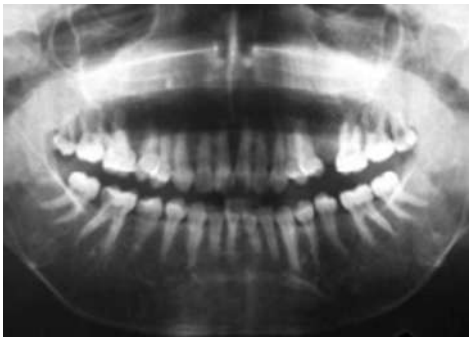
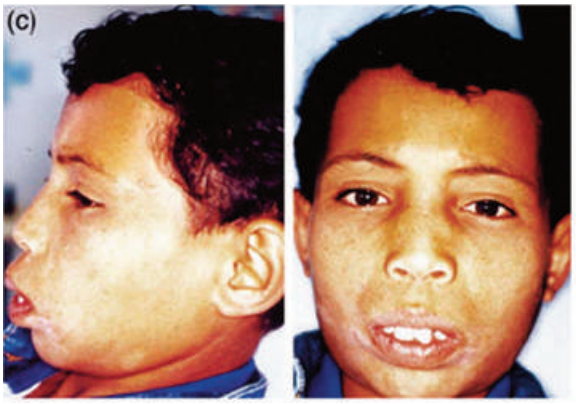


Figure 2. *Panoramic radiograph of β-thalassaemia major patient demonstrating thinned mandibular inferior cortex, enlarged bone marrow spaces, short spiky roots, and faint lamina dura. Reproduced with permission from [1] and [4].*

Clinically, changes in facial profile may range from slight depression of the nasal bridge and minor maxillary overgrowth to forehead bossing, prominent cheekbones, saddle nose, protrusive maxillary anterior teeth; lip incompetence, increased overjet and open bite, and malocclusion [1, 6, 12, 15]. The hyperplasia of bone marrow in the maxilla significantly exceeds that of the mandible, and when significant, can result in a characteristic appearance known as 'chipmunk face' [11] as illustrated in Figure 3. A Class II skeletal relationship, with maxillary protrusion and relatively short mandible has been confirmed by cephalometric analysis [11, 16-20]. Furthermore, a reduced height of the ramus of the mandible, an increase in anterior facial height, and a decrease in posterior facial height has been observed [4, 21].

Figure 3. *Profile view of a 13–year-old boy with β-thalassaemia major showing typical facial features; characterised by frontal bossing, bulging cheekbone, saddle nose, and protrusive premaxilla (chipmunk face). Reproduced with permission from [4].*



2.2. Oral soft tissue/gingival changes

Chronic anaemia as a sequalae to β-thalassaemia major, may result in pallor of the oral mucosa, oral ulceration and burning tongue. Necrotising stomatitis, possibly linked to agranulocytosis due to deferiprone has also been described [22]. Iron deposition in the parotid glands can also result in painful facial swelling but is rare [4, 23].

Patients with β-thalassaemia demonstrate a tendency for higher plaque rates, gingivitis and periodontitis scores than control subjects [24-26]. Several factors are thought to be associated with this increased risk of periodontal disease, including poor oral hygiene; reduced salivary flow rate, reduced salivary IgA levels, impaired function of the salivary glands due to excessive iron deposits, increased number of pathogenic bacteria, malocclusion, mouth breathing, nutritional deficiencies, infection, impaired immune system, and genetic factors [1, 4, 26-28].

2.3. Dental changes

Changes in dental morphology have been consistently noted in β-thalassaemia major and include short roots and taurodonts (enlarged pulp chambers) [13]. Smaller teeth, most notably reduced mesiodistal crown distance, has also been demonstrated, and may cause malocclusion, particularly when associated with a changes in the width, length and form of the dental arches due to jaw bone changes [11, 16, 20, 29, 30].

Dental caries prevalence has been found to be higher in patients with β-thalassaemia than in healthy controls [7, 31-35]. The suggested reasons for this increased caries risk have been attributed to lack of dental awareness, poor oral hygiene, and lack of regular dental care. Additional potential risk factors that have been identified include a reduced salivary flow rate and buffer capacity (phosphorous levels) in patients with β-thalassaemia major [12, 34], lower level of salivary immunoglobulin [31], and a high count of salivary streptococcus mutans [36, 37].

3. MEDICAL RISK ASSESSMENT/CONSIDERATIONS FOR THE DELIVERY OF DENTAL CARE

Patients with β-thalassaemia major have multiple comorbidities associated with their condition which need to be considered when providing dental care:

3.1. Chronic anaemia

In addition to the oro-facial manifestations associated with chronic anaemia, patients may appear to be fatigued, lethargic, and poorly motivated. Dental care should be adapted according to their tolerance of the planned procedure on the day of treatment. If patients are receiving regular exchange transfusion, invasive dental care should be delivered in the week following a planned exchange, as the patient's blood counts will be optimal. Invasive dental procedures should be avoided on the same day as the exchange, as the patient is often fatigued following transfusion.

3.2. Transfusion-transmitted infections

Prior to screening of blood products, people with β-thalassaemia were at increased risk of carriage of hepatitis B, C, G viruses and human immunodeficiency virus. Appropriate cross-infection protocols should be in place and precautions taken when providing care. In the case of associated hepatic disease / liver cirrhosis, caution must be used when prescribing medication.

3.3. Iron overload and tissue deposition

Iron accumulation in hepatic, cardiac, and endocrine tissues is well documented for patients with β-thalassaemia major. Dentists need to take additional precautions to compensate for potential complications such as impaired liver function (increased risk of bleeding, avoid prescribing hepatotoxic medication) and diabetes (increased risk of periodontal disease, infection, delayed healing). Iron deposits have also been found in the gingivae [38].

Incorporation of blood pigment bilirubin, a product of haemoglobin breakdown, has been described in the dentinal tubules resulting in yellow discoloration of teeth [39]. Although the impact of iron deposits on periodontal health is unknown, further studies investigating the use of gingival biopsies for diagnosis of iron overload are needed.

3.4. Infections

Infections are major complications and represent one of the main causes of morbidity in patients with β-thalassaemia. Predisposing factors for infections include severe anaemia, iron overload, splenectomy, and a range of immune abnormalities defective neutrophils, macrophage chemotaxis [40]. Surgical splenectomy may increase the oral colonisation and candidal counts in patients with β-thalassaemia major [15, 37, 41]. This increased infection risk should be taken into account when providing dental care.

3.5. Cardiomyopathy

Chronic anaemia can result in cardiomyopathy and is further exacerbated by cardiac iron overload. Other cardiac related complications include tachycardia, arrythmia and congestive heart failure [42]. Although patients may be asymptomatic with their cardiac dysfunction, when anxious and/or undergoing a stressful dental procedure, they may precipitate their cardiac symptoms. Dentists need to be aware of the degree of cardiac involvement and implement precautions as appropriate.

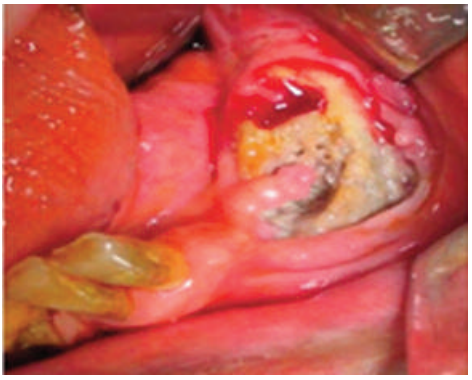
3.6. Depression

Literature examining psychological functioning in individuals with β-thalassaemia indicates that this population is vulnerable to experiencing psychological adjustment problems, such as symptoms of depression and anxiety. A significant difference in symptoms of anxiety by gender has been described, with females reporting more symptoms than males [43]. Lifelong adherence to a complicated medical regimen can potentially impact on the emotional functioning of patients with β-thalassaemia. This can further impact patient motivation and willingness to accept dental interventions.

3.7. Medication-related osteonecrosis of the jaw

Medication-related osteonecrosis of the jaw (MRONJ) is a known risk factor which is associated with anti-resorption therapy used in β-thalassaemia and can develop either spontaneously or due to surgical intervention [44, 45]. This is characterised by trans-mucosal exposure of necrotic bone, often triggered by surgical trauma such as dental extractions (Figure 4) [46]. The incidence of MRONJ for patients treated for osteoporosis with antiresorptive drugs such as bisphosphonates and denosumab is approximately 0.01-0.1% [47]. There is currently no clear evidence for the efficacy of any intervention to manage MRONJ [48]. In view of this, dental extractions are avoided where at all possible.

Figure 4. *Clinical presentation of bisphosphonates-related osteonecrosis of the mandible showing mucosal exposure of the necrotic bone. Reproduced with permission from [46].*



4. ORAL/DENTAL TREATMENT/SURGERY MODIFICATIONS

4.1. Close liaison with the haematology team

Due to the great clinical variability in systemic signs and symptoms with which patients with β-thalassaemia present, the most important aspect of providing dental care for patients with β-thalassaemia is the need to deliver it through a coordinated team approach, ensuring close liaison with the haematologist, and where appropriate the paediatrician. Information from the haematology team on the patient's current clinical status and recent blood test results is required to complete a risk assessment and plan appropriate dental care. The appropriate setting for provision of care should be determined, namely whether in the setting of primary or secondary (hospital-based) care. This decision is made in relation to severity and degree of control of the underlying β-thalassaemia, in addition to the presence of the potential multiple secondary effects of this disorder (Table 5).

Table 5. *Dental care: risk assessment and treatment modifications.*

Medical risk assessment	Treatment modification
Chronic anaemia	• Consider tolerance for the proposed dental procedure • Avoid intravenous sedation and general anaesthesia – if required, a hospital setting/close support from the haematology team is required
Transfusion	• Confirm whether patient receives regular blood transfusions • Avoid treatment on the same day • Time dental appointment within 1 week of transfusion • Consider referral to community dental service or hospital dental services for invasive dental procedures
Blood borne viruses	• Follow cross-infection control protocol • Confirm status of infection and management • Check for presence of liver cirrhosis – caution when prescribing drugs, bleeding risk (preoperative blood test may be required)
Cardiomyopathy	• Assess degree of cardiac involvement; liaise with medical team • Minimise stress/anxiety for the patient
Depression	• Consider tolerance to dental procedure • Adapt treatment plan accordingly

4.2. Preventative programme

Due to the higher risk of oral disease and numerous potential oral manifestations, patients with β-thalassaemia should be encouraged to register with a dentist and attend regularly to identify potential issues as early as possible. A preventive dental programme should be implemented with reinforcement of oral hygiene instructions, including the use of a fluoride toothpaste, dietary advice and preventive measures including prophylaxis, fluoride application, and fissure sealants implemented to minimise the need for invasive dental procedures [49]. This will reduce the risk of dental caries and periodontal disease.

4.3. Early management of oral/dental infections

Due to the increased risk of severe infections, particularly when a splenectomy has been undertaken, patients with β-thalassaemia who present with acute dental infections/abscesses should receive urgent dental care and antimicrobial therapy at the earliest opportunity. If the person is not registered with a dentist, they should seek emergency dental care from the local emergency dental services or hospital so that antibiotics can be prescribed until suitable care can be arranged. Alternatively, their general medical practitioner may be able to assist. Given those invasive dental procedures (most notably dental extractions or deep scaling), are associated with bacteraemia, antibiotic prophylaxis has been proposed, although the risks of antibiotic resistance should also be considered.

4.4. Management of the teeth/dentures

Although orthodontic treatment for children generally commences after the adult teeth have erupted, the jawbone and dental morphology changes in β-thalassaemia major may benefit from earlier assessment and diagnosis so that preventive and interceptive approaches can be used. Early diagnosis and orthodontic treatment help to intercept and correct craniofacial deformities, thus rehabilitating occlusion and restoring function. This can be coupled with cosmetic dentistry if the crowns of the teeth are reduced in dimensions and there is spacing. Correction of drifted maxillary anterior teeth and increased overjet due to maxillary enlargement should be undertaken to improve aesthetics, reduce susceptibility to trauma, avoid gingival inflammation, and improve functional ability if a patient is wearing dentures, these should be reviewed more regularly to check for signs of trauma due to expansion of the maxilla.

4.5. Dental treatment modality

Dental local anaesthetic often contains adrenaline. For those patients with β-thalassaemia who have cardiac disease, maximum dose of adrenaline of 0.04 mg is advised, compared to 0.2 mg for a healthy adult [50]. Intraligamental and intraosseous injection with adrenaline-containing anaesthetics is not recommended for patients with cardiovascular disease as the drug rapidly enters the circulation with these techniques [51]. Consideration may be also given to using a local anaesthetic without a vasoconstrictor for short dental procedures where there is no haemostatic advantage of using adrenaline, with 2% lidocaine and 1/100,000 epinephrine used for longer procedures requiring more profound anaesthesia.

Sedation should be used with caution with patients with β-thalassaemia major referred to a specialist centre due to the presence of chronic, potentially severe anaemia and the risk of respiratory depression. Although inhalation sedation is generally preferable to intravenous

sedation in patients, the potential for nitrous oxide to worsen anaemia by impairing vitamin B12 activity should be considered [52, 53]. The use of general anaesthesia is best avoided due to the risks associated with underlying anaemia, secondary comorbidities and potentially challenging intubation due to changes in the facial/jaw bones. When general anaesthesia is absolutely necessary, it should be carried out as an inpatient procedure, with the patient admitted under the care of the haematology team [54].

4.6. Dental assessment prior to commencement of antiresorptive therapy

All patients should ideally have a comprehensive dental assessment with their local dentist prior to the commencement of antiresorptive therapy, to ensure that they are as dentally fit as feasible [47]. Emphasis is on reduction of mucosal trauma and avoidance of subsequent dental extractions. Preventive dental advice should be given, emphasising the importance of reporting any symptoms such as loose teeth, pain, or swelling, as soon as possible. If a patient has spontaneous or chronic bone exposure, referral to an oral surgery/oral and maxillofacial surgery specialist should be considered. When a patient is already on bisphosphonates and a dental extraction is unavoidable, straightforward extractions can be undertaken in primary care, although a second opinion can be sought when necessary. Surgical extractions should be undertaken by a specialist in oral surgery/maxillofacial surgeon. All patients should be advised of the risk pre-operatively and closely monitored postoperatively. There is no evidence supporting the discontinuation of bisphosphonates temporarily, as the drugs persist in the skeletal tissues for years.

5. DENTAL CARE PATHWAYS

Most people with β-thalassaemia can receive routine dental treatment in the primary care setting, using local anaesthesia as long as a comprehensive risk assessment has been undertaken. Referral may be required to specialist services in the specialised community or hospital dental services may be suitable for specific courses of treatment, such as extractions if the patient receives regular blood transfusions, and/or treatment modalities such as sedation or general anaesthesia. After completing treatment, the patient is discharged to the local dentist for continued care. Some individuals with β-thalassaemia who have significant secondary medical comorbidities, may require more long-term care from a specialist dental service.

KEY POINTS AND RECOMMENDATIONS

1. Regular dental care is essential, including preventative advice **(Grade C).**
2. Dental infections should be managed early and aggressively **(Grade C).**
3. Dental assessment is recommended prior to commencement of medications known to be associated with the risk of medication-related osteonecrosis of the jaw **(Grade B).**

REFERENCES

1. Hattab FN. Orofacial and Systemic Features of Thalassemia Major: Management, and Prevention with Reference to Populations in the Arabian Gulf: Bentham Science Publishers, 2021.
2. Kattamis A, Forni GL, Aydinok Y, Viprakasit V. Changing patterns in the epidemiology of beta-thalassemia. Eur J Haematol 2020;105(6):692-703.
3. Duggal MS, Bedi R, Kinsey SE, Williams SA. The dental management of children with sickle cell disease and beta-thalassaemia: a review. Int J Paediatr Dent 1996;6(4):227-234.
4. Hattab FN. Periodontal condition and orofacial changes in patients with thalassemia major: a clinical and radiographic overview. J Clin Pediatr Dent 2012;36(3):301-307.
5. Elangovan A, Mungara J, Joseph E, Guptha V. Prevalence of dentofacial abnormalities in children and adolescents with beta-thalassaemia major. Indian J Dent Res 2013;24(4):406-410.
6. Nabi AT, Muttu J, Chhaparwal A, Mukhopadhyay A, Pattnaik SJ, Choudhary P. Implications of beta-thalassemia on oral health status in patients: A cross-sectional study. J Family Med Prim Care 2022;11(3):1174-1178.
7. Abbasi F, Tabesh A, Yavari A, Makaremi R, Bizhani O, Mahmood M. Evaluation and relation of oral health-related quality of life and oral health status in Thalassemia Major patients, a cross-sectional study. BMC Oral Health 2023;23(1):493.
8. Olivieri NF. The beta-thalassemias. N Engl J Med 1999;341(2):99-109.
9. Galanello R, Origa R. Beta-thalassemia. Orphanet J Rare Dis 2010;5:11.
10. Viprakasit V, Origa R. Genetic basis, pathophysiology and diagnosis. In: Cappellini MD, Cohen A, Porter J, Taher AT, Viprakasit V, eds. Guidelines for the management of transfusion dependent thalassaemia (TDT). 3rd ed. Nicosia, Cyprus: Thalassaemia International Federation, 2014.
11. Hattab FN. Dental and Orofacial Changes in Thalassemia Major: An Overview. In: Greene E, ed. Thalassemia: Causes, Treatment Options and Long-Term Health Outcomes. New York, USA: Nova Science Publishers Inc, 2014.
12. Hattab FN. Thalassemia major and related dentomaxillofacial complications: Clinical and radiographic overview with reference to dental care. Int J Exp Dent Sci 2017;6:1-10.
13. Ohri N, Khan M, Gupta N, Bhatt G, Malhotra P, Ranjanpati A. A study on the radiographic features of jaws and teeth in patients with thalassemia major using orthopantomograph. J Ind Acad Oral Med Radiol 2015;27(3):343-348.
14. Bayati S, Keikhaei B, Bahadoram M, Mahmoudian-Sani MR, Vaneshani M, Behbahani F. Radiographic Features of the Maxillofacial Anomalies in Beta-Thalassemia Major: With New View. World J Plast Surg 2021;10(3):78-83.
15. Van Dis ML, Langlais RP. The thalassemias: oral manifestations and complications. Oral Surg Oral Med Oral Pathol 1986;62(2):229-233.
16. Hattab FN, Yassin OM. Dental arch dimensions in subjects with beta-thalassemia major. J Contemp Dent Pract 2011;12(6):429-433.
17. Abu Alhaija ES, Hattab FN, al-Omari MA. Cephalometric measurements and facial deformities in subjects with beta-thalassaemia major. Eur J Orthod 2002;24(1):9-19.
18. Amini F, Jafari A, Eslamian L, Sharifzadeh S. A cephalometric study on craniofacial morphology of Iranian children with beta-thalassemia major. Orthod Craniofac Res 2007;10(1):36-44.
19. Gupta DK, Singh SP, Utreja A, Verma S. Prevalence of malocclusion and assessment of treatment needs in beta-thalassemia major children. Prog Orthod 2016;17:7.
20. Hattab FH, Abu-Alhaija ESJ, Yassin OM. Tooth crown size of the permanent dentition in subjects with thalassemia major. Dent Anthropol 2000;14(3):7-13.

21. Takriti M, Dashash M. Craniofacial parameters of Syrian children with beta-thalassemia major. J Investig Clin Dent 2011;2(2):135-143.
22. Tewari S, Tewari S, Sharma RK, Abrol P, Sen R. Necrotizing stomatitis: a possible periodontal manifestation of deferiprone-induced agranulocytosis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009;108(4):e13-19.
23. Goldfarb A, Nitzan DW, Marmary Y. Changes in the parotid salivary gland of beta-thalassemia patients due to hemosiderin deposits. Int J Oral Surg 1983;12(2):115-119.
24. Mehdizadeh M, Mehdizadeh M, Zamani G. Orodental complications in patients with major beta-thalassemia orodental complications in patients with major beta-thalassemia. Dental Res J 2008;5(1):17-20.
25. Arabsolghar M, Mohammadi M, Kaheh A, Norouzifard A, Ahmadzadeh S. Different type of periodontitis and gingivitis in patients with major thalassemia comparing to healthy people. J Oral Health Oral Epidemiol 2015;4(1):24-29.
26. Pedulla E, Scibilia M, Saladdino G, Colletta G, Rapisarda S, Terranova M, Spampinato R. Dental and periodontal condition in patients affected by β-thalassemia major and β-thalassemia intermedia: A study among adults in Sicily, Italy. J Dent Health Oral Disord Ther 2015;3(1):00081.
27. Arabsolghar M, Arbabi P, Mohammadi M, Farsineja A. Evaluation of salivary immunoglobulin A level in thalassemic patients with periodontitis in comparison with thalassemic patients with healthy periodontium. J Oral Health Oral Epidemiol 2016;5(4):205-209.
28. Akcali A, Yildiz MS, Akcali Z, Huck O, Friedmann A. Periodontal condition of patients with Thalassemia Major: A systematic review and meta-analysis. Arch Oral Biol 2019;102:113-121.
29. Al-Wahadni A, Qudeimat MA, Al-Omari M. Dental arch morphological and dimensional characteristics in Jordanian children and young adults with beta-thalassaemia major. Int J Paediatr Dent 2005;15(2):98-104.
30. Hattab FN. Mesiodistal crown diameters and tooth size discrepancy of permanent dentition in thalassemic patients. J Clin Exp Dent 2013;5(5):e239-244.
31. Siamopoulou-Mavridou A, Mavridis A, Galanakis E, Vasakos S, Fatourou H, Lapatsanis P. Flow rate and chemistry of parotid saliva related to dental caries and gingivitis in patients with thalassaemia major. Int J Paediatr Dent 1992;2(2):93-97.
32. De Mattia D, Pettini PL, Sabato V, Rubini G, Laforgia A, Schettini F. [Oromaxillofacial changes in thalassemia major]. Minerva Pediatr 1996;48(1-2):11-20.
33. MukherjeeDas S, Das U, Bhattacherjee S, Datta P. Oral hygiene status and dental caries experience in transfusion dependent thalassemia patients: An analytical cross-sectional study. Indian J Public Health Dev 2024;15(3): 366-370.
34. Hattab FN, Hazza'a AM, Yassin OM, al-Rimawi HS. Caries risk in patients with thalassaemia major. Int Dent J 2001;51(1):35-38.
35. Khan NIH, Kabil NS, Ebeid FSE. Dental status and oral health-related quality of life among Egyptian children with B-thalassemia major:A case-control study. J Oral Health Dent 2019;2:74-82.
36. Luglie PF, Campus G, Deiola C, Mela MG, Gallisai D. Oral condition, chemistry of saliva, and salivary levels of Streptococcus mutans in thalassemic patients. Clin Oral Investig 2002;6(4):223-226.
37. Karayilmaz H, Yalcin-Erman H, Erken-Gungor O, Ozturk Z, Felek R, Kupesiz A. Evaluation the oral hygiene conditions, oral Candida colonization and salivary Streptococcus mutans and Lactobacilli density in a group of beta-thalassemic children and adolescence. Med Oral Patol Oral Cir Bucal 2019;24(6):e712-e718.
38. Caliskan U, Tonguc MO, Ciris M, Balta N, Kirzioglu FY, Caglayan G, Canatan D. The investigation of gingival iron accumulation in thalassemia major patients. J Pediatr Hematol Oncol 2011;33(2):98-102.
39. Hattab FN, Qudeimat MA, al-Rimawi HS. Dental discoloration: an overview. J Esthet Dent 1999;11(6):291-310.

40. Vento S, Cainelli F, Cesario F. Infections and thalassaemia. Lancet Infect Dis 2006;6(4):226-233.
41. Hazza'a AM, Darwazeh AM, Museedi OS. Oral Candida flora in a group of Jordanian patients with beta-thalassemia major. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2010;109(2):252-256.
42. Akiki N, Hodroj MH, Bou-Fakhredin R, Matli K, Taher AT. Cardiovascular complications in β-thalassemia: getting to the heart of it. Thalassemia Rep 2023;13(1):38-50.
43. Mednick L, Yu S, Trachtenberg F, Xu Y, Kleinert DA, Giardina PJ, Kwiatkowski JL, Foote D, Thayalasuthan V, Porter JB, Thompson AA, Schilling L, Quinn CT, Neufeld EJ, Yamashita R, Thalassemia Clinical Research N. Symptoms of depression and anxiety in patients with thalassemia: prevalence and correlates in the thalassemia longitudinal cohort. Am J Hematol 2010;85(10):802-805.
44. Ruggiero SL, Dodson TB, Aghaloo T, Carlson ER, Ward BB, Kademani D. American Association of Oral and Maxillofacial Surgeons' Position Paper on Medication-Related Osteonecrosis of the Jaws-2022 Update. J Oral Maxillofac Surg 2022;80(5):920-943.
45. Radia R, Loughlin A, Adams A. Manifestations of beta thalassaemia in the dental setting: a case report. Dental Update 2022;49(8):707-709.
46. Bakardjiev A. The challenge for the dental community-bisphosphonate-related osteonecrosis of the jaw. J Dent Oro Surg 2014;1(4):1-4.
47. SCDEP. Oral Health Management of Patients at Risk of Medication-related Osteonecrosis of the Jaw. Supplement March 2024. Available at: https://www.sdcep.org.uk/media/d0sb4flf/sdcep-mronj-guidance-supplement_march-2024.pdf. Accessed: 02 Sep 2024.
48. Fedele S, Kumar N, Davies R, Fiske J, Greening S, Porter S. Dental management of patients at risk of osteochemonecrosis of the jaws: a critical review. Oral Dis 2009;15(8):527-537.
49. UK Government. Guidance: Delivering better oral health: an evidence-based toolkit for prevention. Updated 9 November 2021. Available at: https://www.gov.uk/government/publications/delivering-better-oral-health-an-evidence-based-toolkit-for-prevention. Accessed: 02 Sep 2024.
50. Kim C, Hwang KG, Park CJ. Local anesthesia for mandibular third molar extraction. J Dent Anesth Pain Med 2018;18(5):287-294.
51. Meechan JG. Supplementary routes to local anaesthesia. Int Endod J 2002;35(11):885-896.
52. Marschall KE, Hines RL. Stoelting's Anesthesia and co-existing disease. 8th ed: Elservier, 2018.
53. Master Anaesthesia. Thalassemia. Available at: https://masteranesthesia.com/thalassemia/. Accessed: 02 Sep 2024.
54. Staikou C, Stavroulakis E, Karmaniolou I. A narrative review of peri-operative management of patients with thalassaemia. Anaesthesia 2014;69(5):494-510.

10 NUTRITION

Authors: **Ellen B. Fung**[1], **Michael Angastiniotis**[2]

[1.] Division Hematology, Department of Pediatrics, University of California San Francisco (UCSF), San Francisco, CA, USA

[2.] Thalassaemia International Federation (TIF), Nicosia, Cyprus

Competing interest: None to disclose.

1. INTRODUCTION

There is an abundance of nutrition information available to patients from numerous sources. Not all the information available is reputable, some of it contradictory, and in a few instances, potentially dangerous to patients with thalassaemia. As a result, questions relating to nutrition and how nutrition may influence the patient's health and quality of life are often posed by patients and their parents. Recognising this need the Thalassaemia International Federation (TIF) has published a detailed guideline offering treating physicians an insight into the nutritional needs of thalassaemia patients and the kind of advice that may be offered by the caring team as well by professional nutritionists [1]. An accompanying patient-facing guide on nutritional recommendations is also now available [2]. In this Chapter, an overview of the most common macro- and micronutrient deficiencies observed in patients with transfusion-dependent β-thalassaemia (TDT) will be provided. More details regarding the aetiology of nutritional deficiencies in TDT, as well as optimal nutrition assessment and management are provided in the aforementioned nutrition-focused TIF guidelines [1].

Undernutrition occurs in a considerable number of patients with β-thalassaemia, varying greatly by country of origin (from 5.2% to 70%) with low- and middle-income countries having the highest prevalence of malnutrition [3]. Undernutrition in β-thalassaemia is not benign, and is associated with growth failure, delayed pubertal development, inadequate immune function and insulin secretion, and altered lipid profiles [4-7]. For this reason, it is imperative that variables related to growth and nutrition are measured routinely throughout the patient's life [8-14].

When body mass index (BMI) is used as a crude indicator of nutritional status, it is clear that poor nutritional status is related to both quality of care (suboptimal therapeutic indices and complications) and the socio-economic background of the country of residence (impoverishment, reduced energy intake, literacy) (Table 1) [12, 13, 15-18].

It is also clear that dietary adequacy in terms of both caloric intake as well as dietary quality (micronutrient content) is a prerequisite to adequate growth and maintaining optimal health. On the other hand, nutritional inadequacy is multifactorial and may include cultural and economic factors as well as individual preferences and sensitivities.

Table 1. *Body mass index of transfusion-dependent β-thalassaemia patients from different countries.*

Country	BMI (<18.5 kg/m²) Underweight	BMI (>25 kg/m²) Obese/overweight	Age (years) Range: >18	Reference
Greece	1.5%	29.9%	Range: >18	[15]
Greece	7.5%	22.4%	Mean: 37.2 ± 10.3	[13]
USA	5.2%	26.5%	Mean: 23.3 ± 3.6	[12]
India (Kolkata)	48.2%*	0%*	Mean: 8.0 ± 2.3	[16]
Iraq (Baghdad)	23.3%	5%	Median: 25 (18-43)	[17]
Iran	28%	6%	Range: 12-40	[18]

**In children underweight <5% BMI, overweight/obese >85% on growth chart centiles.*
Abbreviations: BMI, body mass index.

2. ENERGY/CALORIC INTAKE

Two decades ago, Soliman and colleagues reported that a short-term high calorie diet partially corrected nutritional status in children with β-thalassaemia major who were unaffected by endocrinopathy or cardiomyopathy [19]. Their results showed that increasing caloric intake significantly increased insulin growth factor 1 (IGF-1), skin fold thickness, mid-arm circumference, and BMI, thus implying growth may also be improved if the diet was sustained. This observation is consistent with other studies [20]. More recently, poor nutritional density, or the reduced concentration of essential nutrients per calorie, has been observed [21, 22]. It is unclear if an increase in caloric quantity would be beneficial for all patients during periods of rapid growth, or simply a re-focus on increasing nutritional density. Regardless, the relationship between caloric intake and energy expenditure (caloric need) should be followed closely even after the growth period since overweight and obesity are possible.

3. VITAMINS AND MINERALS

There are numerous reports of essential vitamin and mineral deficiencies in patients with β-thalassaemia, some of which have linked these deficiencies to poor growth, bone health, and altered glucose homeostasis [23-25]. It is valuable therefore to briefly highlight the vitamins and minerals that appear to be at greatest risk for deficiency and summarise what is known about their importance in patients with thalassaemia.

3.1. Zinc

Zinc, an essential trace mineral, is indispensable for hundreds of enzymes and metalloproteins in the body [26, 27]. Zinc deficiency commonly manifests as growth failure and increased susceptibility to infection in children, while in adults, deficiency is most often associated with hypogonadism, diabetes, and poor bone mineralisation, all of which are coincidentally common complications of β-thalassaemia [26-28]. Deficient circulating levels of zinc have been documented in both TDT and non-transfusion-dependent β-thalassaemia (NTDT) patients [3, 24, 29, 30]. The aetiology of zinc deficiency in β-thalassaemia is likely to be a combination of inadequate intake,

elevated urinary zinc excretion, iron overload, oral chelator use, and reduced zinc binding capacity to serum carrier proteins in the face of increased requirements [3, 23, 31-33]. There is increased urinary losses of zinc with prolonged use of thiazide diuretics in non-thalassaemic populations [34] as well as in diabetic patients with β-thalassaemia [35]. Given diabetes is common in some patients with TDT, and thiazide diuretics are used frequently in patients with cardiac failure, zinc status should be monitored more frequently.

The relationship between zinc status and chelation use is complex and depends not only on chelator type but also chelator dosing regimen, adherence and hepatic iron concentration. Regardless of these conflicting results, some centres provide 50 mg/day of zinc in supplemental form to patients prescribed deferiprone regardless of serum zinc level.

There have been few studies examining the effects of supplementation primarily in TDT patients. These reports show improvement in linear growth [31, 36], bone health [36], as well as improved insulin sensitivity and glucose homeostasis [37, 38] with between 25-40 mg zinc supplementation provided per day.

Monitoring zinc levels annually is recommended for all patients, while considering every 6 months for those on chelation therapy or thiazide diuretics and, if indicated, supplements should be prescribed. Zinc status is most commonly assessed from plasma or serum zinc. Circulating zinc is sensitive to significant deficiency, but can be artificially increased by oestrogen, haemolysis, and contamination during sampling. Circulating zinc can also be artificially depressed by food intake, diurnal variation, chelation, infection, and systemic inflammation [39]. Ideally, samples should be drawn fasting, in the morning, and at least 24 hours after chelation dose. For fasting samples drawn in the morning, adequate serum zinc for adult females is >70 ug/dL and for adult males >74 ug/dL (http://www.izincg.org).

Zinc supplements are available in various formulations, with different contents of elemental zinc. It is recommended that whichever formulation is chosen, 25 mg/day up to a maximum of 40 mg/day of elemental zinc is prescribed, according to individual patient's needs. Caution is needed for high doses of zinc, as gastrointestinal irritation can occur, and may interact with the absorption of other essential minerals (e.g., copper).

3.2. Iron

In TDT patients, the contribution of dietary intake of iron is not significant when compared with transfusional iron intake. However, intestinal absorption becomes more significant at low pretransfusion haemoglobin levels. Globally, many TDT patients do not receive blood transfusions before haemoglobin levels fall to 6 or 7 g/dL, and similar to patients with NTDT, dietary iron absorption may rise 5-fold, or up to 5 mg/day, and therefore contribute to iron loading. In this group of patients, dietary restriction of iron is important. Drinking black tea with meals may reduce non-heme dietary iron absorption, while foods rich in vitamin C will increase non-heme iron absorption. Neither will influence the absorption of iron found in animal products, therefore patients not prescribed regular chelation should also limit consumption of beef, liver, pork, lamb and chicken. Iron supplements have no place in any of the thalassaemia syndromes, TDT or NTDT; this includes during pregnancy when iron supplementation is routinely provided to women to address gestational anaemias.

3.3. Calcium and vitamin D

Calcium and vitamin D are the most commonly prescribed supplements for β-thalassaemia patients. Calcium homeostasis is intimately related to vitamin D. Vitamin D deficiency is defined as a 25-hydroxy (25OH) vitamin D level <20 ng/mL (50 nmol/L), marked deficiency as <10 ng/mL (<25 nmol/L), and insufficiency between 20-30 ng/mL (50-75 nmol/L) [40, 41]. Vitamin D deficiency is associated with poor bone mineralisation, contributing to thalassaemic bone disease, muscle weakness and, more importantly, may affect the heart muscle, causing left ventricular dysfunction associated with cardiac iron uptake [41].

Low consumption of calcium [22], decreased intestinal calcium transport [42], combined with altered vitamin D status all contribute to the disturbance in calcium homeostasis, particularly if hypoparathyroidism is present. Hypocalcaemia and hypercalciuria are frequently observed in TDT [43]. Hypercalciuria has been related to renal tubular dysfunction [44] and possibly to the use of deferasirox [45, 46] with an increasing number of cases of nephrocalcinosis reported [47]. Hypercalciuria with nephrocalcinosis was also previously described in deferoxamine treated patients, although a mechanistic link has not been established. Hypercalciuria does not seem to be related to dietary calcium intake [44] though has been associated within increased 25OH vitamin D levels [45, 48].

Given the importance of calcium to bone health, and the frequency of hypocalcaemia and hypercalciuria observed, at least annual assessment of serum calcium, corrected for albumin level, and urinary calcium is recommended. If increased calcium intake is needed, given the potential risk of cardiovascular disease and nephrolithiasis, dietary sources of calcium (e.g., milk, yogurt, tofu, or kale) are preferred to supplemental calcium. For some patients with critically low dietary calcium intake or hypoparathyroidism, supplemental calcium may be necessary. Vitamin D supplementation is commonly recommended for patients to maintain 25OH vitamin D within an optimal range: between 30 to 50 ng/mL (75-100 nmol/L) [1]. Oral formulations of vitamin D2, ergocalciferol, are preferred when available. It is also recommended that 25OH vitamin D is monitored every 6 months to maintain circulating levels above 30 ng/mL (75 mmol/L) [1]. For patients with marked vitamin D deficiency (serum 25OH vitamin D <10 ng/mL or <25 nmol/L), short-term high dose supplementation is recommended followed by a daily maintenance dose (please refer to Chapter 6 for strategy).

3.4. Folate

Folate, the natural form of the vitamin, also known as folic acid in supplemental form, is an essential vitamin crucial for the synthesis of nucleoproteins. Since these are lost in the cell destruction of ineffective erythropoiesis, increased requirement of folate is expected. Patients on high transfusion regimens, which suppress endogenous erythropoiesis in TDT, rarely develop folate deficiency, in contrast to those on low transfusion regimens. In view of the fact that many patients with TDT are transfused at low haemoglobin levels, as well as possible benefits from folic acid supplementation in reducing risks of thrombosis related to homocysteine levels and atherosclerosis [49, 50], the possibility of providing folic acid supplements, especially to patients who are irregularly transfused, should be considered. This is especially important in older patients β-thalassaemia who may be more prone to thrombotic complications and atherosclerosis [51, 52]. Supplementation with 1 mg/day or 5 mg/week of folic acid appears to be sufficient to maintain optimal folate status for

most patients, along with consumption of foods containing folate [53]. Although the chances of toxic effects of supplementation are low, annual assessment of serum folate is recommended.

3.5. Vitamin E

Vitamin E, also known as α-tocopherol, is a fat-soluble vitamin with antioxidative functions as well as immune boosting properties and activity against non-alcoholic fatty liver and low-grade inflammation. Vitamin E is frequently depleted in patients with β-thalassaemia primarily due to high levels of oxidative stress, hepatic iron overload, and associated liver damage [54], although reduced dietary intake has also been demonstrated [22]. Supplements of vitamin E have been shown to reduce oxidative stress in NTDT and TDT [55-58] and to reduce lipid peroxidation of red cell membranes [59]. However, most of these trials which used 400-600 IU vitamin E/day, were for relatively short durations of treatment and with small patient numbers. Moreover, when antioxidants are given in high quantities, they may exhibit pro-oxidant qualities [60] and vitamin E toxicity has resulted in an increased risk of bleeding and intracranial haemorrhage in animal studies [61]. Therefore, prolonged supplementation, especially at high doses, requires more extensive trials in β-thalassaemia. A diet rich in foods that contain vitamin E can be recommended, with intake of foods including eggs, avocados, vegetable oils (e.g., olive oil, corn oil, safflower, and sunflower oil), nuts and seeds.

3.6. Vitamin C

Vitamin C, also known as ascorbate, is an antioxidant which is integrally involved in iron metabolism, and therefore is of critical importance to patients with β-thalassaemia. Dietary ascorbate enhances non-heme iron absorption in the gut. Ascorbate also modulates iron metabolism by stimulating ferritin synthesis, inhibiting lysosomal ferritin degradation, and decreasing cellular iron efflux. Furthermore, ascorbate cycling across the plasma membrane is responsible for ascorbate-stimulated iron uptake and tissue iron loading.

Vitamin C can be depleted in conditions in which there are increased free iron radicals. Oxidative stress induced by iron overload is one of the major precipitating causes of vitamin C deficiency in TDT patients. As a result, deficiency is frequently demonstrated in patients with elevated iron stores [23, 62]. Scurvy, a severe form of vitamin C deficiency, has been observed in β-thalassaemia, but it is rare [63-65].

Supplementation with 100 mg ascorbate/day has been effective in reducing oxidative stress and improving chelator efficacy [66, 67]. However, supplementation with vitamin C must be used with caution. High doses can cause overt side effects in some susceptible patients (e.g., oxalate renal stones) [68]. Moreover, rebound scurvy type symptoms can occur with abrupt discontinuation of high dose supplementation (typically at doses >500 mg/day).

In order to avoid toxicity and increasing non-transferrin-bound iron, vitamin C must be given at the time of deferoxamine infusion at a dose not exceeding 2-3 mg/kg (typically 100 mg/day). The benefit of vitamin C combined with oral chelating agents has not been studied thoroughly in β-thalassaemia. Therefore, routine supplementation is not recommended, with the exception of heavily iron loaded patients on long term deferasirox where vitamin C supplementation combined with oral chelation may prove beneficial [63].

4. ANTIOXIDANT THERAPY

Iron overload, more specifically circulating non-transferrin-bound iron, results in the production of a variety of reactive oxygen species (ROS), which lead to cell and tissue damage. The evaluation and maintenance of antioxidant defence is useful in protecting patients with TDT from tissue damage. The challenge is whether, beyond iron chelation, the provision of antioxidants in the diet or as supplements can counter this imbalance and have a beneficial effect on patient wellbeing. Various molecules have been tested as antioxidants to scavenge and inactivate ROS, leading to cellular protection against oxidation. Examples of nutrients and substances (botanicals) tested in β-thalassaemia include vitamins C, E, A, zinc, curcumin, silymarin, green tea, N-acetylcysteine, α-lipoic acid, L-carnitine, and fermented papaya [54, 69-72]. Most studies, using combinations of substances, have been tested in small numbers of patients for a short treatment duration (3-12 months). In TDT patients, some benefit is demonstrated but there is also possible long-term toxicity, especially where vitamin C is concerned. For this reason, there is no clear recommendation on supplementation unless more extensive trials are conducted with possible long-term toxicity considered. However, encouraging a diet rich in antioxidants (fruits and vegetables) should be recommended for all patients. While the use of natural antioxidants (botanicals) such as curcuminoids, quercetin, and green tea may be of some help; as described below, the same caution applies to a broad recommendation for the use of many botanicals, given the published studies in patients with TDT are limited in length and sample size, with unknown long-term effects using products that have limited regulation or oversight.

5. BOTANICALS

Various substances, often derived from herbal sources, have been proposed to enhance treatment in β-thalassaemia. These often attract the attention of patients, and professionals should therefore be able to respond to any questions and be aware of the potential benefits, limitations or even dangers of these substances. A few have been investigated through clinical trials and are discussed in more detail.

5.1. Tea

Phenolic compounds including polyphenols, (e.g., tannic acid and tannins) inhibit the absorption of non-heme iron. They are especially high in tea, coffee, cocoa, red wine, and some herb teas. A simple cup of black tea may reduce iron absorption by 40 to 90% and a cup of coffee by 39% [73, 74]. Consumption of green tea (3 cups/day after meals daily for 12 months) in addition to chelation therapy in NTDT adults has been shown to decrease liver iron concentration compared to chelation therapy alone [75]. Slightly higher haemoglobin levels were also observed at the end of the study in those who consumed green tea, possibly related to the antioxidants within tea leading to reduced haemolysis. These findings are most relevant to NTDT patients who may absorb a large percentage of dietary iron; inhibitors of iron absorption, such as tea, may be useful in their management.

Green tea extracts (GTEs) have gained popularity as an ingredient in dietary supplements. They are rich in polyphenolic compounds which have been shown to have iron-chelating properties [76]. GTEs have been shown to be effective in improving ineffective erythropoiesis, iron dysregulation, and oxidative stress in iron-overloaded β-thalassaemic mice [77]. However, hepatotoxicity due to GTE intake has been observed, including serious liver injury, especially when ingested under fasting

conditions or in cases where there is underlying liver vulnerability [78]. These findings resulted in a requirement for powdered GTE products sold in the U.S. to have the label: *"Do not take on an empty stomach. Take with food. Do not use if you have a liver problem and discontinue use and consult a healthcare practitioner if you develop symptoms of liver trouble, such as abdominal pain, dark urine, or jaundice (yellowing of the skin or eyes)."*

5.2. L-Carnitine

Carnitine is a butyrate derivative, beta-hydroxy-gamma-trimethylaminobutyric acid, with potential benefits in β-thalassaemia, since it is believed to have antioxidant and cardioprotective properties. It is known to be essential for the metabolism of long-chain fatty acids and it is present in high energy demanding tissues such as skeletal muscle, cardiac muscle, and the liver.

In clinical trials, L-carnitine at a dose of 50 mg/kg/day given to small groups of patients for 3-6 months, L-carnitine was show to 1) improve heart function [79] and exercise performance [80], 2) improve pulmonary artery systolic pressure in patients with pulmonary hypertension [81], 3) increase transfusion intervals [80], and 4) reduce bone pain and muscle weakness [82]. However, caution is needed in patients with seizures and those with hypothyroidism, since L-carnitine inhibits triiodothyronine (T3) and thyroxine (T4) entry into the cell nuclei [83].

5.3. Wheat grass

This is a popular health food prepared as a juice from the leaf buds of the wheat grass plant. It contains chlorophyll, vitamins, minerals, and several enzymes. A wide range of health benefits have been attributed to the use of wheat grass including its ability to lower cholesterol, regulate blood sugar, aid in digestion, and decrease inflammation [84].

There have been a handful of studies that have explored the potential benefit of wheat grass for patients with β-thalassaemia. The primary outcome considered in most of the studies was the potential to decrease the transfusion requirement [85-88]. The limitations of these studies include limited sample size, lack of generalisability to older patients, inconsistent type of intervention (i.e., juice, pill, powder) and lack of a consistent definition of transfusion requirement reduction. In summary, it appears that wheat grass may have an effect of reducing transfusion requirement in a subset of young TDT patients, although the predicting variables of whom will respond are unclear. More studies would be necessary to elucidate a mechanism of action and determine efficacy in a larger cohort of adult patients with TDT.

It is important to note that wheatgrass is considered a food or nutraceutical, not a drug, and therefore, is not regulated by the Food and Drug Administration (FDA) in the US or other European regulatory agencies. As such, there is no assurance that the amount of wheat grass reported on the label is consistent with composition of the product (tablet, powder, juice) or that the product claims on packaging are true.

5.4. Silymarin

A derivative of milk thistle (*Silybum marianum*), silymarin, is a flavonolignan complex which has antioxidant properties and has been investigated extensively as a hepatoprotective agent in non-thalassaemic populations. Silymarin has also been reported to inhibit hepatitis C virus entry into hepatocytes [89, 90]. Despite optimistic studies performed in patients with drug induced liver injury and non-alcoholic liver disease, few studies have evaluated the effects of silymarin on hepatitis B or C infection in β-thalassaemia.

There are reports that combined treatment with silymarin and conventional iron chelators is effective in reducing iron overload in TDT patients [91, 92]. Moreover, an extensive 2024 systematic review of children and adolescents with β-thalassaemia treated with silymarin found beneficial effects on the reduction of ROS, inhibition of haemolysis, attenuation of inflammation, and reduction in hepatic and cardiac iron overload [93].

Silymarin is available in capsular form and usually dosed at 140 mg three times a day (420 mg) with no recorded side effects. Although silymarin supplementation appears promising, more studies, especially long-term, are required before broad recommendations for all patients can be made.

5.5. Curcumin

Curcumin, an ingredient of turmeric, is claimed to possess several pharmacological properties including antioxidant, iron-chelating, and anti-inflammatory activities [94]. Studies have been limited in patients with β-thalassaemia, which nevertheless claim benefit against oxidative stress. In one study, 21 patients with haemoglobin E/β-thalassaemia were treated with 500 mg daily turmeric in capsular form for 12 months. An improvement in parameters of oxidative stress was noted as well as a reduction in non-transferrin-bound iron; however, all values returned to baseline 3 months after stopping curcumin [95]. Similar findings were confirmed in a 2021 study using doses of 500 and 1000 mg/day for 24 weeks in 29 adult patients with haemoglobin E/β^0-thalassaemia, markers of oxidative stress and inflammation were reduced in both dosage groups [96].

More recent randomised trials have produced less-conclusive results. Saeidnia and colleagues reported that curcumin given for 3 months decreased serum ferritin and increased hepcidin compared to placebo in NTDT patients [97, 98]. In contrast, Eghbali and colleagues [99] published a randomised clinical trial in 171 TDT patients provided curcumin 2 times per day for 6 months. They observed a significant decrease in liver enzymes and bilirubin but no effect on serum ferritin, cardiac, or hepatic iron measured by T2* magnetic resonance imaging. No severe adverse effects were reported. These discrepant results in the recent clinical trials do not support a firm recommendation for consistent use of curcumin.

KEY POINTS AND RECOMMENDATIONS

1. Routine anthropometric, biochemical, and clinical assessments to clarify nutritional status is necessary for patients with β-thalassaemia (Table 2), and nutritional factors such as caloric intake and micronutrient deficiencies should be considered in instances of poor growth. Include a nutrition professional (dietitian) in a comprehensive nutritional assessment and individualised nutrition recommendations (**Class I**).

Table 2. *Anthropometric, biochemical, and clinical assessments recommended β-thalassaemia to clarify nutritional status.*

Routine assessments	Frequency
Anthropometry: height, sitting height, weight	Every 6 months until pubertal maturity, with calculation of body mass index (kg/m2) and growth velocity charted on centile charts for age and gender
Pubertal assessment (Tanner staging)	Every 6 months until pubertal maturity
Urinary and serum calcium (corrected for albumin)	At least annually from 10 years of age
Free T4, TSH	Annually from 9 years of age
25OH vitamin D	Every 6 months to maintain circulating levels above 30 ng/mL (75 mmol/L)
Copper, magnesium, selenium, zinc, folate, vitamin C, vitamin E, cholesterol, triglycerides	Annually from 10 years or earlier if specific nutritional deficiency is suspected
Fasting glucose and insulin, or OGTT	Every 2 years from 10-18 years of age, annually thereafter
Bone densitometry (DXA): • Lumbar spine, total body less head (children, adolescents) • Lumbar spine, femoral neck, total hip (adults)	Every 1-2 years, starting at 10 years of age
Other tests which may be relevant to nutrition assessment but not routinely recommended	
• Bone age assessment (hand wrist x-ray) in relation to chronological age for patients with growth delay or growth failure considering rhGH therapy • GDF15, a stress-induced hormone, which reduces appetite has been suggested as a biomarker of malnutrition. Very high GDF15 levels have been found in β-thalassaemia [8] • Liver steatosis, assessed by abdominal ultrasound, can be related to severe malnutrition [9-11] • Body composition assessment, including diminished lean mass and sarcopenia are indicators of malnutrition. Bioelectrical impedance or whole body DXA exams provide body composition analysis for those centres with the available technology [12-14]	

Abbreviations: TSH, thyroid-stimulating hormone; 25OH, 25-hydroxy; OGTT, oral glucose tolerance test; DXA, Dual-energy X-ray absorptiometry; rhGH, recombinant human growth hormone; GDF15, growth/differentiation factor 15.

2. A varied, nutrient-dense diet rich in antioxidants (fruits and vegetables) is recommended for all patients (**Class II**).

3. Zinc supplementation (25 mg/day up to a maximum of 40 mg/day) may be given in cases of zinc deficiency, poor growth, reduced bone mass, or those at risk of diabetes, but is not recommended as routine for all patients. Monitor zinc levels in serum or plasma annually or every 6 months for those on chelation therapy or thiazide diuretics (**Class IIa**).

4. Dietary iron restriction is recommended in non-transfusion-dependent β-thalassaemia (NTDT) patients or in transfusion-dependent β-thalassaemia (TDT) patients with low pretransfusion haemoglobin level (<8 g/dL). Dietary iron restriction should not be a primary focus of nutritional counselling for TDT patients where the deposition of iron from transfused red cells far outweighs dietary iron absorption (**Class IIa**).

5. Vitamin D supplements are commonly recommended for patients to achieve 25-hydroxy (OH) vitamin D within an optimal range (30-50 ng/mL or 75-100 nmol/L), along with measurements of 25OH vitamin D levels every 6 months. For patients with marked vitamin D deficiency (serum 25OH vitamin D <10 ng/mL or <25 nmol/L), short-term high dose supplementation is recommended followed by a daily maintenance dose of 1000-2000 IU **(Class I)**. Refer to Chapter 6 for dosing strategies.

6. At least annual assessment of serum calcium, corrected for albumin level, and urinary calcium is recommended from the age of 10 years. Assessment can also be done more frequently (e.g., quarterly) in patients at risk along with other routine biochemical analyses. A diet rich in foods high in calcium (e.g., milk, yogurt, tofu, or kale) is preferred over supplemental calcium, unless patients have critically low dietary calcium intake or hypoparathyroidism, supplemental calcium may be necessary (**Class I**).

7. Folic acid supplementation of up to 1 mg/day (or 5 mg/week) is recommended for NTDT patients (**Class I**). Folic acid supplementation for TDT patients may be considered especially for those maintained on low pretransfusion haemoglobin level, since the risk of thrombosis may be reduced, and toxicity is low (**Class IIb**).

8. A diet rich in foods with high vitamin E content (e.g., eggs, vegetable oil, nuts, or seeds) is recommended. Short-term (3 month) supplementation of vitamin E (α-tocopherol) should be provided to those with identified deficiency; however, prolonged use of supplements requires further research (**Class IIb**).

9. If vitamin C deficiency is confirmed, supplementation is recommended in conjunction with deferoxamine infusions at a dose of 2-3 mg/kg/day (**Class IIa**).

10. Vitamin C may be useful for reducing oxidative stress and improving oral chelator efficacy particularly in patients who are heavily iron overloaded, although supplementation must be used with caution to avoid toxicity and inadvertently increasing non-transferrin-bound iron (NTBI). Supplementation may be considered (2-3 mg/kg/day, typically 100 mg/day) in heavily iron overloaded patients when combined with oral deferasirox (**Class IIb**).

11. Although routine supplementation without identification of single nutrient deficiency is not recommended (aside from folate, vitamin D, and zinc), a daily multivitamin/mineral supplement without iron is suggested. Supplementation of any kind, however, should not

take the place of a healthy, well-balanced, nutrient-dense diet or adequate iron chelation (**Class IIb**).

12. Tea consumption, either black or green, to inhibit iron absorption may primarily be of benefit if taken with meals for patients with NTDT. Green tea extracts should be used with caution (**Class IIa**).
13. L-carnitine may be beneficial at a dose of 50 mg/kg/day, although caution should be exercised in patients with thyroid dysfunction (**Class IIb**).
14. There is insufficient evidence on any long-term benefits from wheat grass or curcumin.
15. Silymarin at a dose of 140 mg three times daily is recommended if liver involvement is detected, and on consultation with a hepatologist (**Class IIa**).

REFERENCES

1. Fung EB, Schryver T, Angastiniotis M. Nutrition in Thalassaemia and Pyruvate Kinase Deficiency. A Guideline for Clinicians. Nicosia, Cyprus: Thalassaemia International Federation, 2023.
2. Fung EB, Yardumian A. Nutrition in Thalassaemia. A Guide for Patients, Families and Caregivers. Nicosia, Cyprus: Thalassaemia International Federation, 2024.
3. Soliman AT, Yassin M, Alyafei F, Alaaraj N, Hamed N, Osman S, Soliman N. Nutritional studies in patients with beta-thalassemia major: A short review. Acta Biomed 2023;94(3):e2023187.
4. Soliman AT, elZalabany M, Amer M, Ansari BM. Growth and pubertal development in transfusion-dependent children and adolescents with thalassaemia major and sickle cell disease: a comparative study. J Trop Pediatr 1999;45(1):23-30.
5. Skordis N, Kyriakou A. The multifactorial origin of growth failure in thalassaemia. Pediatr Endocrinol Rev 2011;8 Suppl 2:271-277.
6. Elalfy MS, Ebeid FSE, El Gendy YG, Zaki MM, Kalifa ASA. Body Composition in Egyptian Children With Transfusion-dependent Thalassemia: The Impact of Nutrition and Metabolic Profile. J Pediatr Hematol Oncol 2020;42(5):e334-e339.
7. Raghuwanshi B, Kumari S, Sahoo DP. Clinical and Metabolic Complications in patients with thalassemia undergoing transfusion therapy. J Family Med Prim Care 2020;9(2):973-977.
8. Karusheva Y, Petry CJ, Yasara N, Kottahachchi D, Premawardhena A, Barker P, Burling K, Sattar N, Welsh P, Mettananda S, O'Rahilly S. Association of GDF15 levels with body mass index and endocrine status in beta-thalassaemia. Clin Endocrinol (Oxf) 2023;99(2):182-189.
9. Padeniya P, Ediriweera D, De Silva AP, Niriella M, Premawardhena A. The association between steatosis and liver damage in transfusion-dependent beta thalassaemia patients. Br J Haematol 2023;200(4):517-523.
10. Ricchi P, Pistoia L, Positano V, Spasiano A, Casini T, Putti MC, Borsellino Z, Cossu A, Messina G, Keilberg P, Fatigati C, Costantini S, Renne S, Peritore G, Cademartiri F, Meloni A. Liver steatosis in patients with transfusion-dependent thalassaemia. Br J Haematol 2024;204(6):2458-2467.
11. Fragkou N, Vlachaki E, Goulis I, Sinakos E. Liver disease in patients with transfusion-dependent beta-thalassemia: The emerging role of metabolism dysfunction-associated steatotic liver disease. World J Hepatol 2024;16(5):671-677.
12. Fung EB, Xu Y, Kwiatkowski JL, Vogiatzi MG, Neufeld E, Olivieri N, Vichinsky EP, Giardina PJ, Thalassemia Clinical Research N. Relationship between chronic transfusion therapy and body composition in subjects with thalassemia. J Pediatr 2010;157(4):641-647, 647 e641-642.
13. Lidoriki I, Stavrou G, Schizas D, Frountzas M, Fotis L, Kapelouzou A, Kokkota S, Fyntanidou B, Kotzampassi K. Nutritional Status in a Sample of Patients With beta-Thalassemia Major. Cureus 2022;14(8):e27985.
14. Vlychou M, Alexiou E, Thriskos P, Fezoulidis I, Vassiou K. Body Composition in Adult Patients with Thalassemia Major. Int J Endocrinol 2016;2016:6218437.
15. Kattamis A, Voskaridou E, Delicou S, Klironomos E, Lafiatis I, Petropoulou F, Diamantidis MD, Lafioniatis S, Evliati L, Kapsali E, Karvounis-Marolachakis K, Timotheatou D, Deligianni C, Viktoratos P, Kourakli A. Real-world complication burden and disease management paradigms in transfusion-related beta-thalassaemia in Greece: Results from ULYSSES, an epidemiological, multicentre, retrospective cross-sectional study. EJHaem 2023;4(3):569-581.
16. Biswas B, Naskar NN, Basu K, Dasgupta A, Basu R, Paul B. Malnutrition, Its Attributes, and Impact on Quality of Life: An Epidemiological Study among beta-Thalassemia Major Children. Korean J Fam Med 2021;42(1):66-72.
17. Ibraheem A. Effects of Ramadan Intermittent Fasting on the Severity of beta-Thalassemia Major Patients. Cureus 2023;15(3):e36735.

18. Saki F, Bahadori R, Kashkooli NM, Jazayeri A, Ghahremani N, Omrani GHR. Prevalence of metabolic syndrome in beta thalassemia major adolescents in southern Iran: a cross-sectional study. Int J Diabetes Dev Ctries 2019;39:444–450.
19. Soliman AT, El-Matary W, Fattah MM, Nasr IS, El Alaily RK, Thabet MA. The effect of high-calorie diet on nutritional parameters of children with beta-thalassaemia major. Clin Nutr 2004;23(5):1153-1158.
20. Fuchs GJ, Tienboon P, Khaled MA, Nimsakul S, Linpisarn S, Faruque AS, Yutrabootr Y, Dewier M, Suskind RM. Nutritional support and growth in thalassaemia major. Arch Dis Child 1997;76(6):509-512.
21. Fung EB. The importance of nutrition for health in patients with transfusion-dependent thalassemia. Ann N Y Acad Sci 2016;1368(1):40-48.
22. Fung EB, Xu Y, Trachtenberg F, Odame I, Kwiatkowski JL, Neufeld EJ, Thompson AA, Boudreaux J, Quinn CT, Vichinsky EP, Thalassemia Clinical Research N. Inadequate dietary intake in patients with thalassemia. J Acad Nutr Diet 2012;112(7):980-990.
23. Goldberg EK, Neogi S, Lal A, Higa A, Fung E. Nutritional Deficiencies Are Common in Patients with Transfusion-Dependent Thalassemia and Associated with Iron Overload. J Food Nutr Res (Newark) 2018;6(10):674-681.
24. Goldberg EK, Lal A, Fung EB. Nutrition in Thalassemia: A Systematic Review of Deficiency, Relations to Morbidity, and Supplementation Recommendations. J Pediatr Hematol Oncol 2022;44(1):1-11.
25. Ozturk Z, Genc GE, Gumuslu S. Minerals in thalassaemia major patients: An overview. J Trace Elem Med Biol 2017;41:1-9.
26. Molenda M, Kolmas J. The Role of Zinc in Bone Tissue Health and Regeneration-a Review. Biol Trace Elem Res 2023;201(12):5640-5651.
27. Ahmad R, Shaju R, Atfi A, Razzaque MS. Zinc and Diabetes: A Connection between Micronutrient and Metabolism. Cells 2024;13(16).
28. Bellini T, Bustaffa M, Tubino B, Giordano B, Formigoni C, Fueri E, Casabona F, Vanorio B, Pastorino A, Herzum A, Matucci-Cerinic C, Arrigo S, Viglizzo G, Piccotti E. Acquired and Inherited Zinc Deficiency-Related Diseases in Children: A Case Series and a Narrative Review. Pediatr Rep 2024;16(3):602-617.
29. Shamshirsaz AA, Bekheirnia MR, Kamgar M, Pourzahedgilani N, Bouzari N, Habibzadeh M, Hashemi R, Shamshirsaz AA, Aghakhani S, Homayoun H, Larijani B. Metabolic and endocrinologic complications in beta-thalassemia major: a multicenter study in Tehran. BMC Endocr Disord 2003;3(1):4.
30. Mashhadi MA, Sepehri Z, Heidari Z, Shirzaee E, Kiani Z. The prevalence of zinc deficiency in patients with thalassemia in South East of iran, sistan and baluchistan province. Iran Red Crescent Med J 2014;16(8):e6243.
31. Arcasoy A, Canata D, Sinav B, Kutlay L, Oguz N, Sen M. Serum zinc levels and zinc binding capacity in thalassemia. J Trace Elem Med Biol 2001;15(2-3):85-87.
32. Mousa SO, Abd Alsamia EM, Moness HM, Mohamed OG. The effect of zinc deficiency and iron overload on endocrine and exocrine pancreatic function in children with transfusion-dependent thalassemia: a cross-sectional study. BMC Pediatr 2021;21(1):468.
33. Ridha MAS, Kahlol MK, Al-Hakeim HK. Alterations in trace elements and cation profiles in transfusion-dependent thalassemia patients. Transfus Apher Sci 2024;63(4):103954.
34. Braun LA, Rosenfeldt F. Pharmaco-nutrient interactions - a systematic review of zinc and antihypertensive therapy. Int J Clin Pract 2013;67(8):717-725.
35. al-Refaie FN, Wonke B, Wickens DG, Aydinok Y, Fielding A, Hoffbrand AV. Zinc concentration in patients with iron overload receiving oral iron chelator 1,2-dimethyl-3-hydroxypyrid-4-one or desferrioxamine. J Clin Pathol 1994;47(7):657-660.
36. Swe KM, Abas AB, Bhardwaj A, Barua A, Nair NS. Zinc supplements for treating thalassaemia and sickle cell disease. Cochrane Database Syst Rev 2013;2013(6):CD009415.

37. Matter RM, Elbarbary NS, Ismail EAR, Darwish YW, Nada AS, Banoub VP. Zinc supplementation improves glucose homeostasis in patients with beta-thalassemia major complicated with diabetes mellitus: A randomized controlled trial. Nutrition 2020;73:110702.
38. Fung EB, Ahmad T, Killilea DW, Hussain R, Lal A. Zinc supplementation improves markers of glucose homeostasis in thalassaemia. Br J Haematol 2020;190(3):e162-e166.
39. McDonald CM, Suchdev PS, Krebs NF, Hess SY, Wessells KR, Ismaily S, Rahman S, Wieringa FT, Williams AM, Brown KH, King JC. Adjusting plasma or serum zinc concentrations for inflammation: Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) project. Am J Clin Nutr 2020;111(4):927-937.
40. World Medical Association. WMA Statement on Vitamin D Insufficiency. Adopted by the 66th WMA General Assembly, Moscow, Russia, October 2015. Available at: https://www.wma.net/policies-post/wma-statement-on-vitamin-d-insufficiency. Accessed: 20 Nov 2024.
41. Wood JC, Claster S, Carson S, Menteer JD, Hofstra T, Khanna R, Coates T. Vitamin D deficiency, cardiac iron and cardiac function in thalassaemia major. Br J Haematol 2008;141(6):891-894.
42. Lertsuwan K, Wongdee K, Teerapornpuntakit J, Charoenphandhu N. Intestinal calcium transport and its regulation in thalassemia: interaction between calcium and iron metabolism. J Physiol Sci 2018;68(3):221-232.
43. Bulgurcu SC, Canbolat Ayhan A, Emeksiz HC, Ovali F. Assessment of the Nutritional Status, Bone Mineralization, and Anthropometrics of Children with Thalassemia Major. Medeni Med J 2021;36(4):325-332.
44. Quinn CT, Johnson VL, Kim HY, Trachtenberg F, Vogiatzi MG, Kwiatkowski JL, Neufeld EJ, Fung E, Oliveri N, Kirby M, Giardina PJ, Thalassemia Clinical Research N. Renal dysfunction in patients with thalassaemia. Br J Haematol 2011;153(1):111-117.
45. Wong P, Fuller PJ, Gillespie MT, Kartsogiannis V, Strauss BJ, Bowden D, Milat F. Thalassemia bone disease: the association between nephrolithiasis, bone mineral density and fractures. Osteoporos Int 2013;24(7):1965-1971.
46. Capolongo G, Zacchia M, Beneduci A, Costantini S, Cinque P, Spasiano A, De Luca G, Di Pietro ME, Ricchi P, Trepiccione F, Capasso G, Filosa A. Urinary Metabolic Profile of Patients with Transfusion-Dependent beta-Thalassemia Major Undergoing Deferasirox Therapy. Kidney Blood Press Res 2020;45(3):455-466.
47. Sayani FA, Lal A, Tasian GE, Al-Mukaddam M, Killilea DW, Wasserstein A, Fung EB. Kidney Stones in Transfusion Dependent Thalassemia: Prevalence and Risk Factors. Open J Urol 2022;12:209-227.
48. Vogiatzi MG, Macklin EA, Trachtenberg FL, Fung EB, Cheung AM, Vichinsky E, Olivieri N, Kirby M, Kwiatkowski JL, Cunningham M, Holm IA, Fleisher M, Grady RW, Peterson CM, Giardina PJ, Thalassemia Clinical Research N. Differences in the prevalence of growth, endocrine and vitamin D abnormalities among the various thalassaemia syndromes in North America. Br J Haematol 2009;146(5):546-556.
49. Baghersalimi A, Hemmati Kolachahi H, Darbandi B, Kamran Mavardiani Z, Alizadeh Alinodehi M, Dalili S, Hassanzadeh Rad A. Assessment of Serum Folic Acid and Homocysteine in Thalassemia Major Patients Before and After Folic Acid Supplement Cessation. J Pediatr Hematol Oncol 2018;40(7):504-507.
50. Qin X, Xu M, Zhang Y, Li J, Xu X, Wang X, Xu X, Huo Y. Effect of folic acid supplementation on the progression of carotid intima-media thickness: a meta-analysis of randomized controlled trials. Atherosclerosis 2012;222(2):307-313.
51. Farmakis D, Giakoumis A, Cannon L, Angastiniotis M, Eleftheriou A. COVID-19 and thalassaemia: A position statement of the Thalassaemia International Federation. Eur J Haematol 2020;105(4):378-386.
52. Taher AT, Cappellini MD. How I manage medical complications of beta-thalassemia in adults. Blood 2018;132(17):1781-1791.
53. Agrawal T, Dewan P, Gomber S, Agarwal R, Sharma S, Kotru M. Optimum dose of oral folic acid supplementation in transfusion-dependent thalassemia: a randomized controlled trial. J Trop Pediatr 2022;68(6).

54. Livrea MA, Tesoriere L, Pintaudi AM, Calabrese A, Maggio A, Freisleben HJ, D'Arpa D, D'Anna R, Bongiorno A. Oxidative stress and antioxidant status in beta-thalassemia major: iron overload and depletion of lipid-soluble antioxidants. Blood 1996;88(9):3608-3614.
55. Tesoriere L, D'Arpa D, Butera D, Allegra M, Renda D, Maggio A, Bongiorno A, Livrea MA. Oral supplements of vitamin E improve measures of oxidative stress in plasma and reduce oxidative damage to LDL and erythrocytes in beta-thalassemia intermedia patients. Free Radic Res 2001;34(5):529-540.
56. Das N, Das Chowdhury T, Chattopadhyay A, Datta AG. Attenuation of oxidative stress-induced changes in thalassemic erythrocytes by vitamin E. Pol J Pharmacol 2004;56(1):85-96.
57. Pfeifer WP, Degasperi GR, Almeida MT, Vercesi AE, Costa FF, Saad ST. Vitamin E supplementation reduces oxidative stress in beta thalassaemia intermedia. Acta Haematol 2008;120(4):225-231.
58. Haghpanah S, Cohan N, Bordbar M, Bazrafshan A, Karimi M, Zareifar S, Safaei S, Aramesh A, Moghadam M, Fard SAZ, Zekavat OR. Effects of three months of treatment with vitamin E and N-acetyl cysteine on the oxidative balance in patients with transfusion-dependent beta-thalassemia. Ann Hematol 2021;100(3):635-644.
59. Sutipornpalangkul W, Morales NP, Unchern S, Sanvarinda Y, Chantharaksri U, Fucharoen S. Vitamin E supplement improves erythrocyte membrane fluidity of thalassemia: an ESR spin labeling study. J Med Assoc Thai 2012;95(1):29-36.
60. Das SK, Nerune SM, Das KK. Antioxidant therapy for hepatic diseases: a double-edged sword. J Basic Clin Physiol Pharmacol 2024;35(1-2):7-14.
61. Wheldon GH, Bhatt A, Keller P, Hummler H. d,1-alpha-Tocopheryl acetate (vitamin E): a long term toxicity and carcinogenicity study in rats. Int J Vitam Nutr Res 1983;53(3):287-296.
62. Bhat KV, Sharma RA, Sharma SM, Joshi P, Dias BF, Shah N, Setia M, Manglani MV. Vitamin C Deficiency and Oxidant Levels in Children With Transfusion-Dependent b-Thalassemia. Indian Pediatr 2021;58(7):631-634.
63. Sarantos K, Evans P, Garbowski M, Davis B, Porter JB. Vitamin C Deficiency in Patients on Long-Term Deferasirox without Supplementation [abstract]. Blood 2008;112(11):1858.
64. Lau H, Massasso D, Joshua F. Skin, muscle and joint disease from the 17th century: scurvy. Int J Rheum Dis 2009;12(4):361-365.
65. Maikap D, Padhan P. Scurvy in a patient with thalassaemia. Clin Exp Dermatol 2022;47(3):613-614.
66. Elalfy MS, Saber MM, Adly AA, Ismail EA, Tarif M, Ibrahim F, Elalfy OM. Role of vitamin C as an adjuvant therapy to different iron chelators in young beta-thalassemia major patients: efficacy and safety in relation to tissue iron overload. Eur J Haematol 2016;96(3):318-326.
67. Darvishi Khezri H, Emami Zeydi A, Sharifi H, Jalali H. Is Vitamin C Supplementation in Patients with beta-Thalassemia Major Beneficial or Detrimental? Hemoglobin 2016;40(4):293-294.
68. Dosedel M, Jirkovsky E, Macakova K, Krcmova LK, Javorska L, Pourova J, Mercolini L, Remiao F, Novakova L, Mladenka P, On Behalf Of The O. Vitamin C-Sources, Physiological Role, Kinetics, Deficiency, Use, Toxicity, and Determination. Nutrients 2021;13(2).
69. Yanpanitch OU, Hatairaktham S, Charoensakdi R, Panichkul N, Fucharoen S, Srichairatanakool S, Siritanaratkul N, Kalpravidh RW. Treatment of beta-Thalassemia/Hemoglobin E with Antioxidant Cocktails Results in Decreased Oxidative Stress, Increased Hemoglobin Concentration, and Improvement of the Hypercoagulable State. Oxid Med Cell Longev 2015;2015:537954.
70. Ozdemir ZC, Koc A, Aycicek A, Kocyigit A. N-Acetylcysteine supplementation reduces oxidative stress and DNA damage in children with beta-thalassemia. Hemoglobin 2014;38(5):359-364.
71. Elalfy MS, Adly AA, Attia AA, Ibrahim FA, Mohammed AS, Sayed AM. Effect of antioxidant therapy on hepatic fibrosis and liver iron concentrations in beta-thalassemia major patients. Hemoglobin 2013;37(3):257-276.
72. Dissayabutra T, Tosukhowong P, Seksan P. The benefits of vitamin C and vitamin E in children with beta-thalassemia with high oxidative stress. J Med Assoc Thai 2005;88 Suppl 4:S317-321.

73. de Alarcon PA, Donovan ME, Forbes GB, Landaw SA, Stockman JA, 3rd. Iron absorption in the thalassemia syndromes and its inhibition by tea. N Engl J Med 1979;300(1):5-8.
74. Morck TA, Lynch SR, Cook JD. Inhibition of food iron absorption by coffee. Am J Clin Nutr 1983;37(3):416-420.
75. Al-Momen H, Hussein HK, Al-Attar Z, Hussein MJ. Green tea influence on iron overload in thalassemia intermedia patients: a randomized controlled trial. F1000Res 2020;9:1136.
76. Koonyosying P, Tantiworawit A, Hantrakool S, Utama-Ang N, Cresswell M, Fucharoen S, Porter JB, Srichairatanakool S. Consumption of a green tea extract-curcumin drink decreases blood urea nitrogen and redox iron in beta-thalassemia patients. Food Funct 2020;11(1):932-943.
77. Settakorn K, Kongkarnka S, Chompupoung A, Svasti S, Fucharoen S, Porter JB, Srichairatanakool S, Koonyosying P. Effects of green tea extract treatment on erythropoiesis and iron parameters in iron-overloaded beta-thalassemic mice. Front Physiol 2022;13:1053060.
78. Oketch-Rabah HA, Roe AL, Rider CV, Bonkovsky HL, Giancaspro GI, Navarro V, Paine MF, Betz JM, Marles RJ, Casper S, Gurley B, Jordan SA, He K, Kapoor MP, Rao TP, Sherker AH, Fontana RJ, Rossi S, Vuppalanchi R, Seeff LB, Stolz A, Ahmad J, Koh C, Serrano J, Low Dog T, Ko R. United States Pharmacopeia (USP) comprehensive review of the hepatotoxicity of green tea extracts. Toxicol Rep 2020;7:386-402.
79. Shahidi M, Hashemi SR, Fattahi N, Roshani D, Vahedi S, Sharifi P, Moradveisi B. The Effects of L-Carnitine on Echocardiographic Changes in Patients With beta-Thalassemia Major and Intermedia. J Pediatr Hematol Oncol 2020;42(6):386-390.
80. El-Beshlawy A, El Accaoui R, Abd El-Sattar M, Gamal El-Deen MH, Youssry I, Shaheen N, Hamdy M, El-Ghamrawy M, Taher A. Effect of L-carnitine on the physical fitness of thalassemic patients. Ann Hematol 2007;86(1):31-34.
81. El-Beshlawy A, Youssry I, El-Saidi S, El Accaoui R, Mansi Y, Makhlouf A, Taher A. Pulmonary hypertension in beta-thalassemia major and the role of L-carnitine therapy. Pediatr Hematol Oncol 2008;25(8):734-743.
82. Tabei SM, Mazloom M, Shahriari M, Zareifar S, Azimi A, Hadaegh A, Karimi M. Determining and surveying the role of carnitine and folic acid to decrease fatigue in beta-thalassemia minor subjects. Pediatr Hematol Oncol 2013;30(8):742-747.
83. Benvenga S, Amato A, Calvani M, Trimarchi F. Effects of carnitine on thyroid hormone action. Ann N Y Acad Sci 2004;1033:158-167.
84. Banerjee S, Katiyar P, Kumar V, Waghmode B, Nathani S, Krishnan V, Sircar D, Roy P. Wheatgrass inhibits the lipopolysaccharide-stimulated inflammatory effect in RAW 264.7 macrophages. Curr Res Toxicol 2021;2:116-127.
85. Marawaha RK, Bansal D, Kaur S, Trehan A. Wheat grass juice reduces transfusion requirement in patients with thalassemia major: a pilot study. Indian Pediatr 2004;41(7):716-720.
86. Choudhary DR, Naithani R, Panigrahi I, Kumar R, Mahapatra M, Pati HP, Saxena R, Choudhry VP. Effect of wheat grass therapy on transfusion requirement in beta-thalassemia major. Indian J Pediatr 2009;76(4):375-376.
87. Singh K, Pannu MS, Singh P, Singh J. Effect of wheat grass tablets on the frequency of blood transfusions in Thalassemia Major. Indian J Pediatr 2010;77(1):90-91.
88. Mutha AS, Shah KU, Kinikar AA, Ghongane BB. Efficacy and Safety of Wheat Grass in Thalassemic Children on Regular Blood Transfusion. Cureus 2018;10(3):e2306.
89. Blaising J, Levy PL, Gondeau C, Phelip C, Varbanov M, Teissier E, Ruggiero F, Polyak SJ, Oberlies NH, Ivanovic T, Boulant S, Pecheur EI. Silibinin inhibits hepatitis C virus entry into hepatocytes by hindering clathrin-dependent trafficking. Cell Microbiol 2013;15(11):1866-1882.
90. Polyak SJ, Ferenci P, Pawlotsky JM. Hepatoprotective and antiviral functions of silymarin components in hepatitis C virus infection. Hepatology 2013;57(3):1262-1271.

91. Darvishi-Khezri H, Salehifar E, Kosaryan M, Karami H, Mahdavi M, Alipour A, Aliasgharian A. Iron-chelating effect of silymarin in patients with beta-thalassemia major: A crossover randomised control trial. Phytother Res 2018;32(3):496-503.
92. Hagag AA, Elfaragy MS, Elrifaey SM, Abd El-Lateef AE. Therapeutic value of combined therapy with deferiprone and silymarin as iron chelators in Egyptian children with beta thalassemia major. Infect Disord Drug Targets 2015;15(3):189-195.
93. Rahimi-Dehkordi N, Heidari-Soureshjani S, Sherwin CMT. The Effects and Safety of Silymarin on beta-thalassemia in Children and Adolescents: A Systematic Review based on Clinical Trial Studies. Rev Recent Clin Trials 2024;19(4):242-255.
94. Nunes YC, Mendes NM, Pereira de Lima E, Chehadi AC, Lamas CB, Haber JFS, Dos Santos Bueno M, Araujo AC, Catharin VCS, Detregiachi CRP, Laurindo LF, Tanaka M, Barbalho SM, Marin MJS. Curcumin: A Golden Approach to Healthy Aging: A Systematic Review of the Evidence. Nutrients 2024;16(16).
95. Kalpravidh RW, Siritanaratkul N, Insain P, Charoensakdi R, Panichkul N, Hatairaktham S, Srichairatanakool S, Phisalaphong C, Rachmilewitz E, Fucharoen S. Improvement in oxidative stress and antioxidant parameters in beta-thalassemia/Hb E patients treated with curcuminoids. Clin Biochem 2010;43(4-5):424-429.
96. Hatairaktham S, Masaratana P, Hantaweepant C, Srisawat C, Sirivatanauksorn V, Siritanaratkul N, Panichkul N, Kalpravidh RW. Curcuminoids supplementation ameliorates iron overload, oxidative stress, hypercoagulability, and inflammation in non-transfusion-dependent beta-thalassemia/Hb E patients. Ann Hematol 2021;100(4):891-901.
97. Saeidnia M, Fazeli P, Erfani M, Nowrouzi-Sohrabi P, Tamaddon G, Karimi M. The Effect of Curcumin on Iron Overload in Patients with Beta-Thalassemia Intermedia. Clin Lab 2022;68(3).
98. Saeidnia M, Ghaderi A, Erfani M, Nowrouzi-Sohrabi P, Shokri M, Tamaddon G, Karimi M, Babashahi M. Suppression of Growth Differentiation Factor 15 Gene Expression by Curcumin in Patients with Beta-Thalassemia Intermedia. Clin Lab 2024;70(1).
99. Eghbali A, Nourigheimasi S, Ghasemi A, Afzal RR, Ashayeri N, Eghbali A, Khanzadeh S, Ghaffari K. The effects of curcumin on hepatic T2*MRI and liver enzymes in patients with beta-thalassemia major: a double-blind randomized controlled clinical trial. Front Pharmacol 2023;14:1284326.

11 LIFESTYLE AND QUALITY OF LIFE

Authors: **Michael Angastiniotis[1], Ellen B. Fung[2]**

[1.] Thalassaemia International Federation (TIF), Nicosia, Cyprus

[2.] Division Hematology, Department of Pediatrics, University of California San Francisco (UCSF), San Francisco, CA, USA

Competing interest: None to disclose.

Acknowledgements: The authors would like to thank Angelo Loris Brunetta and George Constantinou (Thalassaemia International Federation, Nicosia, Cyprus) for their review and input on this Chapter.

1. INTRODUCTION

Patients with optimally treated transfusion-dependent β-thalassaemia (TDT) can now enjoy a near-normal life and lifestyle, and experience full physical and emotional development from childhood to adulthood. Across the world, however, treatment is still suboptimal in most locations and life expectancy is well below that of the general population. Quality of life (QOL) is also variable. The aim of promoting best practice guidelines is to point the way for the best possible care resulting in the best possible outcomes for patients. This must be linked to an effort towards achieving equity across the world and promoting prioritisation of these hereditary disorders especially where they are prevalent. Healthy lifestyle includes all activities that encourage physical, spiritual, and mental functions and contributes to decreasing health problems, managing stressful events, and improving the QOL. Life activities that can be influenced by a healthy lifestyle include:

- Physical activity and exercise.
- Self-care and management along with self-efficacy.
- Social integration which includes education, employment, and relationships (including marriage and reproduction).
- Dietary habits and optimal nutrition (see Chapter 10).
- Daily habits such as alcohol intake, smoking, and substance abuse.

All of these issues are considered in this Chapter and may influence the outcomes of patients with TDT, especially with regards to QOL. Holistic care includes patient education and encouragement of a healthy lifestyle.

2. WHAT IS THE AIM OF OPTIMAL CARE?

According to the World Health Organization (WHO) definition (set out in the constitution, [1]), health is a state of complete physical, mental, and social well-being and not merely the absence of disease or infirmity. Furthermore, well-being has been defined as the combination of feeling good and functioning well; the experience of positive emotions as well as the development of one's potential, having some control over one's life, having a sense of purpose, and experiencing positive relationships [2]. Healthcare professionals should, beyond following clinical protocols, have the

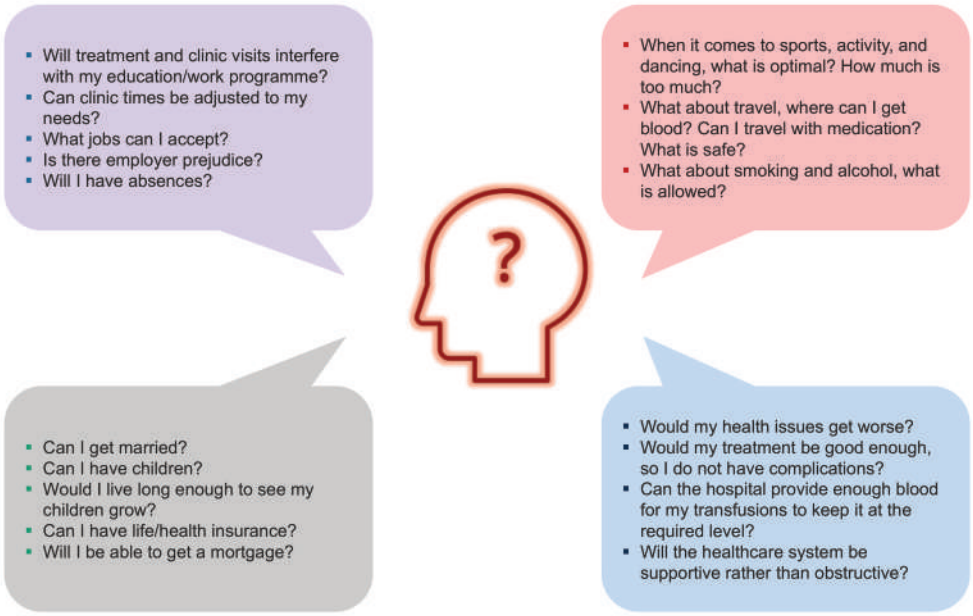
clear aim of reducing as far as possible the degree to which the disease interferes with the patients' personal and social lives. This is achieved by recognising the limitations that the disease imposes but also the effect that the treatment regimens have on the patients' lifestyles, and the time that these treatments steal from normal living. Beyond managing the physical condition, healthcare staff should be willing to listen to patients' concerns and be able to advise on all lifestyle issues. The challenges to well-being in thalassaemia care could include:

- Inadequate clinical care resulting in increased complication rates, reducing the ability to cope with daily activities and stresses.
- The daily demands for treatment and monitoring, interfering with life activities including the need for strict adherence to treatment protocols/regimes [3].
- Individual concerns/fears about the state of health and the future dangers to be faced.
- Family stress which may be made worse by inadequate support, such as in recruiting blood donors, out of pocket expenses and financial catastrophe [4], and ignorance of the disease [5].
- Poor psychological functioning, inability to adopt positive coping mechanisms [6], along with lack of guidance and support, sometimes complicated by cognitive deficits [7].
- Service-related stressors, such as transition from paediatric to adult care [8], system failures in the provision of essential supplies medications and blood, lack of confidence in staff expertise or adherence to guidelines, time lost to extensive administrative processing of hospital visits.
- Poor Social integration, which may be affected by cultural as well as economic influences, as well as school performance, employment, stigmatisation, and relationships including marriage.
- Political instability and war situations. At the time of writing, Syria, Palestine, Lebanon, Yemen, and Myanmar are high prevalence territories affected by such violence and instability. Afghanistan is still facing a humanitarian crisis.

3. THE HOPE: OFFERING HOLISTIC CARE TO PATIENTS

Leading a 'normal' life is an often-expressed priority for patients. This includes social integration [9], connecting and interacting with people and contributing to society, despite counter forces that the disease and its treatment bring, which can lead to isolation and, in some societies, stigmatisation. Marginalisation will lead to depression and possibly increase health risks [10]. These issues, and the broader concept of QOL, become more prominent as longer survival and minimisation of complications are achieved through modern treatment. The concepts of QOL, social integration, living and experiencing life beyond health preservation are interwoven. Psychosocial support as described in Chapter 12 is a necessary component of management, as is high-quality and well-organised holistic care. The healthcare team should have these concepts in mind and be able to guide patients on a variety of lifestyle matters. Figure 1 illustrates some of the issues that patients may seek answers to. This chapter aims to provide a foundation from which healthcare professionals can respond with confident and informed guidance to their patients.

Figure 1. *Common questions from transfusion-dependent β-thalassaemia patients.*



4. SELF-EFFICACY

Patients with TDT face lifelong challenges which are the result of both the anaemia and its treatment. Serious consequences include short stature, bone deformities, delayed puberty, and a varied pathology which can interfere with daily activities and life achievements. Coping with these challenges depends on the encouragement and empowerment of both the family and the healthcare team as well as professional support from psychologists, social services, and educators. The expected endpoint is self-efficacy and self-management. To reach this level of independence, growing TDT patients require knowledge of the condition and acceptance of its challenges. Knowledge and understanding are necessary to promote serious requirements such as adopting positive coping and adherence to treatment (see Chapter 12) and improving the patient's QOL. Moreover, the patient's journey from childhood, through adolescence to adulthood requires adaptation to role changes, family dynamics, and the bid for more independent decision making by the adolescent [11]. Knowledge and skills can be promoted through personal contact in the clinic, through organised seminars and webinars, and the use of other technologies such as social media and mobile apps.

5. EXERCISE AND PARTICIPATION IN SPORTS

A frequent enquiry from patients concerns their ability to work and participate in sports. In general, physical and recreational activities should be encouraged, as this is an important aspect of healthy and normal living, as well as a means towards social integration. In fact, some investigators have gone so far as to propose that exercise is more effective and safer than prescription medications for a number of chronic diseases of adulthood [12, 13]. However, limitations for some patients with

TDT must be recognised. Physical capacity can be influenced primarily by the degree of anaemia, and cardio-circulatory and pulmonary function, which are important to the oxygenation of tissues. Additionally, in a chronic condition such as TDT, other co-morbidities may be present such as diabetes, cardiac, or liver dysfunction. For the most part, clinical evaluation focuses on the contribution of respiratory and cardiovascular function to exercise tolerance.

5.1. Assessing β-thalassaemia patients for exercise capacity

In children who are maintained on optimal transfusion and chelation regimens, assessment of exercise capacity before participating in physical activities is usually not necessary. However, in young patients who are not optimally treated from early life and for all patients considering vigorous physical activities, a general cardiovascular assessment outlined below is strongly recommended [14]:

- Medical history of TDT management and current anaemia (mean haemoglobin level) and iron status.
- The past, current, and proposed activity level, should be quantified, an International Physical Activity Questionnaire (IPAQ) can be helpful (https://sites.google.com/view/ipaq/home).
- Body mass index related to age and gender.
- Cardiac assessment including echocardiography and measuring myocardial iron by magnetic resonance imaging (MRI) T2* MRI, to examine heart function through assessment of maximum heart rate responses, stroke volume reserve, and the effects of iron overload [15-17].
- Ergometry (mainly cycle ergometry or treadmill).
- Respiratory function tests to measure aerobic capacity (e.g., VO2max) and aerobic threshold [18], which are useful guides to assess exercise tolerance.

The global TDT population is not homogeneous, with thousands of patients surviving and functioning with low haemoglobin levels and poor adherence to chelation therapy. A universal and all-encompassing guideline on how much exercise can be tolerated cannot therefore be formulated, and an individualised approach based on a comprehensive clinical assessment (which may include ergometry) is advisable. Many existing studies have concluded that there is exercise limitation in TDT patients. Chronically low haemoglobin levels, either in non-transfusion-dependent patients or prior to transfusion in TDT have been associated with reduced exercise performance [15, 18]. Additional factors that contribute to activity limitations may include the degree of anaemia, pain [19, 20], iron overload affecting heart function – especially through vascular inflammation [18], and even restrictive lung dysfunction [21].

In general, pre-adolescent children are encouraged to exercise without restrictions, if treated according to accepted transfusion and chelation standards. If maintained at low haemoglobin levels, careful cardiorespiratory assessment is necessary. From early adolescence, iron accumulation and tissue damage may be evident in the heart and endocrine glands if chelation has been sub-optimal. For this reason, even though routine daily activity is unrestricted, exercise tolerance should be assessed at regular intervals.

In addition to the above, consideration must be given to all co-morbidities, including bone disease, which is common at all ages in TDT patients. In addition to pain, which may limit mobility, the propensity to fracture must also be considered whenever giving advice concerning exercise and sporting activities involving physical contact.

5.2. Exercise interventions

There are only a handful of published studies exploring the effect of exercise on health in patients with TDT. In 2013, one study randomly assigned 75 adult TDT patients to either an 8-week walking programme or standard activity [22]. Investigators observed an improvement in self-reported QOL measures in those prescribed the walking program. In 2020, another study assigned 40 adult TDT patients to water exercise three times per week for 8 weeks [23]. Similar positive effects on QOL were observed following this exercise regimen, reinforcing the potential beneficial effect of monitored exercise regimens in TDT.

Some have also suggested that exercise may reduce iron burden. Three separate studies in small groups of adolescents and young adults found that an increase in aerobic exercise in 30-90-minute sessions, 5 times per week for 8 weeks, resulted in reductions in serum ferritin level [24-26]. It was suggested that exercise may assist in the release of iron from tissues; therefore, adequate chelation in the presence of exercise is crucial. Relationships between physical activity, pain, and poor bone health have also been explored. In a recent report of 71 patients with thalassaemia (50 adults >18 years, mean age 27.4 years, 82% TDT) nearly half of the patients reported daily somatic pain, and sedentary behaviour was positively associated with pain severity [19]. They also observed better bone health (bone mineral density Z-scores by Dual X-ray Absorptiometry [DXA]) in patients who were more physically active. Given the significant bone deficits observed in TDT, and the relative inactivity for many patients, it appears that regular non-contact weight bearing physical activity has the potential to not only improve bone health but may also have positive effects on bone pain, glucose tolerance, iron load, and overall QOL.

5.3. Limitations of physical activity recommendations

It must be stated that studies in which physical activity was encouraged were conducted in an environment where subjects were evaluated prior to enrolment, and medical supervision was provided. There are no published guidelines regarding how to safely exercise, nor are there recommendations regarding the types of exercises that would result in the greatest benefit for all patients with TDT. As mentioned above, for some patients, cardiac iron overload and spleen size are significant risk factors which should be considered before initiating any new activity. For most patients however, non-contact, weight bearing physical activity can be encouraged (e.g., walking regimens) after consultation with the patient's haematologist and cardiologist. Physical therapists can also be helpful in translating a physician's recommendation for activity into safe daily patient exercises. In a well-publicised case, a patient with TDT ran the London marathon on two occasions. This is an inspiration for all patients across the world, and proof that modern treatment can lead to a normal QOL. However, in the spectrum of TDT care that exists across the world, this example is sadly relevant to only a minority of optimally-treated patients.

6. EDUCATION

Education is a useful step towards social integration and an interesting life in contrast to passive acceptance of TDT as a condition that impairs the patient from achieving a proper education. However, education requires system and family support as well as a good clinical state. One possible negative influence is cognitive impairment recognised in a small proportion of TDT patients. This can reduce the ability of patients to assimilate knowledge and their ability to reason. In various studies β-thalassaemia patients had significantly different scores from healthy controls [27, 28]. The exact pathology behind this phenomenon is not clearly understood, although covert white matter

lesions have been described in small studies of β-thalassaemia [29]. It may also be that cognitive impairment is not due to thalassaemia but rather to other kind of problems that patients may experience (e.g., environmental, family, society restrictions to education) or due to lack of confidence and stress of treatment in countries with low health support. Irrespectively, cognitive assessment should be considered even for asymptomatic patients [7]. In a recent (2024) survey by the Thalassaemia International Federatio, compiling data from 48 countries, 32.6% of respondents were university graduates [30]. In a another review of 251 TDT patients from Cyprus, 29% had completed tertiary education [31], which compares well with the general population of Cyprus in which 39.4% are university graduates. This suggests that despite some reports of a cognitive impairment, full education is achieved by a significant proportion of patients. On the same note, in the USA, 82% percent of school age children were at expected grade level [32]. The main limiting factor expressed by patients is the need to interrupt educational sessions in order to meet clinic and transfusion appointments, which are in most clinical services, during daytime working hours.

Adjusting day transfusion services to include evening and weekend sessions, to suit patients in full-time education or employment, will greatly assist their achievement of social integration and their contribution to society and this should be a priority for healthcare professionals and managers offering the service. In addition, medical teams should be ready to liaise with educational services and especially schoolteachers, to provide information and education concerning TDT, and the ability of patients to perform in school, recognising that concerns and sometimes even prejudice from teachers may adversely affect student performance. Prejudice is also a feature in the playground, where bullying and negative behaviour can make the young TDT patient feel different and isolated, which can have a lasting effect on their self-image. Feeling different can also have personal consequences in countries where thalassaemia is viewed as an 'immigrant disease', potentially leading to racial and ethnic discrimination [33]. These issues require educational intervention by teachers so that understanding the issues affecting pupils with hereditary and chronic disorders are discussed and any recognised deficits in education performance dealt with.

7. EMPLOYMENT

Patients' wellbeing and social adaptation require a feeling that they are contributing to something bigger than self, contributing to society and not being a passive receiver of assistance. Being employed is the active proof of self-efficacy and social integration.

Many adult TDT patients are employed without difficulty. However, problems do still exist due to several factors, which originate in part from patients but also from employers and the social environment more generally, or from an inadequate healthcare system to support patients in getting treated out of hours. Many patients who have not benefitted from adequate psychosocial support still have low self-esteem and feel that 'poor health' does not allow them to work. In a recent patient survey with over 2000 respondents from 48 countries, only 32% were fully or part-time employed [30]. This indicates that a significant number of patients are having difficulties and a proportion of the global community regard themselves as disabled. On the other hand, prejudice from employers is still an issue in many parts of the world [34]. The reasons given include repeated absences from work and easy fatigue [35], reinforcing the need for out of hours clinic and transfusion sessions. This is a need not satisfied even in well-resourced settings [36] contributing to low work performance and employer reluctance.

If TDT is considered a disability which 'hinders patient's full participation in society on an equal basis with others', then the United Nations (UN) Convention on the Rights of Persons with Disabilities [37] clearly states in Article 27 that parties must recognise the right of persons with disabilities to work on an equal basis with others, prohibiting discrimination on the basis of disability, assuring equal remuneration for work of equal value as well as safe and healthy working conditions. In view of this, the thalassaemia care team and patient organisations have a duty to adequately prepare patients along with family and educators and to advocate on behalf of their patients, educating the public in general but also individual potential employers. The team should also instil a positive attitude in their patients concerning their ability to work.

The patient's parents and siblings also have a great influence on their education and employment. Healthcare professionals must help, support, and direct the family to the fact that their child is not disabled, and can be an achiever in life, just as all their other children.

8. MARRIAGE AND REPRODUCTIVE LIFE

Identifying a life partner, getting married, and having a family are widely accepted as key goals in one's life. Patients with TDT should have no inherent reason to limit their relationships. Nevertheless, there is worry about being rejected by partners or their families because of their thalassaemia diagnosis. The possibility of developing a permanent relationship is influenced by cultural factors as well as societal understanding of the condition. In an updated report from Cyprus [31], of 318 patients over the age of 18 years, only 35% had not married. In the same group of patients, 64% had children. In a much larger international cohort of 966 adult patients, 240 (24.8%) were married or lived with partners [38] which probably reflects more accurately the global picture. These figures cannot be achieved where the majority of patients are children, and where stigmatisation limits patient opportunities to form stable relationships. Several studies have reported that as many as 51% to 80% of TDT patients may have pubertal failure, sexual dysfunction, and/or infertility, due to hypogonadism [39]. From an early age, patients should be seen by an endocrinologist to avoid or correct hypogonadism (see Chapter 7).

Healthcare professionals should also provide general support and encouragement to patients and have a duty to coordinate the multidisciplinary team which oversees both the preconception optimisation of both men and women and cares for the woman through her pregnancy and delivery. Guidelines for the management of thalassaemia patients who wish to have children are published nationally in several countries and should be referred to by those caring for these patients. Chapter 7 in these Guidelines gives adequate description of the issues faced concerning fertility and pregnancy; guidelines for the management of pregnancy are also provided by the British Society for Haematology [40] and matters concerning preservation of gametes following haematopoietic stem-cell transplantation must also be discussed [41].

9. ALCOHOL CONSUMPTION

Patients with TDT should be discouraged from consuming alcohol. Alcohol can potentiate the oxidative damage of iron and aggravates the effect of the hepatitis viruses on liver tissue. If the liver is iron loaded and/or infected by hepatitis C or B virus, alcohol consumption may further promote progression to cirrhosis and hepatocellular carcinoma. Excessive alcohol consumption may also affect bone formation and is a risk factor for osteoporosis. In addition, alcohol may have unexpected interactions with medications.

10. SMOKING

Tobacco must also be avoided in TDT since it may directly affect bone remodelling, which is associated with osteoporosis. Moreover, in view of the doubts concerning cardiorespiratory fitness for exercise, it can be assumed that smoking will make matters worse and, of course, it carries all the adverse effects described in the general population.

11. DRUG ABUSE

Substance abuse is common in many societies and a special danger among adolescents and young people. TDT patients attempting to 'fit in' and be accepted into peer groups are potentially vulnerable to experimentation with these drugs. There are no published studies on the prevalence of drug abuse in this cohort, but many clinicians have encountered isolated cases. Treating staff should be able to recognise patients who have a problem and be ready for transparent and frank discussions around these issues. Substance abuse can have serious consequences in TDT patients with tissue damage affecting many vital organs.

12. QUALITY OF LIFE

All the issues discussed in this Chapter are expected to be addressed by a service which offers patient-centred and holistic care, alongside accepted clinical standards of care. The aim is to achieve autonomy in life and to allow patients to satisfy and fulfil their personal ambitions. The goal of holistic care is not just to achieve freedom from symptoms and reduce complications in these lifelong conditions but to cater for the need for well-being, reducing stress, and understanding the socio-psychological environment. This includes often neglected concerns such as the family environment and economic support for all treatment modalities, access to clinical care made difficult by distance, and the expense of travel. Another concern is the loss of time from education or work due to the frequent visits for treatment. These are examples of avoidable stressors which the healthcare team must be aware of and be ready to assist in removing, if necessary, with the help of professionals such as social services or psychologists. In addition, the healthcare team should be ready to advocate with authorities for support for their patients to resolve such matters through understanding and policy making for improved service development. In addition to these psychosocial consequences of TDT and its treatment, physical symptoms, mainly fatigue and pain often persist. In fact, chronic pain is becoming a major feature in adult patients, related to bone disease and affecting over half of the adult patient population [19, 36].

Awareness of each patient's needs cannot be possible without asking and listening to patient concerns. Too often there is staff avoidance and the inability or unwillingness to address these concerns while clinical management is given priority. There may be physician/nursing factors such as time or work pressures, poor interpersonal communication, and other situational issues. The practice of labelling patient types, including 'the difficult patient', can lead to disparities in care and patient harm which could be avoided. Patients should also be viewed as individuals with 'a life' who happen to have thalassaemia and as a central part of the management team.

In order for the health team to be aware and to be able to monitor progress over time, measures of the QOL have been developed. These depend on the patient's perception of his/her own life and well-being which includes psychological wellness and social functions. They are influenced by the physical state of health and so by the quality of medical care as well as other social influences.

12.1. Assessing quality of life

Several measures, as questionnaires, have been developed to evaluate health-related QOL, which explore domains such as the physical state of health, emotional state, and social circumstances. These are patient reported outcome (PRO) measures. They help to understand the health burden of thalassaemia and inform and support advocacy to service providers and policy planners [42]. They have also been used in iron chelation therapy trials, especially to understand the impact of transition from subcutaneous to oral formulations on patient convenience, satisfaction, and QOL [43-47]. In more recent years, PRO instruments measuring QOL or functional status have also been used in trials of curative or disease-modifying therapies to measure the impact of reduction or anaemia or transfusion requirements [47-55]. These instruments can be classified as *generic*, which can be applied to any/other disease condition, and *disease-specific* questionnaires.

12.1.1. Generic Instruments

These allow comparison of the patient group with the general population. The main examples that have been applied in thalassaemia are:

- WHOQOL (https://www.who.int/tools/whoqol): developed by the WHO as a QOL assessment that would be applicable cross-culturally. It is available as a 100-item questionnaire (WHOQOL-100), but also a shorter 'brief' version (WHOQOL-BREF). It has been used to assess thalassaemia populations but not extensively [35, 49, 56].
- The 36-item Short Form Survey (SF-36, version 2): SF-36 is a similar measure for use in clinical practice and research, health policy evaluations, and general population surveys. Developed by the Rand Corporation in the USA in the 1990s [57], it has been used extensively in thalassaemia research including observational studies and clinical trials [7, 42, 48, 49, 58].
- Pediatric Quality of Life Inventory (PedsQL, https://www.pedsql.org/about_pedsql.html): this is a measure of health-related QOL that can be used for healthy children and adolescents as well as those with acute and chronic health conditions. Some disease-specific modules for the PedsQoL have been developed to assess particular health issues; for example, for sickle cell disease (the PedsQL-SCD Module) but not for thalassaemia. It can be used for paediatric patient self-report or a parent proxy report. The benefit of exploring the attitude of children and their parents cannot be overemphasised [49, 59].
- Patient-Reported Outcomes Measurement Information System (PROMIS): a measure that allows for the assessment of many PRO domains including pain, fatigue, emotional distress, physical functioning and social role participation [49, 60].
- Other generic tools are also used like the Dartmouth Primary Care Cooperative Information Chart System (Dartmouth COOP) questionnaire which was used in one study concerning thalassaemia in children and adolescents [61], the Functional Assessment of Chronic Illness Therapy (FACIT) – especially the Fatigue Scale in trials of novel therapies targeting anaemia [47, 55], and various versions of the EuroQol (EQ) [49, 50].

12.1.2. Disease-specific tools

These were designed specifically for thalassaemia and include:

- Transfusion-dependent QoL Questionnaire (TranQol): the most frequently used instrument [62].
- Specific Thalassemia Quality of Life Instrument (STQOLI) [63].
- Thalassaemia Adult Life Index (ThALI): more recently developed [64].
- Non-transfusion-dependent thalassemia (NTDT)-PRO: developed to measure tiredness, weakness, and shortness of breath [52-54].

Even though these questionnaires explore patients' perceptions of their life, they do not explore patient attitudes and responses to the service and treatment received. QOL is related to the quality of care and to patient-centred care [65]. These are related to patient outcomes so that patient experience is important to be expressed and understood by service providers. Measures of patient experience are distinct from the measures described above. They explore patient experience and satisfaction with healthcare delivery. To our knowledge such tools have not been applied to thalassaemia patients.

12.2. Qualitative investigation

Another method which allows patients to express their thoughts and feelings is 'qualitative research'. Patients express feelings and attitudes in their own words allowing the listener to gather in-depth insights and better understand the patient view.

Common qualitative methods include interviews with open-ended questions. Data are collected through semi-structured or unstructured face-to-face interviews [36, 66]. This methodology offers freedom to the patient to express feelings and experience much in the same way as can be offered in the consultation room, yet doctors rarely offer the time needed to listen to the patient. In the research setting, the patient is prompted by a small number of general questions or prompts that allow him/her to talk about what is of interest. Small groups of people who participate together in interviews which are focused on a particular topic or issue are often referred to as focus groups. The conversation is usually recorded and then coded according to how often a response or quote is repeated by the individual or other participants. Interpretation of the responses leads to an understanding of patient attitudes. The method has been used in thalassaemia research and is suitable for all ages and situations [67].

These various measures and methodologies provide useful indicators for improving care both for individuals and for service improvement. They do not indicate factors or actions that can achieve the desired improvements. They do not collect impressions about adequacy of care as perceived by patients. Also, they do not detect deeper psychological disturbance such as anxiety, depression, which have been shown to affect thalassaemia patients more than the general population [68, 69]. To detect and deal with these issues it is important to include professional psychologists in the multidisciplinary team caring for these chronic conditions (see Chapter 12). A qualitative approach and discussion sessions with families are also needed if they are to contribute effectively to patient support.

KEY POINTS AND RECOMMENDATIONS

1. A holistic approach to patient care includes recognition of the need for social integration and a 'normal' life (**Class I**).
2. The treating physician should be able to provide advice on lifestyle issues (**Class I**).
3. Physical activity, particularly weight-bearing, non-contact activity should be encouraged, but the condition of each individual patient should be recognised and co-morbidities identified through careful cardiovascular assessment and ergometry, if available, before recommending additional physical activity or an exercise regime (**Class I**).
4. Clinic and transfusion times should be flexible and take into consideration the patient's commitments, such as their education and work. Availability of some evening and weekend clinic and transfusion sessions is highly recommended (**Class IIb**).
5. Liaison with teachers and employers to provide understanding of the condition and its management may be necessary (**Class IIb**).
6. Consumption of tobacco and substance abuse should be avoided. Consumption of alcohol is discouraged, especially in patients with iron overload or those at risk of liver or bone disease (**Class IIa**).
7. A quality of life assessment should form part of the regular evaluation of each patient's progress and may be useful to highlight issues in service delivery (**Class IIa**).

REFERENCES

1. WHO. Constitution. Available at: https://www.who.int/about/governance/constitution. Accessed: 21 Nov 2024.
2. Ruggeri K, Garcia-Garzon E, Maguire A, Matz S, Huppert FA. Well-being is more than happiness and life satisfaction: a multidimensional analysis of 21 countries. Health Qual Life Outcomes 2020;18(1):192.
3. Lee WJ, Mohd Tahir NA, Chun GY, Li SC. The impact of chelation compliance in health outcome and health related quality of life in thalassaemia patients: a systematic review. Health Qual Life Outcomes 2024;22(1):14.
4. Othman A, Abdul Ghani MSA, Taib F, Mohamad N. Psychological distress and coping strategies among the caretakers of children with transfusion-dependent thalassemia. Front Pediatr 2022;10:941202.
5. Yousuf R, Akter S, Wasek SM, Sinha S, Ahmad R, Haque M. Thalassemia: A Review of the Challenges to the Families and Caregivers. Cureus 2022;14(12):e32491.
6. Kasi PM, Naqvi HA, Afghan AK, Khawar T, Khan FH, Khan UZ, Khuwaja UB, Kiani J, Khan HM. Coping styles in patients with anxiety and depression. ISRN Psychiatry 2012;2012:128672.
7. Bizri M, Koleilat R, Akiki N, Dergham R, Mihailescu AM, Bou-Fakhredin R, Musallam KM, Taher AT. Quality of life, mood disorders, and cognitive impairment in adults with beta-thalassemia. Blood Rev 2024;65:101181.
8. Chandra J, Sahi PK. Transition of Care for Patients with Thalassemia. Indian J Pediatr 2023;90(12):1227-1231.
9. Politis C, Di Palma A, Fisfis M, Giasanti A, Richardson SC, Vullo C, Masera G. Social integration of the older thalassaemic patient. Arch Dis Child 1990;65(9):984-986.
10. Atkin K, Ahmad WI. Living a 'normal' life: young people coping with thalassaemia major or sickle cell disorder. Soc Sci Med 2001;53(5):615-626.
11. Catarino M, Charepe Z, Festas C. Promotion of Self-Management of Chronic Disease in Children and Teenagers: Scoping Review. Healthcare (Basel) 2021;9(12).
12. Pedersen BK, Saltin B. Exercise as medicine - evidence for prescribing exercise as therapy in 26 different chronic diseases. Scand J Med Sci Sports 2015;25 Suppl 3:1-72.
13. Fiuza-Luces C, Garatachea N, Berger NA, Lucia A. Exercise is the real polypill. Physiology (Bethesda) 2013;28(5):330-358.
14. Piatti G, Giuditta M, Consonni D, Cassinerio E, Cappellini MD. Cardiopulmonary testing in adult patients with beta-thalassemia major in comparison to healthy subjects. Ann Hematol 2022;101(11):2445-2452.
15. Marinov BI, Terziyski KV, Sapunarova KG, Kostianev SS. Exercise performance in children with severe beta-thalassemia before and after transfusion. Folia Med (Plovdiv) 2008;50(4):48-54.
16. Nanas S, Vasileiadis I, Dimopoulos S, Sakellariou D, Kapsimalakou S, Papazachou O, Tasoulis A, Ladis V, Pangalis G, Aessopos A. New insights into the exercise intolerance of beta-thalassemia major patients. Scand J Med Sci Sports 2009;19(1):96-102.
17. Vasileiadis I, Roditis P, Dimopoulos S, Ladis V, Pangalis G, Aessopos A, Nanas S. Impaired oxygen kinetics in beta-thalassaemia major patients. Acta Physiol (Oxf) 2009;196(3):357-363.
18. Sohn EY, Kato R, Noetzli LJ, Gera A, Coates T, Harmatz P, Keens TG, Wood JC. Exercise performance in thalassemia major: correlation with cardiac iron burden. Am J Hematol 2013;88(3):193-197.
19. Fung EB, Goldberg EK, Bambot S, Manzo R, Lal A. Relationships among Physical Activity, Pain, and Bone Health in Youth and Adults with Thalassemia: An Observational Study. Thalassemia Rep 2022;12(3):90-100.
20. Oliveros O, Trachtenberg F, Haines D, Gerstenberger E, Martin M, Carson S, Green S, Calamaras D, Hess P, Yamashita R, Vichinsky E, Thalassemia Clinical Research N. Pain over time and its effects on life in thalassemia. Am J Hematol 2013;88(11):939-943.
21. Piatti G, Allegra L, Fasano V, Gambardella C, Bisaccia M, Cappellini MD. Lung function in beta-thalassemia patients: a longitudinal study. Acta Haematol 2006;116(1):25-29.
22. Arian M, Memarian R, Vakilian F, Badiei Z. Effects of a designed walking program on mental health, functional ability and Cardiac symptoms on Patients with thalassemia major. Evid Based Care J 2013;3(2):17-26.

23. Hasanpour Dehkordi A, Hasani T, Fekri K, Deris F, Etemadifar S. Effects of Aquatic Exercise on Dimensions of Quality of Life and Blood Indicators in Patients with Beta-Thalassemia Major. Int J Prev Med 2020;11:128.
24. Molazem Z, Noormohammadi R, Dokouhaki R, Zakerinia M, Bagheri Z. The Effects of Nutrition, Exercise, and a Praying Program on Reducing Iron Overload in Patients With Beta-Thalassemia Major: A Randomized Clinical Trial. Iran J Pediatr 2016;26(5):e3869.
25. Heidary H, Bijeh N, Saah J, Abrishami F. The effect of eight weeks of aerobic training on hematological indices in β-thalassemia minor patients in kermanshah. Horiz Med Sci 2011;17(3):20-28.
26. Vashtani SH, Nazem F, Bordar F. he effect of aerobic rehabilitation program on concentration of ferritin, iron, TIBC and cardiovascular operation in the young patients suffering from major thalassemia. J Guilan Univ Med Sci 2009;18(71):95-102.
27. Tartaglione I, Manara R, Caiazza M, Carafa PA, Caserta V, Ferrantino T, Granato I, Ippolito N, Maietta C, Oliveto T, Casale M, Di Concilio R, Ciancio A, De Michele E, Russo C, Elefante A, Ponticorvo S, Russo AG, Femina G, Canna A, Ermani M, Cirillo M, Esposito F, Centanni A, Gritti P, Perrotta S. Brain functional impairment in beta-thalassaemia: the cognitive profile in Italian neurologically asymptomatic adult patients in comparison to the reported literature. Br J Haematol 2019;186(4):592-607.
28. Daar S, Al Saadoon M, Wali Y, Al Mujaini R, Al Rahbi S, Chan MF, Al-Siyabi A, Alansary N, Mahadevan S, Al-Adawi S. Cognitive Function in Adults with Beta-Thalassemia Major in Oman: A Pilot Study. Oman Med J 2021;36(6):e322.
29. Musallam KM, Taher AT, Karimi M, Rachmilewitz EA. Cerebral infarction in beta-thalassemia intermedia: breaking the silence. Thromb Res 2012;130(5):695-702.
30. Economidou EC, Angastiniotis M, Avraam D, Soteriades ES, Eleftheriou A. Addressing Thalassaemia Management from Patients' Perspectives: An International Collaborative Assessment. Medicina (Kaunas) 2024;60(4).
31. Angastiniotis M, Christou S, Kolnakou A, Pangalou E, Savvidou I, Farmakis D, Eleftheriou A. The Outcomes of Patients with Haemoglobin Disorders in Cyprus: A Joined Report of the Thalassaemia International Federation and the Nicosia and Paphos Thalassaemia Centres (State Health Services Organisation). Thalassemia Rep 2022;12(4):143-156.
32. Pakbaz Z, Treadwell M, Kim HY, Trachtenberg F, Parmar N, Kwiatkowski JL, Cunningham MJ, Martin M, Sweeters N, Neufeld EJ, Giardina PJ, Olivieri N, Yamashita RC, Vichinsky E. Education and employment status of children and adults with thalassemia in North America. Pediatr Blood Cancer 2010;55(4):678-683.
33. Dyson SM, Atkin K, Culley LA, Dyson SE, Evans H, Rowley DT. Disclosure and sickle cell disorder: a mixed methods study of the young person with sickle cell at school. Soc Sci Med 2010;70(12):2036-2044.
34. Wahab IA, Naznin M, Nora MZ, Suzanah AR, Zulaiho M, Faszrul AR, Kamaruzaman WS. Thalassaemia: a study on the perception of patients and family members. Med J Malaysia 2011;66(4):326-334.
35. Foong WC, Chean KY, Rahim FF, Goh AS, Yeoh SL, Yeoh AAC. Quality of life and challenges experienced by the surviving adults with transfusion dependent thalassaemia in Malaysia: a cross sectional study. Health Qual Life Outcomes 2022;20(1):2.
36. Drahos J, Boateng-Kuffour A, Calvert M, Levine L, Dongha N, Li N, Pakbaz Z, Shah F, Martin AP. Health-Related Quality-of-Life Impacts Associated with Transfusion-Dependent beta-Thalassemia in the USA and UK: A Qualitative Assessment. Patient 2024;17(4):421-439.
37. United Nations. Convention on the Rights of Persons with Disabilities and Optional Protocol. 2006. Available at: https://www.un.org/disabilities/documents/convention/convoptprot-e.pdf. Accessed: 22 Nov 2024.
38. De Sanctis V, Soliman AT, El-Hakim I, Christou S, Mariannis D, Karimi M, Ladis V, Kattamis A, Daar S, Yassin M, Canatan D, Galati MC, Raiola G, Campisi S, Kakkar S, Kaleva V, Saki F, Ellinides A, Pikis G, Christodoulides C, Abdulla M, Di Maio S, Theodoridis C, Elsedfy H, Kattamis C. Marital status and paternity in patients with Transfusion- Dependent Thalassemia (TDT) and Non Transfusion-Dependent Thalassemia (NTDT): an ICET - A survey in different countries. Acta Biomed 2019;90(3):225-237.

39. De Sanctis V, Soliman AT, Yassin MA, Di Maio S, Daar S, Elsedfy H, Soliman N, Kattamis C. Hypogonadism in male thalassemia major patients: pathophysiology, diagnosis and treatment. Acta Biomed 2018;89(2-S):6-15.
40. Shah FT, Nicolle S, Garg M, Pancham S, Lieberman G, Anthony K, Mensah AK. Guideline for the management of conception and pregnancy in thalassaemia syndromes: A British Society for Haematology Guideline. Br J Haematol 2024;204(6):2194-2209.
41. Bedrick BS, Kohn TP, Pecker LH, Christianson MS. Fertility preservation for pediatric patients with hemoglobinopathies: Multidisciplinary counseling needed to optimize outcomes. Front Endocrinol (Lausanne) 2022;13:985525.
42. Sobota A, Yamashita R, Xu Y, Trachtenberg F, Kohlbry P, Kleinert DA, Giardina PJ, Kwiatkowski JL, Foote D, Thayalasuthan V, Porter JB, Thompson AA, Schilling L, Quinn CT, Neufeld EJ, Thalassemia Clinical Research N. Quality of life in thalassemia: a comparison of SF-36 results from the thalassemia longitudinal cohort to reported literature and the US norms. Am J Hematol 2011;86(1):92-95.
43. Payne KA, Desrosiers MP, Caro JJ, Baladi JF, Lordan N, Proskorovsky I, Ishak K, Rofail D. Clinical and economic burden of infused iron chelation therapy in the United States. Transfusion 2007;47(10):1820-1829.
44. Porter J, Bowden DK, Economou M, Troncy J, Ganser A, Habr D, Martin N, Gater A, Rofail D, Abetz-Webb L, Lau H, Cappellini MD. Health-Related Quality of Life, Treatment Satisfaction, Adherence and Persistence in beta-Thalassemia and Myelodysplastic Syndrome Patients with Iron Overload Receiving Deferasirox: Results from the EPIC Clinical Trial. Anemia 2012;2012:297641.
45. Taher A, Al Jefri A, Elalfy MS, Al Zir K, Daar S, Rofail D, Baladi JF, Habr D, Kriemler-Krahn U, El-Beshlawy A. Improved treatment satisfaction and convenience with deferasirox in iron-overloaded patients with beta-Thalassemia: Results from the ESCALATOR Trial. Acta Haematol 2010;123(4):220-225.
46. Taher AT, Origa R, Perrotta S, Kouraklis A, Ruffo GB, Kattamis A, Goh AS, Huang V, Zia A, Herranz RM, Porter JB. Patient-reported outcomes from a randomized phase II study of the deferasirox film-coated tablet in patients with transfusion-dependent anemias. Health Qual Life Outcomes 2018;16(1):216.
47. Musallam K, Taher AT, Hnoosh A, Eliason L, Pelligra CG, Guo S, Mu Y, Cappellini MD. Luspatercept Improves Fatigue-Related Quality of Life through 5 Years of Treatment in Non-Transfusion Dependent Beta-Thalassemia [abstract]. Blood 2024;144(Suppl 1): 3857-3858.
48. Cappellini MD, Taher AT, Piga A, Shah F, Voskaridou E, Viprakasit V, Porter JB, Hermine O, Neufeld EJ, Thompson AA, Tang D, Yucel A, Lord-Bessen J, Yu P, Guo S, Shetty JK, Miteva D, Zinger T, Backstrom JT, Oliva EN. Health-related quality of life in patients with beta-thalassemia: Data from the phase 3 BELIEVE trial of luspatercept. Eur J Haematol 2023;111(1):113-124.
49. Badawy SM, Beg U, Liem RI, Chaudhury S, Thompson AA. A systematic review of quality of life in sickle cell disease and thalassemia after stem cell transplant or gene therapy. Blood Adv 2021;5(2):570-583.
50. Locatelli F, Lang P, Wall D, Meisel R, Corbacioglu S, Li AM, de la Fuente J, Shah AJ, Carpenter B, Kwiatkowski JL, Mapara M, Liem RI, Cappellini MD, Algeri M, Kattamis A, Sheth S, Grupp S, Handgretinger R, Kohli P, Shi D, Ross L, Bobruff Y, Simard C, Zhang L, Morrow PK, Hobbs WE, Frangoul H, Group CT-S. Exagamglogene Autotemcel for Transfusion-Dependent beta-Thalassemia. N Engl J Med 2024;390(18):1663-1676.
51. Cappellini MD, Sheth S, Taher AT, Al-Samkari H, Antmen AB, Beneitez D, Cannas G, Coates TD, Czapla L, Dahlin JL, Estepp JH, Feenstra E, Georgiev P, Gheuens S, Gilroy KS, Glenthøj A, Musallam K, Osman K, Porter JB, Shao H, Uhlig K, Van Beers EJ, Viprakasit V, Kuo KHM, Kattamis A. ENERGIZE-T: A Global, Phase 3, Double-Blind, Randomized, Placebo-Controlled Study of Mitapivat in Adults with Transfusion-Dependent Alpha- or Beta-Thalassemia. Blood 2024;144(Suppl 1): 409-411.
52. Taher AT, Musallam KM, Viprakasit V, Kattamis A, Lord-Bessen J, Yucel A, Guo S, Pelligra CG, Shields AL, Shetty JK, Glassberg MB, Bueno LM, Cappellini MD. Identifying thresholds for meaningful improvements in NTDT-PRO scores to support conclusions about treatment benefit in clinical studies of patients with non-transfusion-dependent beta-thalassaemia: analysis of pooled data from a phase 2, double-blind, placebo-controlled, randomised trial. BMJ Open 2024;14(11):e085234.

53. Taher AT, Musallam KM, Viprakasit V, Kattamis A, Lord-Bessen J, Yucel A, Guo S, Pelligra C, Shields AL, Shetty JK, Miteva D, Bueno LM, Cappellini MD. Psychometric evaluation of the NTDT-PRO questionnaire for assessing symptoms in patients with non-transfusion-dependent beta-thalassaemia. BMJ Open 2023;13(3):e066683.
54. Taher A, Cappellini MD, Viprakasit V, Sutcharitchan P, Mahmoud D, Laadem A, Khan A, Gwaltney C, Harding G, Attie K, Zhang X, Zou J, Pariseau J, Henry Hu X, Kattamis A. Validation of a patient-reported outcomes symptom measure for patients with nontransfusion-dependent thalassemia (NTDT-PRO((c))). Am J Hematol 2019;94(2):177-183.
55. Taher A, Al-Samkari H, Aydinok Y, Bartolucci P, Besser M, Dahlin JL, Estepp JH, Gheuens S, Gilroy KS, Glenthøj A, Goh AS, Iyer V, Kattamis A, Loggetto SR, Morris S, Musallam KM, Osman K, Ricchi P, Salido-Fiérrez E, Sheth S, Tai F, Tevich H, Uhlig K, Urbstonaitis R, Viprakasit V, Cappellini MD, Kuo KHM. ENERGIZE: A Global Phase 3 Study of Mitapivat Demonstrating Efficacy and Safety in Adults with Alpha- or Beta- Non–Transfusion-Dependent Thalassemia [abstract]. Hemasphere 2024;8(S1):11-12.
56. Telfer P, Constantinidou G, Andreou P, Christou S, Modell B, Angastiniotis M. Quality of life in thalassemia. Ann N Y Acad Sci 2005;1054:273-282.
57. Jenkinson C, Coulter A, Wright L. Short form 36 (SF36) health survey questionnaire: normative data for adults of working age. BMJ 1993;306(6890):1437-1440.
58. Musallam KM, Khoury B, Abi-Habib R, Bazzi L, Succar J, Halawi R, Hankir A, Koussa S, Taher AT. Health-related quality of life in adults with transfusion-independent thalassaemia intermedia compared to regularly transfused thalassaemia major: new insights. Eur J Haematol 2011;87(1):73-79.
59. Wang M, Huang M, Hong Y. Psychological intervention in children with transfusion-dependent beta-thalassaemia. Vox Sang 2022;117(3):386-392.
60. Rodigari F, Brugnera G, Colombatti R. Health-related quality of life in hemoglobinopathies: A systematic review from a global perspective. Front Pediatr 2022;10:886674.
61. Pakbaz Z, Treadwell M, Yamashita R, Quirolo K, Foote D, Quill L, Singer T, Vichinsky EP. Quality of life in patients with thalassemia intermedia compared to thalassemia major. Ann N Y Acad Sci 2005;1054:457-461.
62. Klaassen R, Alibhai S, Kirby Allen M, Moreau K, Merelles-Pulcini M, Forgie M, Blanchette V, Buckstein R, Odame I, Quirt I, Yee K. Introducing the TranQol: a new disease-specific quality of life measure for children and adults with thalassemia major. J Blood Disord Transfus 2013;4(4):1000150.
63. Lyrakos GN, Vini D, Aslani H, Drosou-Servou M. Psychometric properties of the Specific Thalassemia Quality of Life Instrument for adults. Patient Prefer Adherence 2012;6:477-497.
64. Kantaris X, Shevlin M, Porter J, Myers L. Development of the Thalassaemia Adult Life Index (ThALI). Health Qual Life Outcomes 2020;18(1):180.
65. Doyle C, Lennox L, Bell D. A systematic review of evidence on the links between patient experience and clinical safety and effectiveness. BMJ Open 2013;3(1).
66. Arbabisarjou A, Karimzaei T, Jamalzaei A. The perception of biological experience in patients with major Thalassemia: a qualitative research. Glob J Health Sci 2014;7(1):79-87.
67. Roy T, Chatterjee SC. The experiences of adolescents with thalassemia in West Bengal, India. Qual Health Res 2007;17(1):85-93.
68. Mednick L, Yu S, Trachtenberg F, Xu Y, Kleinert DA, Giardina PJ, Kwiatkowski JL, Foote D, Thayalasuthan V, Porter JB, Thompson AA, Schilling L, Quinn CT, Neufeld EJ, Yamashita R, Thalassemia Clinical Research N. Symptoms of depression and anxiety in patients with thalassemia: prevalence and correlates in the thalassemia longitudinal cohort. Am J Hematol 2010;85(10):802-805.
69. Khoury B, Musallam KM, Abi-Habib R, Bazzi L, Ward ZA, Succar J, Halawi R, Hankir A, Koussa S, Taher AT. Prevalence of depression and anxiety in adult patients with beta-thalassemia major and intermedia. Int J Psychiatry Med 2012;44(4):291-303.

12 PSYCHOLOGICAL SUPPORT

Authors: **Robert Yamashita[1], Dru Haines[1], Manel Ratnayake[2], Marisa Schweber-Koren[1], Mary Peoples[1], Prantar Chakrabarti[3]**

[1.] Comprehensive Thalassemia Center, Benioff Children's Hospital Oakland, University of San Francisco (UCSF), Oakland, CA, USA

[2.] National Thalassaemia Centre, Kurunegala, Sri Lanka

[3.] Zoho Corporation, Tenkasi, Tamil Nadu, India

Competing interest: None to disclose.

Acknowledgements: The authors would like to thank Angelo Loris Brunetta and George Constantinou (Thalassaemia International Federation, Nicosia, Cyprus) for their review and input on this Chapter.

1. INTRODUCTION

The need for continuity of care and psychological support for chronic diseases is widely accepted [1, 2], as is the negative impact of psychological issues on iron chelation adherence [3-6] and quality of life (QOL) [7] in transfusion-dependent β-thalassaemia (TDT). This Chapter will (1) provide a comprehensive review of the published social and behavioural problems in TDT, with a specific focus on any suggested interventions, and (2) articulate the social and psychological support interventions that have been successfully used for similar problems in other diseases.

Unfortunately, there is a surprising lack of published evidence for psychological support interventions in thalassaemia. A 2001 Cochrane Review of psychological therapies for thalassaemia [8], assessed as 'up-to-date' in 2014 [9], concluded that "no randomised, controlled trials employing psychological therapies … were identified" and "no trials, where quasi-randomisation methods such as alteration are used, were found." This is particularly concerning since a standard observation in many clinical reviews of thalassaemia over the past 25 years is that patient behaviour, primarily with adherence to iron chelation therapy, is a significant variable in long-term outcome and increased financial burden due to management of associated long-term health complications [10-15]. Non-adherence is episodic in many patients, but over time, is probably just as damaging, and further research is needed to see how this influences interventions [16].

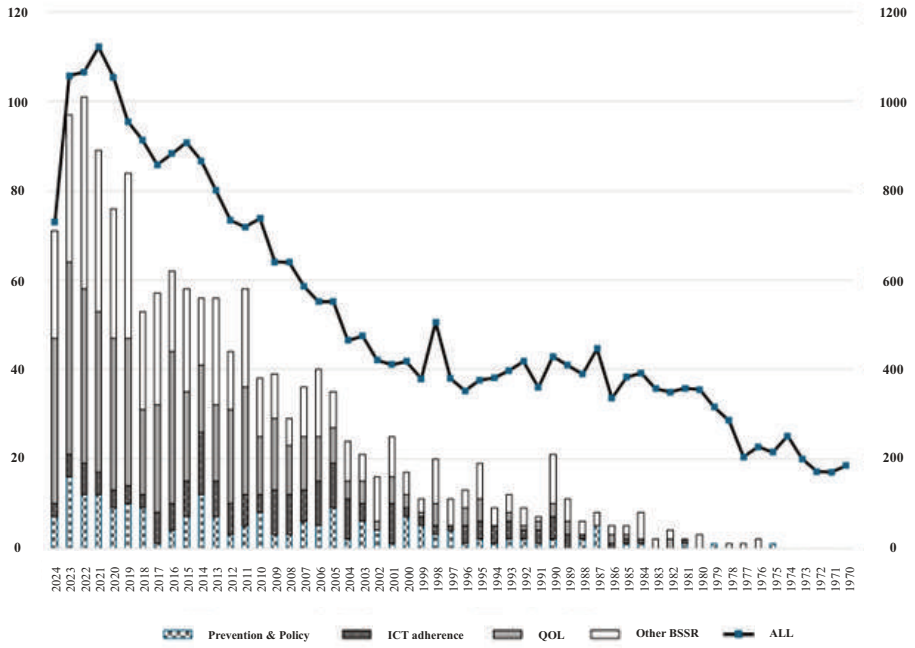
2. THE CHALLENGE OF PSYCHOLOGICAL SUPPORT: WHAT DOES THE LITERATURE TELL US?

The challenge of 'psychological support' in thalassaemia is not a simple construct. Psychological support encompasses a complex set of defined responses to a diverse set of problems that have become apparent in thalassaemia over the past 30 years. This is illustrated by a simple PubMed review (August 2024) using a Title/Abstract search for 'thalassaemia or thalassaemia' and only 'psychological' and 'support'. This only yields 42 reports. The first appears in 1985 and looks to address the need for psychological support in a thalassaemia childcare centre [17]. Almost a decade later, a second report describes how psychological support can mediate the incidence of low birth weight in an urban clinic [18]. This is followed by other observations of psychosocial distress and its impact on iron chelation adherence [19, 20]. In 2001, a systematic review outlines the types of therapeutic services that could apply to thalassaemia patients [8]. In the following decade (up to

2010), following a characterisation of the adult patient [21] and a cross-sectional patient survey [22], a cluster of articles looked at 'psychological burdens' in different patient groups including children and caregivers [23, 24], adolescents [25], and adults [26, 27]. Consequently, several other studies tried to further assess patient reported outcome (PRO) and psychological adaptation of patients and caregivers. Only a single, non-randomised interventional study in 2009 used cognitive behavioural family therapy to try and alter adherence to chelation therapy [28]. These results suggested a wide diversity in the application of psychological support in the clinical effort to manage the patient developmental pathway and their long-term survival associated with iron chelation adherence.

This initial review suggests that 'psychological support' is an undefined response to a clinical need that requires specification. In order to develop a more complete understanding of the component elements of psychological support in thalassaemia, we conducted a comprehensive review of the 1,427 articles identified by a broad search of the 'behavioural and social science research' (BSSR) literature conducted on 18 August 2024 (Figure 1). Our review identified four major groups of articles:

Figure 1. *MEDLINE thalassaemia literature (1970-2024) showing data for core areas of interest (prevention and policy, ICT adherence, QOL, and other BSSR domains). A comprehensive database of the available literature was constructed from a PubMed search (8/18/2024) using EndNote as the search interface. A broad search started with 'all field' search for the term Thalass* that identified 36,584 citations. We then developed a working database of 32,577 items by searching and removing earth science items (thalassio*, seagrass, fish, greenhouse, etc.). A further reduction was used through an abstract search for Sickle Cell (items that were sickle cell disease only or were focused on sickle cell disease were eliminated. The dataset of 30,841 articles is presented by year (solid line). We then searched for key areas of BSSR research. Abbreviations: ICT, iron chelation therapy; QOL, quality of life; BSSR, behavioural and social science research.*



- **Antenatal screening (prevention and policy)** (14% of articles): these articles showed a well-organised response to the problem of introducing antenatal screening in an at-risk population. They illustrate the complexity of creating a comprehensive solution that includes governmental support, legislation, community education, and face-to-face interaction. These reports tend to be post-hoc celebrations of an arduous ad-hoc process (**Grade C**). The efforts to replicate this success have yielded some articles that identify specific complications associated with community demographic diversity in migrant populations. These articles identify the challenges this presents for implementing interventional strategies (**Grade C**). Cases of 'successful' implementation of antenatal screening appeared in small, homogenous environments [29]. However, the approaches were challenged when implemented in complex heterogeneous populations. A few articles have addressed elements of this complex environment [30] by looking at the economics of iron chelation therapy [31, 32], clinical outreach to the communities of affected patients [33], and addressing the needs of culturally different patients [34] (**Grade C**).
- **Iron chelation therapy** (14% of articles): most investigations either tried to measure adherence [35, 36] or assess patient experience with treatment [31, 37-39] (**Grade B**). Over half of these articles appeared during a brief 10-year span that cantered on the introduction of new oral chelators. They began to lay a scientific foundation for assessing PRO as one step in understanding the patient's iron chelation practices [4, 6, 27, 37, 40]. These reports tend to have a very good scientific basis (**Grade A**), however, because they are associated with other kinds of clinical investigations, they do not attempt to solve observed behavioural or social problems.
- **QOL** (38% of articles): prior to 2010, articles invoked 'QOL' as a goal and rarely defined or measured what was meant by patient QOL (**Grade C**). From 2010 onwards, there was an effort to formally measure patient QOL using standardised BSSR instruments [40]. The effort to measure PROs in 2010 using the widely used Medical Outcomes Study (MOS) 36-Item Short Form Survey (SF36, version 2) [41] instrument was part of a broader effort to assess TDT patients under the US National Institute of Health's Thalassemia Clinical Research Network (TCRN) [40]. This effort demonstrated that PRO data could be gathered under broader health research mandates [40, 42-45]. This is followed by 377 articles that highlight SF36 and a range of standardised QOL instruments in publications between 2010 and 2024 (**Grade C**), including WHOQOL, Pediatric Quality of Life Inventory (PedsQL), Patient-Reported Outcomes Measurement Information System (PROMIS), the EuroQol (EQ), Functional Assessment of Chronic Illness Therapy (FACIT), and Dartmouth Primary Care Cooperative Information Chart System (Dartmouth COOP). Other thalassaemia-specific questionnaires have also been developed through observational studies (e.g., Transfusion-dependent QoL Questionnaire [TranQol] [46], Specific Thalassemia Quality of Life Instrument [STQOLI] [47], Thalassaemia Adult Life Index [ThALI] [48]) or in the context of clinical trials (e.g., Non-Transfusion-Dependent Thalassemia-Patient Reported Outcomes [NTDT-PRO] [49-51]). Beyond use in iron chelation therapy trials, SF-36 and other PRO measures of functional status are now also commonly used in disease-modifying or curative therapy trials targeting anaemia and transfusion burden [52-55]. The ability to use the published data to begin to characterise the QOL of TDT patients is an important first step to improve patient responses to their care. PRO QOL data are only useful if linked to clinical data. For example, explaining the approximate 10-point difference in physical function scores between patients from high-income countries and patients from low- and middle-income countries could be instructive. This may be

attributed to transfusion goals. For instance, if the transfusion target haemoglobin is >12 g/dL, we would expect a significant difference from sites where the transfusion goal haemoglobin level is 'somewhere close' to 10 g/dL or where transfusion is not offered to patients until their haemoglobin level is <4 g/dL. In order to make QOL data useful, there are two primary barriers that need to be addressed in the use of standardised QOL instruments. First is the need to address the differences between paediatric and adult reports, where paediatric assessments are most commonly done through parental proxies. Second is overcoming different regional perceptions that limit data presentation [56]. Addressing these two problems requires informed BSSR.

- **BSSR** (35% of articles): this is a residual grouping of articles that addressed a range of research interests ranging from psychological problems (14%), psychosocial issues (13%), and related concerns (5%). Psychological problems include a wide-ranging cross-national recognition that patients with thalassaemia (and their caregivers) are vulnerable to psychiatric problems [23, 27, 57-66]. These articles look at the psychological problems within the context of coping, burden of chronic disease and treatment, and patient adherence to iron chelation therapy while implying that failure to adhere reflects a patient's psychological or cognitive makeup. The early reports tended to be at the level of clinical descriptive studies (**Grade C**). More recent studies have shifted to identifying the neuropsychological investigation of cognitive deficits, which have often been linked to chronic anaemia and brain iron content [67-75] (**Grade B**). Others suggest that the observed psychological problems could be a function of the level and nature of support services [76] and not simply a problem of patient's psychological makeup. Social support (20%) addresses the range of needs of families and patients. The effort to specify these needs began with Ratip's work to develop disease-specific standardised assessments of these domains [77-81]. Other efforts include interventional studies that target changes in institutional organisation practices [82], patient group sessions [82, 83], family therapy [28], interventions with coping strategies, emotional intelligence, mindfulness, and cognitive behavioural therapy [84-87], and patient chelation camps [88]. While these reports suggest some success, most lack a robust analytical assessment (**Grade C**). Most studies appear to address issues identified in a single clinic. There needs to be a concerted effort to take the most promising efforts and move them to multicentre, hypothesis-driven studies.

As a whole, this literature suggests that patients with thalassaemia and their caregivers are faced with many distinct psychological and social challenges which have an impact on emotional functioning and may result in increased vulnerability to symptoms of psychiatric illnesses, such as depression and anxiety. Psychological support appears to be a loose reference to a broad mix of organisational responses to clinical needs, and not a coherent interventional strategy. Thus, there are limited well-developed interventional trials aimed at providing psychological support to improve overall well-being of patients and their families. The few, small interventional studies are mostly descriptive reports of clinic-level responses. They lack analytical rigor because standardised BSSR instruments were not used. Recent reports show an effort to develop the needed rigorous, scientific, understanding of PROs within the context of longitudinal cohorts or iron chelation and other disease-modifying/curative therapy trials. Most are designed to inform a clinical response to underlying clinical problems. These efforts should establish the analytical foundation for future interventional studies in psychological support, with systematic analyses of their impact on patients. This would require further investment in adult care facilities, and devising strategies that

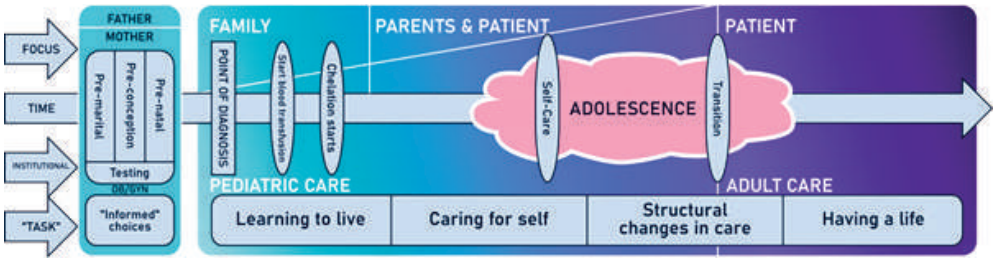
can be equally relevant to and affordable in non-Western countries. It should also be empowered by longitudinal collection of both QOL and clinical endpoint data, to support the design of appropriate interventions.

In the meantime, we can only offer recommendations for psychological support based on existing best practices and research done with other disease populations. It is also important to recognise that TDT is no longer a disease of childhood – and if the patient receives the appropriate care, they can live a relatively normal life. Today, for patients, thalassaemia has to be viewed as a life-long chronic problem.

3. PRACTICAL CONSIDERATIONS

Recommendations for standards of care for psychological support require a practical organisational model. As the specific challenges associated with being a patient with TDT differ throughout development, a clinical pathway model that starts with the functional landmarks that define the patient and family experience is helpful (diagnosis-treatment). There are two modifiers to the clinical experience. Firstly, because TDT is a chronic disease presenting shortly after birth, the natural growth from infant to adult will shape how patients learn to live with their disease. In the early stages, patients are dependent on their family caregivers, and as they develop, the patient must learn to successfully manage their own care. The second is the institutional organisation of clinical medicine. Paediatrics typically works with the patient and their family while adult medicine works with the individual patient – although regional variations in practice could exist. This situation complicates any psychological support recommendations. At each of the landmarks along the pathway (e.g., point of diagnosis, start of transfusion, initiation of chelation, transition into more self-care in adolescence, and transition to adult care), patients and families may be more vulnerable to experiencing psychological sequelae associated with disease management and developmental challenges commonly experienced during that period of time. Our model of the 'clinical pathway of TDT' is illustrated in Figure 2.

Figure 2. *Clinical pathway of transfusion-dependent β-thalassaemia. Abbreviations: OB/GYN, obstetrics and gynaecology.*



Over the past 10 years, the understanding of TDT has shifted. In high-income countries with well-developed western medical systems, the disease is no longer viewed as a 'disease of childhood' but rather as a chronic disorder that starts at birth. Because TDT is well-described, with a known set of symptoms and clinical response, long-term patient survival requires a knowledgeable and experienced clinical team. The recent availability of gene therapy only accentuates the importance of a knowledgeable and experienced clinical team, because event-free survival requires a healthy patient.

Treatment in TDT is a two-step process. The first step is blood transfusions that begins shortly after birth. Blood transfusions are needed because of ineffective erythropoiesis and low haemoglobin, which impacts oxygen circulation. The lack of oxygen makes a patient lethargic leading to a failure to thrive and can lead to long-term end-organ damage. The impact of receiving blood with functioning haemoglobin is immediately felt by patients. However, blood cells have an estimated 30-day life span, so a TDT patient needs regular or chronic blood transfusions. Regular transfusion therapy will lead to iron buildup, that can lead to tissue iron overload, organ damage, and early patient death. Iron chelation therapy works slowly and requires daily drug intake, where the patient does not receive an immediate response. In TDT, patients are usually compliant with regular transfusions but compliance with iron chelation therapy is uneven (see Chapters 2 and 3).

Patient adherence to their iron chelation therapy is the single-most important action that improves long-term outcome in TDT. Advances in iron monitoring and chelation have changed the expectation of patient long-term survival [89, 90], and published studies suggest adherence rates for oral iron chelation as high as 90% [91], more typically 63% [15] and as low as 42% [92]. These data appear to be higher than the previous generation of iron chelation that required subcutaneous drug infusions for 8-12 hours 5-7 days a week. A recent Cochrane review of 'Interventions for improving adherence to iron chelation therapy in people with sickle cell disease or thalassaemia' suggests that the higher adherence reports with iron chelation could be an artefact of trial participation. Critically, the 'certainty of evidence' for adherence interventions was 'low to very low across all' outcomes in the review, and only four trials 'measured quality of life with validated instruments' and they 'reported no difference in QOL' [93].

Regardless of any flaws in the published data, the introduction of new interventions has changed the life-expectancy for TDT patients. For patients who need lifesaving regular blood transfusions, it is incumbent on practitioners to be alert to changes in the patient's iron chelation therapy adherence practices. Change needs to be evaluated at several levels.

First, evaluation needs to start with measurable organic changes (for further details see Chapter 3):

- Evaluating trends in iron loading: such as serial increases in serum ferritin levels in conjunction with increasing indications of organ involvement (using magnetic resonance imaging [MRI] reports).
- Other clinical tests that can be a useful indicator of iron burden: for example, measuring labile plasma iron (LPI), even though this is largely a research tool, can indicate iron burden [94].
- Bioassays of chelator drug levels can provide an 'objective' measure of actual chelator use, but the tests are expensive for routine application.

Second, patient reported adherence needs to be evaluated. Self-reports of adherence are inherently unreliable since patient subjective responses are highly variable and dependent on the patient-

provider relationship. Most reported assessment methods are described in research studies but can be adjusted for clinical use:

- Basic standardised questionnaires have been devised to assess patient medication adherence. The most well-known is probably the Morisky Medication Adherence Scale (MMAS) [95].
- Pill counts or empty vial counts (in the case of deferoxamine) have been practiced with variable results. A refinement on this approach is to use the medication possession ratio (MPR), which is the proportion of days of medication supply a given drug in a particular time period, divided by the number of days in the time period [96].
- Medication monitoring devices have also been introduced. These include 'digital pillboxes' that have a 'smart' vial cap that electronically records the date and time of bottle opening. This is an expensive tool, and studies have failed to show superiority to self-reporting questionnaires [97-99].
- Text messaging and mobile phone app interventions are another approach but there is a need to establish the long-term benefit.

Third, and most important, the patient needs to be engaged in developing solutions for their practices. A paternalistic (top down) approach, where the physician tells a patient that they have to do their therapy, will not work. As with parenting, where every parent knows that telling an adolescent or young adult what they have to do rarely yields the appropriate long-term action. With TDT, clinicians need to recognise that patients live their thalassaemia every day, many (especially older patients) know more about the disease burden than clinical practitioners. The lived experience frames their understanding and ultimately their actions. Finding effective long-term solutions requires building the patient/provider relationship. The relationship is built around trust and respect and allows for creative solutions aimed at helping a patient develop a coherent response to a long-term problem:

- Taking time to listen to concerns and providing support is the most essential action supporting patient adherence [100].
- Increasing patient knowledge and understanding of their condition results in patient empowerment which can encourage adherence to prescribed treatment [101]. As patients age, discussing laboratory results such as serum ferritin or MRI results, can help engage patients. These discussions not only enable patients to understand why the tests are needed, but they also inform them about their overall health trajectory.
- Utilising and trusting the input of the clinical team (other physicians, nurses, and allied healthcare workers). The team gets to know the patient in different ways. Their insights into the social factors that shape adherence practice (illustrated, in part, in Table 1) provide a more comprehensive understanding of the patient's circumstance.

Table 1. *Common barriers to adherence and suggested interventions.*

Barrier	Intervention
Lack of understanding concerning regimen implementation or importance	Provide age-appropriate education
Forgetfulness	Set alarms; use visual reminders
Inconvenience	Work with the medical team to change the regimen to fit better with the patient's lifestyle
Inconsistent schedule of medication	Implement a reminder system (e.g., alarms); use a self-monitoring chart to document completion of tasks
Side effects of treatment	Find ways to help minimise or cope with the side effects
Length of treatment	Help the patient find activities to do to during the treatment
Complicated regimen	Simplify regimen (with medical team); create a self-monitoring chart to document completion of each task
Social Stigma	Engage the patient in treatment aimed at improving self-esteem; encourage the patient to meet other individuals with similar medical conditions
Poor supervision	Increase adult involvement and monitoring
Cultural or religious beliefs	Work with family to understand their beliefs and when possible, adapt treatments to fit within their values
Psychiatric illness	Treat underlying psychiatric illness
Family psychopathology	Work with caretakers to create an environment that is conducive to encouraging adherence (e.g., decreased conflict, increased communication)
Poor social support	Help the patient/family find resources within their community; encourage the patient to meet other individuals with similar medical conditions

Whatever other method is used to engage patient adherence, it is important to remember that β-thalassaemia is no longer a 'disease of childhood', it is a lifelong chronic disease. Patient adherence requires constant, periodic assessment. This lifelong relationship has negative side effects on the provider and the provider team. The amount of time required to build and sustain the relationship, can result in staff fatigue, burnout, and information overload. Often, the time commitment to build and sustain the patient-provider relationship runs against the artificial limits imposed by the healthcare reimbursement system. Developing a workable solution requires a non-combative and

collaborative 'monitoring' and 'quality control' approach by healthcare administrators [102]. Devising approaches that help maintain a close relationship with the adult patient is essential to ensure compliance.

Systematic studies to examine different intervention modalities that may help patients and families effectively cope with the particular challenges inherent at each time point are needed. These should address how early 'upstream' familial experiences impact 'downstream' patient adherence adaptations and long-term survival. As most of the existing literature consists of descriptive reports and cross-sectional studies, the following practical recommendations are largely based on what we know from our clinical work and/or research with other chronic illnesses.

3.1. Point of diagnosis

Parents will undergo a series of changes after their child is diagnosed with β-thalassaemia (shock, denial, sadness/anger, adaptation, reorganisation) [103]. One of their most important immediate concerns is getting reliable information [104]. Learning the additional tasks associated with caring for a child with β-thalassaemia can be overwhelming to the parent and lead to psychological distress [19, 21, 83]. Importantly, if parents feel overwhelmed with caring for their child, effective management of the illness may become compromised [105]. To minimise these feelings, effective psychological support of parents around the time of diagnosis should include:

- Providing necessary information about β-thalassaemia. This needs to be repeated several times for full comprehension. Efforts at providing leaflets or links [QR codes] to more information may improve the understanding.
- Opportunities to ask questions and share concerns.
- Occasions to meet parents of older children diagnosed with β-thalassaemia, as this can help increase social support and confidence, while decreasing feelings of helplessness and hopelessness.
- Enabling families to interact with each other may help them realise that they are not alone in this journey.
- Access to psychosocial clinicians who can help them explore and manage their feelings of loss in a constructive manner.

It is especially important to help parents accept and learn to effectively cope with their child's chronic medical condition at this early stage. This is because parental behaviours and attitudes throughout development will lay the groundwork for how children will cope with their condition. Parents who demonstrate healthy coping and understand that a well-managed patient who adheres to his/her therapy can live a successful life [106] will help their children to learn to make β-thalassaemia a piece of who they are, rather than what defines them. Introducing the family to an appropriately experienced family with a child who has β-thalassaemia can be a helpful learning experience for parents of young children.

3.2. Start of blood transfusion

The best ways to provide psychological support aimed at helping children effectively cope with invasive medical procedures has been widely studied [107-112]. It is essential to help parents and children engage in effective coping strategies as soon as developmentally appropriate, as the experience of distress during a medical procedure has been found to be predictive of distress during future procedures [113].

Starting at a very young age, children often look to their parents for signals on how they should react in anxiety-provoking, novel situations. In one study, parent behaviour during an invasive medical procedure accounted for 53% of the variance in child distress behaviour [113]. Providing information about the procedure prior to the actual procedure and giving the parent a job to do (e.g., distract the child), is likely to reduce parental anxiety, with positive indirect benefits for their children. However, if parents are not able to remain calm in front of their children during procedures such as blood transfusion, it is helpful for clinicians to give parents' 'permission' to leave the room and instead consider including the presence of another supportive adult.

Specific coping strategies aimed directly at the child have been particularly useful in helping children cope effectively with invasive medical procedures. In a meta-analysis of psychological interventions for needle-related procedural distress in children and adolescents, 'distraction' was found to be one of the most efficacious coping techniques [114]. In fact, a recent study conducted with patients with thalassaemia found that bubble blowing during an injection helped reduce anxiety [115]. Importantly, distraction techniques should be adapted to the child's interest and age/developmental level keeping in mind their socioeconomic background. It is particularly useful to encourage parents who engage in excessive reassurance to instead focus on distracting their child, as reassurance often amplifies fear and distress [116], likely due to refocusing the child's attention on the fearful and painful aspects of the situation.

As children get older, they may ask for more information about transfusions or other invasive medical procedures (e.g., MRI). Fostering trust, reducing uncertainty, correcting misconceptions, enhancing the belief in their ability to cope with a procedure, and minimising distress are some of the potential benefits of providing advance information about a procedure to a child [117, 118]. Effective pre-procedural information should include:

- A developmentally appropriate verbal explanation of what the child will see, hear, feel, and smell during, before, and after the procedure.
- Minimally threatening, but accurate information, as children who are given information that turns out not to be true (e.g., "you will not feel a thing" when in fact the child is liable to experience some pain), are more likely to distrust their parents and/or the medical team, which may affect future interactions.
- Use of visual aids (e.g., books, pictures, models, videos).
- Where possible, medical play can help young children understand their therapeutic regimen [119-122].
- Time for the child to ask questions.

In ideal clinical settings, a child-life specialist should be included on the treatment team. Child life specialists are health care professionals who have the specific training to understand the impact TDT has on children and can help them and their families navigate the complex processes needed to not only understand their treatment, but provide them with the necessary life-long skills not just to manage their clinical needs but to live with their disease.

3.3. Initiation of iron chelation

Parents need to be provided with support and guidance about choosing which type of chelation is best for their child. For example, although oral chelators are associated with less distress and better QOL in older patients, due to specific developmental characteristics of very young children (e.g., transient food preference, oppositional behaviour, unpredictability), this may not be true for some children in this age group [123]. Parents of very young children need to be encouraged to carefully consider their chelation options and determine which option best fits with their own capacities and their child's personality characteristics.

When starting iron chelation therapy, parents should be encouraged to develop consistent routines around medication taking. Developing predictable routines around a child's medical regimen makes these tasks part of the typical daily schedule, thereby fostering good adherence by minimising several of the problems often associated with adherence difficulties (e.g., forgetting, conflicts about when to take the medication) [123, 124].

Behavioural interventions which include increased monitoring and incentives for meeting goals have been shown to be successful at improving adherence in patients with thalassaemia [125]. The use of incentives may be particularly useful for paediatric patients who do not yet understand the intrinsic value of adhering to an undesirable medical regimen. These may include verbal praise, stickers or small toys or other incentives earned either immediately or over time, for cooperating with daily chelation. By pairing a positive outcome (e.g., sticker, toy) with an aversive stimulus (chelation) the child develops a positive association with the aversive event, increasing the likelihood that the child will perform the behaviours again in the future.

At various times along the clinical pathway, patients may struggle with chelation adherence [4]. When this occurs, it is essential to identify *why* the patient is having difficulty following the prescribed plan. Interventions that do not consider the specific barrier to adherence will have limited success (see Table 1 for common barriers and suggested interventions). In general, effective interventions aimed at improving adherence usually:

- Incorporate behavioural or multiple strategies.
- Include patients (and parents) in the development.
- Start from where the patient is at, gradually increasing goals.
- Revise over time.

Older patients often make remarks such as "I am tired of doing this", "I don't want to", or "I need a break". Many often take the break. This 'voluntary non-adherence' is difficult to assess, and many treating physicians do not attempt to study this in their patients. The 5 Whys analysis is a problem-solving technique that involves repeatedly asking "why" to identify the root cause of an issue and may help in such situations. It is important to continually monitor every patient's adherence to iron chelation therapy.

3.4. Adolescence and transition to increased self-care

Adolescence is a time when adherence to daily medical regimens often declines [43]. Frequently, the transition of responsibility from the parent to the adolescent occurs before the patient is emotionally ready, resulting in poor adherence. Because adolescents are vulnerable to having their decision-making driven by their desire to be independent and to fit-in with peers, parents need to

continue to play an active role in monitoring adolescents self-care. Shared responsibility between the patient and caregiver has been found to be associated with better adherence [4, 88].

Also, to avoid the negative consequences of abrupt shifts in responsibility, the transition of responsibility needs to:

- Occur gradually over time, starting when children are young (e.g., help gathering supplies) and increasing their involvement as they mature.
- Teach older patients how to take over responsibility for often-overlooked tasks, such as ordering supplies and making medical appointments.

3.5. Transition to adult care

One reason why adherence may be lowest in young adults [43] is because of insufficient psychosocial support as patients transition from paediatric to adult medical providers. Often the transition to adult care providers happens in an abrupt manner, leaving the patient unprepared for the shift to adult medicine [126, 127]. Discussions about transitions should occur well in advance of the actual transfer in care and should include an exploration of the patients concerns and how they will prepare for and manage the changes inherent in moving from a paediatric to an adult medicine clinic [128]. This plan can result in fewer cases being 'lost to follow-up' [129]. A well-coordinated transition plan includes:

- Long-term plan that starts with orienting pre-adolescent patients for the change.
- Annual assessments of a patient's preparation for transition.
- Multiple opportunities to orient the patient to an adult clinic, adult care practices, and the adult care system.
- Multiple overlapping visits with paediatric and adult haematologists.

4. PAIN IN THALASSAEMIA PATIENTS

Pain is a concern for patients with β-thalassaemia. However, reports of pain in TDT are relatively recent [130]. Pain appears to increase in intensity and frequency with age [42, 131]. Because pain appears more frequently in older TDT patients, it presents a new problem.

The presence of pain usually indicates an underlying organic source, but in β-thalassaemia there is a lack of understanding of pain symptoms and its organic cause. The presence of pain in non-thalassaemic adults is associated with decreased social function and increased depression [132-136]. For most patients who report the presence of pain, their response and choice of clinical intervention solutions is predicated on how they understand the source of the pain. If chronic pain symptoms are not modulated, patients are at risk of choosing non-clinically managed pharmacological solutions.

Because there is a lack of understanding of the relationship between TDT pain symptoms and its organic cause, effective pain management strategies do not exist. If thalassaemia presents with pain symptoms, an effective treatment plan requires conducting a careful clinical evaluation to identify the underlying cause. Depending on the site of pain, there should be a consideration of clinical studies such as: bone mineral density assessment, MRI/X-ray evaluation to assess

musculoskeletal deformities or recent injuries, and an evaluation of average haemoglobin levels (since medullary expansion and extramedullary haematopoiesis can cause bone pain) to elucidate the sources of pain [130, 137, 138]. Bone pain is also a common side effect of some novel disease-modifying agents such as luspatercept [139]. Based on these findings, a pain management strategy can be developed. Pain management plans can include pharmacological and nonpharmacological interventions; however, because most thalassaemia pain appears to be chronic, strong consideration should be given to nonpharmacological interventions such as:

- Physical Therapy
- Acupuncture/acupressure
- Massage
- Deep breathing
- Guided imagery
- Progressive muscle relaxation
- Hypnosis
- Biofeedback
- Mindfulness training

Until the underlying organic studies of thalassaemia pain are conducted, clinicians need to routinely ask if the TDT patient has the presence of pain. If they report on having the presence of pain, then patient pain symptoms management strategies need to be routinely assessed. Clinicians should encourage patients with pain to engage in a variety of empirically validated [140-143] cognitive and behavioural coping strategies which have been shown to successfully help patients manage their pain and distress through learning how to regulate their emotional and physical responses to pain.

5. IMPORTANCE OF SOCIAL SUPPORT THROUGHOUT DEVELOPMENT

As social support has been found to play an important role in the psychological functioning of children and their families [144], starting from an early age, patients and their families would benefit from:

- Deciding how to present information about the patient's medical condition to friends and family.
- Learning about the harmful effects (e.g., feelings of shame) of keeping thalassaemia a secret.
- Relying on existing friend, family, religious, and community supports.
- Meeting other patients and families with chronic medical conditions through attending camps, events sponsored by specific illness foundations, or one-to-one meeting facilitated by a clinician.

There are many other opportunities to provide psychological support. For example, as children with TDT frequently miss school for medical appointments and transfusions [26], which can negatively impact school functioning [145], parents should be encouraged to educate the school about their child's condition and to set up plans which support the child when he/she

needs to miss school. Further, patients with TDT may be vulnerable to experiencing cognitive deficits [67-75, 146-149]. If there are concerns from parents or the school, it may be valuable for patients to participate in neuropsychological testing to assess for any concerns and provide recommendations that could help support the patients learning potential.

6. PSYCHOSOCIAL SUPPORT THROUGHOUT THE LIFESPAN AS PART OF STANDARD CARE

As social and emotional concerns can occur anywhere along the lifespan and such concerns can have an impact on the patient's QOL and physical health, opportunities for regular psychological support should be part of the treatment plan of all patients with TDT. This is best accomplished through a comprehensive team approach used by thalassaemia centres of excellence found in high-income countries. The teams include multidisciplinary allied health specialists including skilled nursing staff, psychologists, social workers, and other specialists such as family or child-life specialists. These health providers are better equipped to assess any social, emotional, or cognitive concerns and intervene with additional support when necessary. If the team members regularly meet with patients and their families, it allows multiple communication pathways to be built. They would be able to actively investigate and address key patient concerns such as body image issues, fear of co-morbidities, and fear early death. An effective team that is centred on improving patient response to therapy can take advantage of the multi-dimensional understanding of a patient, to create interventions that work for the patient. This is especially important when the team tries to build a novel adherence strategy for a patient. The different information pathways are also important for monitoring commonly seen symptoms of anxiety and depression and determining when those symptoms become psychiatric disorders that need early psychotherapy or psychiatry referrals, in order to prevent long-term health consequences. Importantly, through the inclusion of psychological support as part of the standard care, some of the stigmatisation associated with seeing a therapist may be removed.

Because TDT has become a chronic condition that begins at birth, there is a need for a comprehensive care framework that offers multidisciplinary care that spans both paediatrics and adult care. Because costs are a challenge, there are generally distinct choices of where national investments are made. Paediatric multidisciplinary teams are common in the US model of healthcare delivery for TDT. The US paediatric centres exert a significant level of energy to provide young patients with the tools they need to survive into adulthood. As young adults, they are then transitioned to systems where comprehensive care is not available where patients become 'lost to follow up'. In the UK, healthcare resources are aimed at supporting the workforce. Multidisciplinary teams are accessible for adults, but non-existent in paediatrics. Centres in the UK have to commit a high level of psychological resources aimed at providing the TDT patient with the necessary tools that allow them to learn to become a functional (working) adult. For many, learning the skills are often challenging, because they were not introduced to them at an earlier age. Efforts to bridge the UK and US models can be found, but finite resources usually require rationing access to multidisciplinary centres of excellence, or by forcing a limit on the services provided by these 'centres. This situation is clearly suboptimal because TDT is no longer a disease of childhood, but a clearly described life-long chronic condition that begins at birth. In low- and middle-income countries, providing regular blood transfusion and iron chelation are themselves a challenge, and

hence the focus on psychosocial support takes a back seat. Innovative, out-of-box thinking incorporating team-building and social integration skills with the help of volunteers can sometimes lead to improvements and merits further experimentation [150]. We need a new model for healthcare resource allocation and delivery that can address the new reality of TDT, a genetic condition that impacts patients across the lifespan.

The system needs to address the distinct needs of the impact TDT has at the paediatric level (where patient care is mediated by families), and care provided to individual adults. In paediatrics, we know that emotional, financial, and psychological burdens caused by frequent visits and hospitalisations for blood transfusions can overwhelm a family leading to family conflicts, relationship breakdowns, and harmful coping mechanisms like substance and alcohol addiction. Early intervention and familial support can help families prepare for helping their child become an independent and productive societal member. In high-income countries, well-supported TDT patients are attending colleges and universities and training to become highly productive professionals in the workforce. Patient success requires institutional support from a comprehensive system of coordinated care that includes not only members of the immediate clinical team but also allied health team that includes psychological and social support professionals. Long-term patient support also extends beyond the clinic to support groups that provide patients and their families with emotional and practical support. While these groups can collaborate with treatment centres, they also offer a helping hand during critical times, making both patients and their families feel less isolated and better prepared to manage the demands of TDT care.

Despite thalassaemia being the most common genetic disorder, it remains a rare condition. Successful management requires a public health team that oversees care across a geographic region. Public health authorities can act as a bridge between the family and the treatment centre, ensuring that the family's medical and psychosocial needs are consistently met. This requires regular communication and collaboration with the treatment centre to ensure that families are properly guided, their concerns are addressed, and they can access additional resources when necessary. The integration of psychosocial support into thalassaemia care, allows families to navigate the complex challenges of managing the disease, that reduce the risk of family disputes, emotional distress, and maladaptive coping mechanisms and will enable TDT patients to become productive members of society.

KEY POINTS AND RECOMMENDATIONS

1. Despite a general lack of large scale, randomised, controlled trial evidence conducted with patients with thalassaemia, there are innumerable cohorts or case-controlled analytical studies to suggest that psychological well-being has an impact on adherence to treatment for chronic diseases in general (**Grade B**).

2. In thalassaemia, the published reports to demonstrate this linkage are mainly descriptive studies (**Grade C**). A systematic review would suggest that recent efforts are more towards **Grade B** investigations (usually ancillary studies attached to robust controlled trials in other clinical areas). However, the lack of uniform instruments and standardised measurements weakens this assessment. The findings to date suggest that:

   - Psychological well-being impacts on adherence to iron chelation therapy in transfusion-dependent β-thalassaemia (TDT) and hence on survival (**Grade C**).
   - Patients with TDT are vulnerable to experiencing psychological challenges (**Grade C**).
   - Patient reported outcomes (PROs) show that oral chelation therapy has a beneficial impact, relative to parenteral chelation (**Grade B**).
   - Neuropsychological investigation of cognitive deficits shows that there are clear intellectual and psychopathological problems in a very limited number of TDT patients (**Grade B**).
   - Benefits of psychological support have been suggested using a variety of approaches (**Grade C**) which include:
     - Targeting changes in institutional organisation practices
     - Patient group sessions
     - Family therapy
     - Interventions with coping strategies, emotional intelligence, mindfulness, and cognitive behavioural therapy
     - Patient chelation camps
   - In all chronic illness, continuity of comprehensive care across the lifespan is essential for long-term, beneficial health outcome (**Grade A**).
   - Institutional organisational support for multidisciplinary teams is essential (**Grade A**).
   - There is a growing body of evidence that highlights the problems associated with transition from paediatric care to adult medicine in inherited chronic disease (**Grade B**).
   - Rare and neglected diseases complicate resource allocation models and lead to notable health disparities (**Grade A**).
   - In thalassaemia, these problems are known and reports from expert committees recommend addressing them, but there are no formal studies of the problems, much less any standardised evidence (**Grade C**).

3. While **Grade A** and **Grade B** evidence for psychological support in thalassaemia is scarce, experience in several large thalassaemia centres strongly suggests that psychological well-being is key to adherence and to outcome:

   - Expert psychological support has to be available at all centres specialising in TDT care (**Grade C**).

- Additional psychosocial support provided by trained specialists (e.g., social workers or family or child health specialists) should be tailored to the patient's age:
  - Children (in general **Grade A**, TDT **Grade C**)
  - Adolescents - transition (in general **Grade B**, TDT **Grade C**)
  - Older adults - pain issues (in general, **Grade A**, TDT **Grade C**)

4. Funding for clinical psychological support services could be more widely achieved if well-designed, multi-centre, interventional studies using common standardised instruments were undertaken to evaluate the benefit of psychological and psychosocial support to treatment adherence. The use of established behavioural and social science approaches in such studies needs to identify the active components of 'psychological support' that are most applicable to patients with TDT.

REFERENCES

1. Falvo DR. Medical and psychosocial aspects of chronic illness and disability. 5th ed. Burlington, MA: Jones & Bartlett Learning, 2014.
2. Lubkin IM, Larsen PD. Chronic illness: impact and intervention. Burlington, MA: Jones & Bartlett Learning, 2013.
3. Beratis S. Noncompliance with iron chelation therapy in patients with beta thalassaemia. J Psychosom Res 1989;33(6):739-745.
4. Evangeli M, Mughal K, Porter JB. Which psychosocial factors are related to chelation adherence in thalassemia? A systematic review. Hemoglobin 2010;34(3):305-321.
5. Panitz D. Difficulties encountered by adolescent thalassemia patients. In: Sugar M, ed. Trauma and adolescence. Madison, CT: nternational Universities Press, Inc, 1999.
6. Porter JB, Evangeli M, El-Beshlawy A. Challenges of adherence and persistence with iron chelation therapy. Int J Hematol 2011;94(5):453-460.
7. Rikos N, Giannadaki G-K, Spontidaki A, Tzagkaraki M, Linardakis M. Health status, anxiety, depression, and quality of life of patients with thalassemia. J Public Health 2021;29:1313-1320.
8. Anie KA, Massaglia P. Psychological therapies for thalassaemia. Cochrane Database Syst Rev 2001;(3):CD002890.
9. Anie KA, Massaglia P. Psychological therapies for thalassaemia. Cochrane Database Syst Rev 2014;2014(3):CD002890.
10. Borgna-Pignatti C, Rugolotto S, De Stefano P, Zhao H, Cappellini MD, Del Vecchio GC, Romeo MA, Forni GL, Gamberini MR, Ghilardi R, Piga A, Cnaan A. Survival and complications in patients with thalassemia major treated with transfusion and deferoxamine. Haematologica 2004;89(10):1187-1193.
11. Efthimiadis GK, Hassapopoulou HP, Tsikaderis DD, Karvounis HI, Giannakoulas GA, Parharidis GE, Louridas GE. Survival in thalassaemia major patients. Circ J 2006;70(8):1037-1042.
12. Modell B, Khan M, Darlison M. Survival in beta-thalassaemia major in the UK: data from the UK Thalassaemia Register. Lancet 2000;355(9220):2051-2052.
13. Olivieri NF, Nathan DG, MacMillan JH, Wayne AS, Liu PP, McGee A, Martin M, Koren G, Cohen AR. Survival in medically treated patients with homozygous beta-thalassemia. N Engl J Med 1994;331(9):574-578.
14. Porter JB, Davis BA. Monitoring chelation therapy to achieve optimal outcome in the treatment of thalassaemia. Best Pract Res Clin Haematol 2002;15(2):329-368.
15. Vekeman F, Sasane M, Cheng WY, Ramanakumar AV, Fortier J, Qiu Y, Duh MS, Paley C, Adams-Graves P. Adherence to iron chelation therapy and associated healthcare resource utilization and costs in Medicaid patients with sickle cell disease and thalassemia. J Med Econ 2016;19(3):292-303.
16. Vosper J, Evangeli M, Porter JB, Shah F. Psychological Factors Associated with Episodic Chelation Adherence in Thalassemia. Hemoglobin 2018;42(1):30-36.
17. Colombino G, Bonzano L. [Assistance for the thalassemic child in a child care center]. Minerva Med 1985;76(6):209-211.
18. Edwards CH, Knight EM, Johnson AA, Oyemade UJ, Cole OJ, Laryea H, Westney OE, Westney LS. Multiple factors as mediators of the reduced incidence of low birth weight in an urban clinic population. J Nutr 1994;124(6 Suppl):927S-935S.
19. Politis C. The psychosocial impact of chronic illness. Ann N Y Acad Sci 1998;850:349-354.
20. Aydin B, Yaprak I, Akarsu D, Okten N, Ulgen M. Psychosocial aspects and psychiatric disorders in children with thalassemia major. Acta Paediatr Jpn 1997;39(3):354-357.
21. Galanello R. A thalassemic child becomes adult. Rev Clin Exp Hematol 2003;7(1):4-21.
22. Vardaki MA, Philalithis AE, Vlachonikolis I. Factors associated with the attitudes and expectations of patients suffering from beta-thalassaemia: a cross-sectional study. Scand J Caring Sci 2004;18(2):177-187.

23. Aydinok Y, Erermis S, Bukusoglu N, Yilmaz D, Solak U. Psychosocial implications of Thalassemia Major. Pediatr Int 2005;47(1):84-89.
24. Prasomsuk S, Jetsrisuparp A, Ratanasiri T, Ratanasiri A. Lived experiences of mothers caring for children with thalassemia major in Thailand. J Spec Pediatr Nurs 2007;12(1):13-23.
25. Roy T, Chatterjee SC. The experiences of adolescents with thalassemia in West Bengal, India. Qual Health Res 2007;17(1):85-93.
26. Gharaibeh H, Amarneh BH, Zamzam SZ. The psychological burden of patients with beta thalassemia major in Syria. Pediatr Int 2009;51(5):630-636.
27. Mednick L, Yu S, Trachtenberg F, Xu Y, Kleinert DA, Giardina PJ, Kwiatkowski JL, Foote D, Thayalasuthan V, Porter JB, Thompson AA, Schilling L, Quinn CT, Neufeld EJ, Yamashita R, Thalassemia Clinical Research N. Symptoms of depression and anxiety in patients with thalassemia: prevalence and correlates in the thalassemia longitudinal cohort. Am J Hematol 2010;85(10):802-805.
28. Mazzone L, Battaglia L, Andreozzi F, Romeo MA, Mazzone D. Emotional impact in beta-thalassaemia major children following cognitive-behavioural family therapy and quality of life of caregiving mothers. Clin Pract Epidemiol Ment Health 2009;5:5.
29. Angastiniotis M, Petrou M, Loukopoulos D, Modell B, Farmakis D, Englezos P, Eleftheriou A. The Prevention of Thalassemia Revisited: A Historical and Ethical Perspective by the Thalassemia International Federation. Hemoglobin 2021;45(1):5-12.
30. Vichinsky EP, MacKlin EA, Waye JS, Lorey F, Olivieri NF. Changes in the epidemiology of thalassemia in North America: a new minority disease. Pediatrics 2005;116(6):e818-825.
31. Payne KA, Desrosiers MP, Caro JJ, Baladi JF, Lordan N, Proskorovsky I, Ishak K, Rofail D. Clinical and economic burden of infused iron chelation therapy in the United States. Transfusion 2007;47(10):1820-1829.
32. Riewpaiboon A, Nuchprayoon I, Torcharus K, Indaratna K, Thavorncharoensap M, Ubol BO. Economic burden of beta-thalassemia/Hb E and beta-thalassemia major in Thai children. BMC Res Notes 2010;3:29.
33. Choy J, Foote D, Bojanowski J, Yamashita R, Vichinsky E. Outreach strategies for Southeast Asian communities: experience, practice, and suggestions for approaching Southeast Asian immigrant and refugee communities to provide thalassemia education and trait testing. J Pediatr Hematol Oncol 2000;22(6):588-592.
34. Banerjee AT, Watt L, Gulati S, Sung L, Dix D, Klassen R, Klassen AF. Cultural beliefs and coping strategies related to childhood cancer: the perceptions of South Asian immigrant parents in Canada. J Pediatr Oncol Nurs 2011;28(3):169-178.
35. Matsui D, Hermann C, Klein J, Berkovitch M, Olivieri N, Koren G. Critical comparison of novel and existing methods of compliance assessment during a clinical trial of an oral iron chelator. J Clin Pharmacol 1994;34(9):944-949.
36. Lee WJ, Mohd Tahir NA, Chun GY, Li SC. The impact of chelation compliance in health outcome and health related quality of life in thalassaemia patients: a systematic review. Health Qual Life Outcomes 2024;22(1):14.
37. Porter J, Bowden DK, Economou M, Troncy J, Ganser A, Habr D, Martin N, Gater A, Rofail D, Abetz-Webb L, Lau H, Cappellini MD. Health-Related Quality of Life, Treatment Satisfaction, Adherence and Persistence in beta-Thalassemia and Myelodysplastic Syndrome Patients with Iron Overload Receiving Deferasirox: Results from the EPIC Clinical Trial. Anemia 2012;2012:297641.
38. Taher A, Al Jefri A, Elalfy MS, Al Zir K, Daar S, Rofail D, Baladi JF, Habr D, Kriemler-Krahn U, El-Beshlawy A. Improved treatment satisfaction and convenience with deferasirox in iron-overloaded patients with beta-Thalassemia: Results from the ESCALATOR Trial. Acta Haematol 2010;123(4):220-225.
39. Taher AT, Origa R, Perrotta S, Kouraklis A, Ruffo GB, Kattamis A, Goh AS, Huang V, Zia A, Herranz RM, Porter JB. Patient-reported outcomes from a randomized phase II study of the deferasirox film-coated tablet in patients with transfusion-dependent anemias. Health Qual Life Outcomes 2018;16(1):216.

40. Sobota A, Yamashita R, Xu Y, Trachtenberg F, Kohlbry P, Kleinert DA, Giardina PJ, Kwiatkowski JL, Foote D, Thayalasuthan V, Porter JB, Thompson AA, Schilling L, Quinn CT, Neufeld EJ, Thalassemia Clinical Research N. Quality of life in thalassemia: a comparison of SF-36 results from the thalassemia longitudinal cohort to reported literature and the US norms. Am J Hematol 2011;86(1):92-95.
41. Jenkinson C, Coulter A, Wright L. Short form 36 (SF36) health survey questionnaire: normative data for adults of working age. BMJ 1993;306(6890):1437-1440.
42. Haines D, Martin M, Carson S, Oliveros O, Green S, Coates T, Eile J, Schilling L, Dinu B, Mendoza T, Gerstenberger E, Trachtenberg F, Vichinsky E, Thalassemia Clinical Research N. Pain in thalassaemia: the effects of age on pain frequency and severity. Br J Haematol 2013;160(5):680-687.
43. Trachtenberg F, Vichinsky E, Haines D, Pakbaz Z, Mednick L, Sobota A, Kwiatkowski J, Thompson AA, Porter J, Coates T, Giardina PJ, Olivieri N, Yamashita R, Neufeld EJ, Thalassemia Clinical Research N. Iron chelation adherence to deferoxamine and deferasirox in thalassemia. Am J Hematol 2011;86(5):433-436.
44. Trachtenberg FL, Martin M, Green S, Oliveros O, Carson S, Gerstenberger E, Allen R, Eile J, Haines D. Use of electronic data collection to assess pain in thalassaemia: a feasibility study. Int J Palliat Nurs 2012;18(9):441-445.
45. Trachtenberg FL, Mednick L, Kwiatkowski JL, Neufeld EJ, Haines D, Pakbaz Z, Thompson AA, Quinn CT, Grady R, Sobota A, Olivieri N, Horne R, Yamashita R, Thalassemia Clinical Research N. Beliefs about chelation among thalassemia patients. Health Qual Life Outcomes 2012;10:148.
46. Klaassen R, Alibhai S, Kirby Allen M, Moreau K, Merelles-Pulcini M, Forgie M, Blanchette V, Buckstein R, Odame I, Quirt I, Yee K. Introducing the TranQol: a new disease-specific quality of life measure for children and adults with thalassemia major. J Blood Disord Transfus 2013;4(4):1000150.
47. Lyrakos GN, Vini D, Aslani H, Drosou-Servou M. Psychometric properties of the Specific Thalassemia Quality of Life Instrument for adults. Patient Prefer Adherence 2012;6:477-497.
48. Kantaris X, Shevlin M, Porter J, Myers L. Development of the Thalassaemia Adult Life Index (ThALI). Health Qual Life Outcomes 2020;18(1):180.
49. Taher AT, Musallam KM, Viprakasit V, Kattamis A, Lord-Bessen J, Yucel A, Guo S, Pelligra CG, Shields AL, Shetty JK, Glassberg MB, Bueno LM, Cappellini MD. Identifying thresholds for meaningful improvements in NTDT-PRO scores to support conclusions about treatment benefit in clinical studies of patients with non-transfusion-dependent beta-thalassaemia: analysis of pooled data from a phase 2, double-blind, placebo-controlled, randomised trial. BMJ Open 2024;14(11):e085234.
50. Taher AT, Musallam KM, Viprakasit V, Kattamis A, Lord-Bessen J, Yucel A, Guo S, Pelligra C, Shields AL, Shetty JK, Miteva D, Bueno LM, Cappellini MD. Psychometric evaluation of the NTDT-PRO questionnaire for assessing symptoms in patients with non-transfusion-dependent beta-thalassaemia. BMJ Open 2023;13(3):e066683.
51. Taher A, Cappellini MD, Viprakasit V, Sutcharitchan P, Mahmoud D, Laadem A, Khan A, Gwaltney C, Harding G, Attie K, Zhang X, Zou J, Pariseau J, Henry Hu X, Kattamis A. Validation of a patient-reported outcomes symptom measure for patients with nontransfusion-dependent thalassemia (NTDT-PRO((c))). Am J Hematol 2019;94(2):177-183.
52. Cappellini MD, Taher AT, Piga A, Shah F, Voskaridou E, Viprakasit V, Porter JB, Hermine O, Neufeld EJ, Thompson AA, Tang D, Yucel A, Lord-Bessen J, Yu P, Guo S, Shetty JK, Miteva D, Zinger T, Backstrom JT, Oliva EN. Health-related quality of life in patients with beta-thalassemia: Data from the phase 3 BELIEVE trial of luspatercept. Eur J Haematol 2023;111(1):113-124.
53. Badawy SM, Beg U, Liem RI, Chaudhury S, Thompson AA. A systematic review of quality of life in sickle cell disease and thalassemia after stem cell transplant or gene therapy. Blood Adv 2021;5(2):570-583.
54. Locatelli F, Lang P, Wall D, Meisel R, Corbacioglu S, Li AM, de la Fuente J, Shah AJ, Carpenter B, Kwiatkowski JL, Mapara M, Liem RI, Cappellini MD, Algeri M, Kattamis A, Sheth S, Grupp S, Handgretinger R, Kohli P, Shi D, Ross L, Bobruff Y, Simard C, Zhang L, Morrow PK, Hobbs WE, Frangoul H, Group CT-S. Exagamglogene Autotemcel for Transfusion-Dependent beta-Thalassemia. N Engl J Med 2024;390(18):1663-1676.

55. Cappellini MD, Sheth S, Taher AT, Al-Samkari H, Antmen AB, Beneitez D, Cannas G, Coates TD, Czapla L, Dahlin JL, Estepp JH, Feenstra E, Georgiev P, Gheuens S, Gilroy KS, Glenthøj A, Musallam K, Osman K, Porter JB, Shao H, Uhlig K, Van Beers EJ, Viprakasit V, Kuo KHM, Kattamis A. ENERGIZE-T: A Global, Phase 3, Double-Blind, Randomized, Placebo-Controlled Study of Mitapivat in Adults with Transfusion-Dependent Alpha- or Beta-Thalassemia. Blood 2024;144(Suppl 1):409-411.
56. Kohlbry P, Al-Karmi B, Yamashita R. Quality-of-life of patients living with thalassaemia in the West Bank and Gaza. East Mediterr Health J 2023;29(6):425-435.
57. Cakaloz B, Cakaloz I, Polat A, Inan M, Oguzhanoglu NK. Psychopathology in thalassemia major. Pediatr Int 2009;51(6):825-828.
58. Pradhan PV, Shah H, Rao P, Ashturkar D, Ghaisas P. Psychopathology and self-esteem in chronic illness. Indian J Pediatr 2003;70(2):135-138.
59. Clemente C, Tsiantis J, Sadowski H, Lee C, Baharaki S, Ba G, Kolvin I, Taylor B. Psychopathology in children from families with blood disorders: a cross-national study. Eur Child Adolesc Psychiatry 2002;11(4):151-161.
60. Saini A, Chandra J, Goswami U, Singh V, Dutta AK. Case control study of psychosocial morbidity in beta thalassemia major. J Pediatr 2007;150(5):516-520.
61. Shaligram D, Girimaji SC, Chaturvedi SK. Quality of life issues in caregivers of youngsters with thalassemia. Indian J Pediatr 2007;74(3):275-278.
62. Shaligram D, Girimaji SC, Chaturvedi SK. Psychological problems and quality of life in children with thalassemia. Indian J Pediatr 2007;74(8):727-730.
63. Khoury B, Musallam KM, Abi-Habib R, Bazzi L, Ward ZA, Succar J, Halawi R, Hankir A, Koussa S, Taher AT. Prevalence of depression and anxiety in adult patients with beta-thalassemia major and intermedia. Int J Psychiatry Med 2012;44(4):291-303.
64. Yetim Sahin A, Kandemir I, Dag H, Turkkan E, Tugrul Aksakal M, Sahin M, Bas F, Karakas Z. Assessing Psychological Disorders in Turkish Adolescents with Transfusion-Dependent Thalassemia. Children (Basel) 2024;11(7).
65. Othman A, Abdul Ghani MSA, Taib F, Mohamad N. Psychological distress and coping strategies among the caretakers of children with transfusion-dependent thalassemia. Front Pediatr 2022;10:941202.
66. Angane AY, Kadam KS, Ghorpade GS, Unnithan VB. Who will guard the guardians? Cross-sectional study on prevalence of psychiatric morbidity, quality of life, and coping skills in caregivers of children with thalassemia major. J Postgrad Med 2022;68(2):72-77.
67. Armstrong FD. Thalassemia and learning: Neurocognitive functioning in children. Ann N Y Acad Sci 2005;1054:283-289.
68. Duman O, Arayici S, Fettahoglu C, Eryilmaz N, Ozkaynak S, Yesilipek A, Hazar V. Neurocognitive function in patients with beta-thalassemia major. Pediatr Int 2011;53(4):519-523.
69. Monastero R, Monastero G, Ciaccio C, Padovani A, Camarda R. Cognitive deficits in beta-thalassemia major. Acta Neurol Scand 2000;102(3):162-168.
70. Zafeiriou DI, Prengler M, Gombakis N, Kouskouras K, Economou M, Kardoulas A, Tsantali C, Dimitriadis A, Athanasiou M, Kirkham FJ. Central nervous system abnormalities in asymptomatic young patients with Sbeta-thalassemia. Ann Neurol 2004;55(6):835-839.
71. Bizri M, Koleilat R, Akiki N, Dergham R, Mihailescu AM, Bou-Fakhredin R, Musallam KM, Taher AT. Quality of life, mood disorders, and cognitive impairment in adults with beta-thalassemia. Blood Rev 2024;65:101181.
72. Bu M, Deng X, Zhang Y, Chen SW, Jiang M, Chen BT. Brain iron content and cognitive function in patients with beta-thalassemia. Ther Adv Hematol 2023;14:20406207231167050.
73. Daar S, Al Saadoon M, Wali Y, Al Mujaini R, Al Rahbi S, Chan MF, Al-Siyabi A, Alansary N, Mahadevan S, Al-Adawi S. Cognitive Function in Adults with Beta-Thalassemia Major in Oman: A Pilot Study. Oman Med J 2021;36(6):e322.

74. Choi S, Leahy RM, Wood JC. Lower white matter volume in beta-thalassemia associated with anemia and cognitive performance. Am J Hematol 2020;95(6):E144-E146.
75. Tartaglione I, Manara R, Caiazza M, Carafa PA, Caserta V, Ferrantino T, Granato I, Ippolito N, Maietta C, Oliveto T, Casale M, Di Concilio R, Ciancio A, De Michele E, Russo C, Elefante A, Ponticorvo S, Russo AG, Femina G, Canna A, Ermani M, Cirillo M, Esposito F, Centanni A, Gritti P, Perrotta S. Brain functional impairment in beta-thalassaemia: the cognitive profile in Italian neurologically asymptomatic adult patients in comparison to the reported literature. Br J Haematol 2019;186(4):592-607.
76. Angastiniotis M. The adolescent thalassemic. The complicant rebel. Minerva Pediatr 2002;54(6):511-515.
77. Canatan D, Ratip S, Kaptan S, Cosan R. Psychosocial burden of beta-thalassaemia major in Antalya, south Turkey. Soc Sci Med 2003;56(4):815-819.
78. Ratip S, Skuse D, Porter J, Wonke B, Yardumian A, Modell B. Psychosocial and clinical burden of thalassaemia intermedia and its implications for prenatal diagnosis. Arch Dis Child 1995;72(5):408-412.
79. Ratip S, Modell B. Psychological and sociological aspects of the thalassemias. Semin Hematol 1996;33(1):53-65.
80. Tsiantis J, Dragonas T, Richardson C, Anastasopoulos D, Masera G, Spinetta J. Psychosocial problems and adjustment of children with beta-thalassemia and their families. Eur Child Adolesc Psychiatry 1996;5(4):193-203.
81. Zani B, Di Palma A, Vullo C. Psychosocial aspects of chronic illness in adolescents with thalassaemia major. J Adolesc 1995;18(4):387-402.
82. Marovic S, Snyders F. Addressing complexities of medical noncompliance in serious childhood illness: Collaborating at the interface of providers, families, and health care systems. Fam Syst Health 2008;26(3):237-249.
83. Yamashita RC, Foote D, Weissman L. Patient cultures: thalassemia service delivery and patient compliance. Ann N Y Acad Sci 1998;850:521-522.
84. Ahmadian B, Ramezani M, Badiei Z, Vaghee S. The effect of applying emotional intelligence components on coping strategies in adolescents with beta-thalassemia major: a randomized clinical trial. BMC Pediatr 2024;24(1):591.
85. Ghonchehpour A, Forouzi MA, Dehghan M, Ahmadi A, Okou G, Tirgari B. The effect of mindfulness-based stress reduction on rejection sensitivity and resilience in patients with thalassemia: a randomized controlled trial. BMC Psychiatry 2023;23(1):281.
86. Mohamadian F, Bagheri M, Hashemi MS, Komeili Sani H. The Effects of Cognitive Behavioral Therapy on Depression and Anxiety among Patients with Thalassemia: a Randomized Controlled Trial. J Caring Sci 2018;7(4):219-224.
87. Hashemi F, Naderi Darshori A, Sharif F, Karimi M, Zare N. Effect of coping strategies training on its use by thalassemia major adolescents: a randomized controlled clinical trial. Int J Community Based Nurs Midwifery 2015;3(1):67-74.
88. Treadwell MJ, Weissman L. Improving adherence with deferoxamine regimens for patients receiving chronic transfusion therapy. Semin Hematol 2001;38(1 Suppl 1):77-84.
89. Forni GL, Gianesin B, Musallam KM, Longo F, Rosso R, Lisi R, Gamberini MR, Pinto VM, Graziadei G, Vitucci A, Bonetti F, Musto P, Piga A, Cappellini MD, Borgna-Pignatti C, Webthal p. Overall and complication-free survival in a large cohort of patients with beta-thalassemia major followed over 50 years. Am J Hematol 2023;98(3):381-387.
90. Modell B, Khan M, Darlison M, Westwood MA, Ingram D, Pennell DJ. Improved survival of thalassaemia major in the UK and relation to T2* cardiovascular magnetic resonance. J Cardiovasc Magn Reson 2008;10:42.
91. Pinto VM, Balocco M, Quintino S, Bacigalupo L, Gianesin B, Rizzi M, Malago R, De Franceschi L, Forni GL. Daily alternating deferasirox and deferiprone therapy successfully controls iron accumulation in untreatable transfusion-dependent thalassemia patients. Am J Hematol 2018;93(10):E338-E340.
92. Rofail D, Abetz L, Viala M, Gait C, Baladi JF, Payne K. Satisfaction and adherence in patients with iron overload receiving iron chelation therapy as assessed by a newly developed patient instrument. Value Health 2009;12(1):109-117.

93. Geneen LJ, Doree C, Estcourt LJ. Interventions for improving adherence to iron chelation therapy in people with sickle cell disease or thalassaemia. Cochrane Database Syst Rev 2023;3(3):CD012349.
94. Steinberg-Shemer O, Yacobovich J, Cohen M, Cabantchik IZ, Tamary H. Labile plasma iron as an indicator of patient adherence to iron chelation treatment. Blood Cells Mol Dis 2018;71:1-4.
95. Moon SJ, Lee WY, Hwang JS, Hong YP, Morisky DE. Accuracy of a screening tool for medication adherence: A systematic review and meta-analysis of the Morisky Medication Adherence Scale-8. PLoS One 2017;12(11):e0187139.
96. Sperber CM, Samarasinghe SR, Lomax GP. An upper and lower bound of the Medication Possession Ratio. Patient Prefer Adherence 2017;11:1469-1478.
97. Badawy SM, Morrone K, Thompson A, Palermo TM. Computer and mobile technology interventions to promote medication adherence and disease management in people with thalassemia. Cochrane Database Syst Rev 2019;6(6):CD012900.
98. Monnette A, Zhang Y, Shao H, Shi L. Concordance of Adherence Measurement Using Self-Reported Adherence Questionnaires and Medication Monitoring Devices: An Updated Review. Pharmacoeconomics 2018;36(1):17-27.
99. Shi L, Liu J, Fonseca V, Walker P, Kalsekar A, Pawaskar M. Correlation between adherence rates measured by MEMS and self-reported questionnaires: a meta-analysis. Health Qual Life Outcomes 2010;8:99.
100. Zolnierek KB, Dimatteo MR. Physician communication and patient adherence to treatment: a meta-analysis. Med Care 2009;47(8):826-834.
101. Nafradi L, Nakamoto K, Schulz PJ. Is patient empowerment the key to promote adherence? A systematic review of the relationship between self-efficacy, health locus of control and medication adherence. PLoS One 2017;12(10):e0186458.
102. Wagner EH, Austin BT, Davis C, Hindmarsh M, Schaefer J, Bonomi A. Improving chronic illness care: translating evidence into action. Health Aff (Millwood) 2001;20(6):64-78.
103. Drotar D, Baskiewicz A, Irvin N, Kennell J, Klaus M. The adaptation of parents to the birth of an infant with a congenital malformation: a hypothetical model. Pediatrics 1975;56(5):710-717.
104. Starke M, Moller A. Parents' needs for knowledge concerning the medical diagnosis of their children. J Child Health Care 2002;6(4):245-257.
105. Otsuki M, Eakin MN, Arceneaux LL, Rand CS, Butz AM, Riekert KA. Prospective relationship between maternal depressive symptoms and asthma morbidity among inner-city African American children. J Pediatr Psychol 2010;35(7):758-767.
106. Pakbaz Z, Treadwell M, Kim HY, Trachtenberg F, Parmar N, Kwiatkowski JL, Cunningham MJ, Martin M, Sweeters N, Neufeld EJ, Giardina PJ, Olivieri N, Yamashita RC, Vichinsky E. Education and employment status of children and adults with thalassemia in North America. Pediatr Blood Cancer 2010;55(4):678-683.
107. Brown RT. Cognitive aspects of chronic illness in children. New York: Guilford Press, 1999.
108. Brown RT, Rickel AU, Daly BP. Chronic illness in children and adolescents. Toronto: Hogrefe & Huber, 2007.
109. Edwards M, Titman P. Promoting Psychological Well-Being in Children with Acute and Chronic Illness: Jessica Kingsley Publishers, 2010.
110. Hayman LL, Mahon MM, Turner JR. Chronic Illness In Children: An Evidence-Based Approach. New York: Springer Publishing Company, 2002.
111. Hymovich DP, Hagopian GA. Chronic illness in children and adults: A psychosocial approach. 1st ed: Saunders, 1991.
112. Thompson RH. The handbook of child life: a guide for pediatric psychosocial care: Charles C Thomas Pub Ltd, 2009.

113. Frank NC, Blount RL, Smith AJ, Manimala MR, Martin JK. Parent and staff behavior, previous child medical experience, and maternal anxiety as they relate to child procedural distress and coping. J Pediatr Psychol 1995;20(3):277-289.
114. Uman LS, Chambers CT, McGrath PJ, Kisely S. A systematic review of randomized controlled trials examining psychological interventions for needle-related procedural pain and distress in children and adolescents: an abbreviated cochrane review. J Pediatr Psychol 2008;33(8):842-854.
115. Bagherain S, Borhani F, Abbaszadeh A, Ranjbar H, Solaimani F. [The effect of distraction by bubble-making on the procedural anxiety of injection in Thalassemic school- age children in Kerman Thalasemia center]. J Nurs Midwifery Q -Shaheed Beheshti Univ Med Sci Health Serv 2012;22(76):52-59.
116. Manimala MR, Blount RL, Cohen LL. The effects of parental reassurance versus distraction on child distress and coping during immunizations. Child Health Care 2000;29(3):161-177.
117. Jaaniste T, Hayes B, Baeyer CV. Providing Children With Information About Forthcoming Medical Procedures: A Review and Synthesis. Clin Psychol Sci Pract 2007;14(2):124-143.
118. Jipson JL, Melamed BG. New Approaches on the Horizon: Comments on Jaaniste, Hayes, and von Baeyer's "Providing Children With Information About Forthcoming Medical Procedures: A Review and Synthesis". Clin Psychol Sci Pract 2007;14(2):149-156.
119. Bandstra NF, Skinner L, Leblanc C, Chambers CT, Hollon EC, Brennan D, Beaver C. The role of child life in pediatric pain management: a survey of child life specialists. J Pain 2008;9(4):320-329.
120. Bolig R, Yolton KA, Nissen HL. Medical play and preparation: questions and issues. Child Health Care 1991;20(4):225-229.
121. Burns-Nader ES. The effects of medical play on reducing fear, anxiety, and procedure distress in school-aged children going to visit the doctor: University of Alabama Libraries; 2011.
122. McCue K. Medical Play: An Expanded Perspective. Child Health Care 1998;16(3):157-161.
123. Fiese BH, Wamboldt FS, Anbar RD. Family asthma management routines: connections to medical adherence and quality of life. J Pediatr 2005;146(2):171-176.
124. Rand CS. Non-adherence with asthma therapy: more than just forgetting. J Pediatr 2005;146(2):157-159.
125. Koch DA, Giardina PJ, Ryan M, MacQueen M, Hilgartner MW. Behavioral contracting to improve adherence in patients with thalassemia. J Pediatr Nurs 1993;8(2):106-111.
126. Bryant R, Walsh T. Transition of the chronically ill youth with hemoglobinopathy to adult health care: an integrative review of the literature. J Pediatr Health Care 2009;23(1):37-48.
127. Chandra J, Sahi PK. Transition of Care for Patients with Thalassemia. Indian J Pediatr 2023;90(12):1227-1231.
128. Morse LA, Sawh RN. Transfer of Care for People with Severe Forms of Thalassemia: Learning from Past Experiences to Create a Transition Plan. J Pediatr Nurs 2021;61:378-386.
129. Allemang B, Allan K, Johnson C, Cheong M, Cheung P, Odame I, Ward R, Williams S, Mukerji G, Kuo KHM. Impact of a transition program with navigator on loss to follow-up, medication adherence, and appointment attendance in hemoglobinopathies. Pediatr Blood Cancer 2019;66(8):e27781.
130. Piga A. Impact of bone disease and pain in thalassemia. Hematology Am Soc Hematol Educ Program 2017;2017(1):272-277.
131. Trachtenberg F, Foote D, Martin M, Carson S, Coates T, Beams O, Vega O, Merelles-Pulcini M, Giardina PJ, Kleinert DA, Kwiatkowski J, Thompson AA, Neufeld EJ, Schilling L, Thayalasuthan V, Pakbaz Z, Yamashita R, Thalassemia Clinical Research N. Pain as an emergent issue in thalassemia. Am J Hematol 2010;85(5):367-370.
132. Avlund K, Rantanen T, Schroll M. Factors underlying tiredness in older adults. Aging Clin Exp Res 2007;19(1):16-25.
133. Burckhardt CS. The impact of arthritis on quality of life. Nurs Res 1985;34(1):11-16.

134. Dunn KM, Croft PR. Epidemiology and natural history of low back pain. Eura Medicophys 2004;40(1):9-13.
135. Garber CE, Greaney ML, Riebe D, Nigg CR, Burbank PA, Clark PG. Physical and mental health-related correlates of physical function in community dwelling older adults: a cross sectional study. BMC Geriatr 2010;10:6.
136. Ozminkowski RJ, Musich S, Bottone FG, Jr., Hawkins K, Bai M, Unutzer J, Hommer CE, Migliori RJ, Yeh CS. The burden of depressive symptoms and various chronic conditions and health concerns on the quality of life among those with Medicare Supplement Insurance. Int J Geriatr Psychiatry 2012;27(9):948-958.
137. Lal A. Assessment and treatment of pain in thalassemia. Ann N Y Acad Sci 2016;1368(1):65-72.
138. Haidar R, Musallam KM, Taher AT. Bone disease and skeletal complications in patients with beta thalassemia major. Bone 2011;48(3):425-432.
139. Cappellini MD, Viprakasit V, Taher AT, Georgiev P, Kuo KHM, Coates T, Voskaridou E, Liew HK, Pazgal-Kobrowski I, Forni GL, Perrotta S, Khelif A, Lal A, Kattamis A, Vlachaki E, Origa R, Aydinok Y, Bejaoui M, Ho PJ, Chew LP, Bee PC, Lim SM, Lu MY, Tantiworawit A, Ganeva P, Gercheva L, Shah F, Neufeld EJ, Thompson A, Laadem A, Shetty JK, Zou J, Zhang J, Miteva D, Zinger T, Linde PG, Sherman ML, Hermine O, Porter J, Piga A, Investigators B. A Phase 3 Trial of Luspatercept in Patients with Transfusion-Dependent beta-Thalassemia. N Engl J Med 2020;382(13):1219-1231.
140. Eccleston C, Palermo TM, Williams AC, Lewandowski A, Morley S. Psychological therapies for the management of chronic and recurrent pain in children and adolescents. Cochrane Database Syst Rev 2009;(2):CD003968.
141. Palermo TM, Eccleston C, Lewandowski AS, de CWAC, Morley S. Randomized controlled trials of psychological therapies for management of chronic pain in children and adolescents: an updated meta-analytic review. Pain 2010;148(3):387-397.
142. Shega JW, Andrew M, Hemmerich J, Cagney KA, Ersek M, Weiner DK, Dale W. The relationship of pain and cognitive impairment with social vulnerability--an analysis of the Canadian Study of Health and Aging. Pain Med 2012;13(2):190-197.
143. Fisher E, Law E, Dudeney J, Palermo TM, Stewart G, Eccleston C. Psychological therapies for the management of chronic and recurrent pain in children and adolescents. Cochrane Database Syst Rev 2018;9(9):CD003968.
144. Lewandowski A, Drotar D. The relationship between parent-reported social support and adherence to medical treatment in families of adolescents with type 1 diabetes. J Pediatr Psychol 2007;32(4):427-436.
145. Thavorncharoensap M, Torcharus K, Nuchprayoon I, Riewpaiboon A, Indaratna K, Ubol BO. Factors affecting health-related quality of life in Thai children with thalassemia. BMC Blood Disord 2010;10:1.
146. Economou M, Zafeiriou DI, Kontopoulos E, Gompakis N, Koussi A, Perifanis V, Athanassiou-Metaxa M. Neurophysiologic and intellectual evaluation of beta-thalassemia patients. Brain Dev 2006;28(1):14-18.
147. Lucke T, Pfister S, Durken M. Neurodevelopmental outcome and haematological course of a long-time survivor with homozygous alpha-thalassaemia: case report and review of the literature. Acta Paediatr 2005;94(9):1330-1333.
148. Nevruz O, Ulas U, Cetin T, Kutukcu Y, Kurekci A. Cognitive dysfunction in beta-thalassemia minor. Am J Hematol 2007;82(3):203-207.
149. Zafeiriou DI, Economou M, Athanasiou-Metaxa M. Neurological complications in beta-thalassemia. Brain Dev 2006;28(8):477-481.
150. Chakrabarti P. Life beyond blood disorders. Lancet Haematol 2023;10(5):e320-e321.

13 MULTIDISCIPLINARY CARE AND REFERENCE CENTRES IN ADDRESSING HAEMOGLOBIN DISORDERS

Authors: **Androulla Eleftheriou[1], Gian Luca Forni[2,3], Farzana Sayani[4,5]**

[1.] Thalassaemia International Federation (TIF), Nicosia, Cyprus
[2.] For Anemia Foundation ETS, Genoa, Italy
[3.] Hematology Unit, IRCCS Giannina Gaslini, Genoa, Italy
[4.] Red Blood Cell Disorders Program, Division of Medical Oncology and Haematology, Department of Medicine, University Health Network, University of Toronto, Toronto, Canada
[5.] Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

Competing interest: None to disclose.

Acknowledgements: The authors would like to thank Dr. Michael Angastiniotis (Thalassaemia International Federation, Nicosia, Cyprus) and Dr. Elpidoforos S. Soteriades (Open University of Cyprus, Latsia, Cyprus) for their review and input on this Chapter.

INTRODUCTION

The lifelong and multiorgan nature of transfusion-dependent β-thalassaemia (TDT) are well reflected in the very content of these guidelines. As patients advance in years, the basic needs in blood transfusion and iron chelation, even if and when provided appropriately and in accordance with international standards, gradually become inadequate to sustain life, maintain wellbeing, and achieve social integration. For this reason, specialists in several medical disciplines including but not confined to heart, liver, and endocrine, are called upon to contribute by monitoring and offering proactive management of iron toxicity and organ dysfunction in their field of expertise. These considerations and needs lead to significant and multiple challenges in the organisation of integrated services so that the best possible conditions for patient care are achieved.

Historically, efforts to recognise and address the unique and evolving needs in managing β-thalassaemia and other haemoglobin disorders have led to a variety of tailored approaches (Table 1). Initially, these strategies focused on regions and countries with high disease prevalence, promoting specialised, targeted services for patients. Over time, collaboration across countries strengthened these efforts, advancing the effectiveness of care for haemoglobin disorders worldwide.

Provision of specialised care extended beyond transfusion and iron chelation, even before they began to be recognised as an absolute necessity, in those centres and countries where:

1. There was high disease prevalence amongst the population whether in the indigenous (e.g., Cyprus, Greece, Italy) or in the well-established and integrated migrant population (e.g. United Kingdom [UK], France).
2. The scientific community and the national health competent authorities recognised the extent of the medical, public health, social, and economic repercussions of the disorders in the absence of any effective national policies for their prevention and appropriate management.

Table 1. *Different approaches for organising care in β-thalassaemia.*

Approach
1. Within haematology departments: development of red blood cell (non-malignant) clinics separate from malignant haematology services
2. Dedicated space within paediatric units where patients remained well into their adolescence and in many cases into their adulthood
3. Dedicated space within haematology units (amongst malignant haematology/oncology patients) or transfusion centres of hospitals
4. Development of "independent" β-thalassaemia units/centres based on outpatient principle attached to/associated with tertiary level hospitals
5. Within general paediatric or paediatric/oncology or general haematology wards/clinics without any arrangement for a dedicated space or development of specialised services
6. Development of centres run by patient non-governmental organisations in collaboration with pharmacists, the Red Cross, and health care professionals

Under these circumstances, haemoglobinopathies were prioritised on the country's national health agenda. As a result, disease-specific policies and programmes were developed with national coordination and full financial coverage. This approach enabled patients to live longer, age, and enjoy a good quality of life.

As prognosis improved significantly, it became obvious that more was needed to be done to continue addressing patients' evolving needs effectively:

1. **Commitment from governments and competent authorities:** there was a need for continued commitment from governments and relevant authorities to continue strengthening the prevention programmes for these disorders as it was fully recognised that by limiting the number of new annual affected births, more resources could be directed towards enhancing the quality and range of services provided to existing patients to cover both medical and social needs.

2. **Engagement of medical and scientific communities:** it became essential for medical and scientific communities to accumulate knowledge and experience in managing the long-term morbidities patients faced as they aged. Recognising this need, other medical specialties, particularly cardiologists (due to heart-related complications often being a leading cause of morbidity), and endocrinologists initially, hepatologists, psychologists, and other specialists began to come to the forefront of care. This marked the beginning of a multidisciplinary approach to effectively address the comprehensive needs of patients with these disorders. Over time, the effectiveness of this approach became evident, as increased knowledge and collaboration across various medical disciplines led to significant improvements in patient outcomes, increased survival, reduction in the development of morbidity, and achieved social inclusion.

The global β-thalassaemia patient community is extremely grateful to these countries for accumulating valuable data and in bringing medical specialities into their work, which supported the development of the multidisciplinary approach globally; and indeed the first medical

professionals who were involved in this approach constituted the first authors of published work and guidelines of the Thalassaemia International Federation (TIF).

The integration of specialised multidisciplinary care services into the management of patients with TDT brought about the need for specialised, disease-specific training/education of the different medical specialties, along with multiple organisational challenges to ensure appropriate coordination. Where such steps were taken, mainly in those countries that initially demonstrated political commitment to effectively address these disorders and where these, led to successful, meaningful integration of services (including active research activity), 'Reference Centres' began to spring up. The terms 'Reference Centre', 'Centre of Excellence' and 'Expert Centre' are often used interchangeably. TIF is using the term 'Reference Centres' to define those that fulfil the criteria discussed in this Chapter. The European Union (EU) and other bodies may have different interpretations.

The first of such centres were developed in the 1980s in Mediterranean countries including Cyprus, Greece, and Italy, and subsequently in the UK and France; with the latter more in the context of wider national strategies for rare disease. In all cases, at least initially, the centres established in those countries were developed without any structured or specific criteria. The patient care pathways and quality standards that these centres developed through the years became the core and solid basis for setting the criteria in later years for defining the role a Reference Centre for haemoglobin disorders should be fulfilling. Gradually, these first centres were officially, and not only by reputation, assigned by the country's national competent authorities as Reference Centres undergoing regular and professional reviews of their quality standards. Patients with haemoglobin disorders within, and gradually from outside these countries began to be referred to these centres by their treating physicians for consultation, second opinion, or for receiving specialised services that did not exist in their own area, region, or country.

In most countries, particularly in low- and middle-income regions where over 80% of patients with haemoglobin disorders live, several challenges have hindered progress in effectively managing these conditions. These challenges include underdeveloped healthcare and public health infrastructures (such as haematology, primary healthcare, and transfusion services), a lack of universal health and social care coverage, and economic constraints. Additionally, many of these countries prioritise other urgent health issues, including communicable diseases and prevalent non-communicable diseases, which limits resources available for advancing management of haemoglobin disorders.

In some cases, national authorities have encouraged non-governmental organisations, such as the Red Cross and patient advocacy groups, to support efforts in managing haemoglobin disorders, helping to address gaps in the healthcare system. These have evolved throughout the years in collaboration with medical and other healthcare professionals' essential services for patients, although mainly confined to the provision of transfusion services and iron chelation therapies. Certainly, in such setting, the provision of any extended specialised treatment is not possible. Therefore, patients would need to be referred to hospital settings; sadly, in an uncoordinated manner, and to medical professionals who (in the greatest majority) do not have specialised knowledge of the treatment of medical complications related to haemoglobin disorders.

The work of TIF for more than 35 years in over 60 countries across all regions of the world has exposed the naked truth: the development and integration of multidisciplinary care services into the management of patients with these disorders and the promotion and establishment of Reference Centres are to-date components of care that are far from being adopted or implemented. Such components are provided to less than 2% of the patients globally, constituting a severe violation of their rights both as humans and as patients. Such advances can only happen if and when the basic, essential medical care in a centre or across a country has reached appropriate quality levels and this can only happen when the medical and public health infrastructures and quality standards are adequately strengthened. The young age of patients in the great majority of these countries as demonstrated by TIF in its Global Thalassaemia Review [1], a deliverable of the joint work of TIF with the World Health Organisation (WHO), and who commonly do not reach ages beyond twenty or thirty years, confirms the fact that they are still receiving suboptimal basic care.

2. MULTIDISCIPLINARY CARE

Bringing fragmented services together in an organised, collaborative manner and adopting international guidelines and standards of care is indeed very challenging, but it has been demonstrated beyond any doubt that such an effort maximises patients' benefits and facilitates their very cumbersome treatment pathways with timely interventions. The value of such an approach is reflected in the improvement of outcomes [2], including both clinical and social outcomes as demonstrated in some countries; while at the same time, leading to the creation of cost-effective services which are of benefit to both the healthcare system and public health [3].

In many centres, the multidisciplinary approach to β-thalassaemia care is even misunderstood as simply having a specialist to refer to, only when a complication has manifested. On the contrary, the whole concept of multidisciplinary care as an essential component of a Reference Centre relies on the availability of proactive, regular, qualitative, and specialised surveillance and interventions. In this context, different medical specialists should be involved well before the appearance of complications, i.e. through regular involvement early in the patient's life. In a multiorgan disease like TDT, there is no doubt as to the necessity and benefits of this approach and TIF has focused considerable attention on promoting this concept and on creating those tools and advisory groups that could support it.

Since the issue of communication is central to the functioning of the multidisciplinary care team, ensuring its effectiveness is important and this can be achieved through expert leadership and in a number of ways including:

- Joint clinics
- Regular team meetings
- Case conferences
- Sharing of results, along with interpretation and discussion for joint decisions
- Using electronic disease-specific medical records with full access to all members of the team
- Involving patients in the discussions and decisions which concern their lives is critical and every effort should be made by all to establish a well-structured and regular programme of active and meaningful patients' engagement

An example of the structure of an interdisciplinary team for the care of TDT as extracted from some well-established Reference Centres in the UK and EU with successful patient outcomes [4] is presented in Table 2. Many other important healthcare specialists are needed particularly when the centres are treating sickle cell disease patients as well, which is the usual case in most centres across countries. Several French and UK multidisciplinary groups (e.g., UK Forum for Haemoglobin Disorder) now exist and fully dedicated to care of haemoglobin disorders. The Italian Society of Thalassaemia and Haemoglobinopathies (SITE) has also published multiple guides on various aspects of multidisciplinary care and good clinical practice for patients with haemoglobinopathies [5, 6].

Table 2. *Structure of a multidisciplinary team for the care of transfusion-dependent β-thalassaemia.*

Specialty	Description
Haematologist/ paediatrician/internist	The physician in charge of routine care, including monitoring of iron overload. Usually coordinates the multidisciplinary team. Supported by other more junior physicians according to the number of patients.
Specialised nurses	Specially trained and experienced haemoglobinopathy nurses who, apart from routine duties like supervising blood transfusions and triage of patients, because of their closer contact with patients, have a significant role in counselling and psychosocial support.
Cardiologist	With special interest and experience in the cardiac complications of β-thalassaemia. Monitors all patients from childhood, collaborates with the lead physician on any management modifications and takes charge when complications like arrhythmias arise.
Endocrinologist	Monitors all patients from early adolescence for these very common complications of β-thalassaemia. Apart from liaison with the multidisciplinary team, there is collaboration with gynaecologists in the case of infertility and pregnancy. Also, part of the team managing bone disease.
Hepatologist	Liver function from is monitored from an early age by the clinic team, but persisting disturbance of liver function, iron overload, viral hepatitis, and overt hepatic disease (increasingly common in older patients) require a consultation with a liver specialist.
Psychologist/social worker	Essential supportive services and should always be part of the team. Many issues may not be recognised by other physicians or nurses and periodic visits to the psychologist could bring matters to the surface for all patients. Referral should not be restricted to when acute emotional problems arise. Especially where universal health coverage is not available, financial hardship and social isolation can contribute to negative patient outcomes. Support for the healthcare team may also be necessary.

Obstetrician	A member of the collaborative team in intended and ongoing pregnancies. Pre-pregnancy counselling along with the haematologist, endocrinologist, and cardiologist is essential but teamwork during gestation is also necessary for good outcomes.
Oral and dental care specialist	All patients should be routinely monitored for dental and maxillary complications at least yearly.
Radiology	Equipped with magnetic resonance imaging for tissue iron quantification of the heart and liver with calibrated software to ensure accuracy and reliability of iron measurement.
Bone marrow transplantation	Specialised unit with experience in haematopoietic transplantation for haemoglobin disorders. Pre- and post-transplant close follow up with the core team.

3. PAEDIATRIC-TO-ADULT TRANSITION

A significant challenge recognised globally throughout the years is the paediatric to adult transition. In chronic lifelong diseases, including β-thalassaemia, there is a need for continuity of comprehensive multidisciplinary care across the lifespan for best long-term outcomes. The period between 12-25 years of age is a time of significant change developmentally and socially. Young adults face challenges of managing their disease, while exploring new opportunities that come with becoming an adult including relationships, education, and work. As young adults begin to navigate these changes, it is important that they are supported and empowered to continue to manage their disease. Often as seen in many chronic illnesses including sickle cell disease, the move from a paediatric to an adult setting can be stressful and is associated with medication non-adherence, loss to follow up, poor clinical outcomes, reduced quality of life, and increased mortality [7].

In TDT, very little has been published on transition and a very small percentage (less than half) of patients participate in a dedicated transition programme between age 12-25 years [8]. TIF's experience and knowledge on this matter confirm that a significant percentage of the patient population across country members are in fact still being looked after by paediatricians in paediatric centres/clinics beyond the age 12-25 years. Most individuals' experiences would be termed 'transfer', which is the simple movement of the patient and medical records from one healthcare provider and facility to another. 'Transition' instead is a planned process that supports adolescents and young adults with chronic health conditions to move from a child-centred to adult-oriented healthcare providers and facilities [9]. The main goal of transition is to empower and enable young adults to manage their own health care and access health services effectively. An organised transition process also allows young adults to prepare for the transition, transfer, and finally integrate into adult-centred health systems.

Barriers to transition may result from patient factors, health system factors, and disease-specific factors [10]. When asked, adult patients usually highlight the lack of β-thalassaemia knowledge by adult providers, fewer β-thalassaemia-specific resources, limited transfusion facilities, and poor communication between provider and patient, amongst others [11, 12]. There is much variability in paediatric to adult transition when it exists, with no standardised guidelines or health care plans, programme organisation, resources, and accessibility across and within countries.

One approach to transition is the GOT TRANSITION®'s Six Core Elements of Health Care TransitionTM, which defines the key components of a structured paediatric to adult transition [9]. This approach identifies six core elements of healthcare transition: i) developing, discussing, and sharing a transition policy, ii) tracking and monitoring transition progress, iii) assessing self-care skills and transition readiness, iv) transition planning, v) transfer and integration with adult-centred care, and vi) transition completion and care with adult physicians. The key to transition is to begin early around 12 years of age, with frequent opportunities for patient and family skill building and preparedness assessment. Adolescents and young adults should be provided with age-appropriate disease information, support and encouragement on taking responsibility of their own care. Before the physical move to the adult centre occurs, individuals should be provided with opportunities to meet with the adult team to help alleviate any stressors. The patient's records and transition treatment plan should be reviewed with the patient and the adult care team to facilitate information sharing and clarification of questions. The lack of adult expertise and poor communication are key barriers to a successful transition. Hence, investment in the development of an adult β-thalassaemia programme together with a healthcare transition plan is important to successfully help patients maintain their quality of life and best clinical outcomes into adulthood.

Therefore, when establishing Reference Centres, it is essential to address the needs of paediatric, adolescent, and adult patient populations, including a well-structured transition plan aligned with the national strategy for managing and monitoring β-thalassaemia and related conditions. If creating separate units or clinics for paediatric and adult patients, the adult unit must also accommodate the unique needs of paediatric and young adult patients transitioning into adult care. This approach may be particularly beneficial in countries with low disease prevalence or highly effective prevention programmes, where the annual number of affected births is minimal, and the paediatric patient population is expected to be small.

4. REFERENCE CENTRES FOR HAEMOGLOBIN DISORDERS: THE EU RARE DISEASES EXAMPLE

Considerable work on this topic has been conducted mainly by the European Commission in the context of its work on promoting quality services for rare diseases across the EU. The many and complex challenges faced by patients/families and treating physicians in the early and accurate diagnosis, and management of rare diseases are similar to those that characterise haemoglobin disorders, which in many EU countries, are classified as rare disorders. However, contrary to the many thousands of other rare diseases, for β-thalassaemia and other haemoglobin disorders, there is (and has existed for some time now) ample experience, scientific evidence, and knowledge on how to implement early and accurate diagnosis, on how to provide specialised monitoring, implement appropriate management and monitoring of the disease progression, treatment effectiveness and prevention.

The European Commission recognised rare diseases as a priority action area since the mid- 1990s and since then the different EU initiatives addressing rare diseases have predominantly focused on bringing together scattered resources and expertise across Member States. This is an effort that is certainly needed in the case of haemoglobin disorders as well – both across Europe, and more importantly, across countries with developing economies. In the context of this work, the European Commission established a special committee of experts, the European Union Committee of Experts on Rare Diseases (EUCERD, from 2010-2013), which focused on developing quality criteria for centres of expertise for rare diseases in Member States [13] and recommendations on establishing European Reference Networks (ERNs) [14] integrated into an EU Directive (2011/24/EU) related to the application of EU patients' rights in cross-border healthcare. This was a major step towards more effectively promoting the sharing of knowledge/expertise and best practices and the creation of clearer structures and networks in the area of rare diseases by bringing together highly specialised providers across the EU [15].

Within the 24 ERNs that were established to cover 24 different rare diseases or families of rare diseases aiming to share best practices for their care and cure, the ERN on haematological diseases (EuroBloodNet, https//eurobloodnet.eu) is the one focused on rare blood disorders including haemoglobin disorders.

Through related EU funded projects, TIF, representing the patient community, contributed along with other European and international medical and scientific experts in rare anaemias (part of the European Network for Rare and Congenital Anaemias [ENERCA]) to the completion, amongst other important deliverables, of a white book titled: "Recommendations for Centres of Expertise in Rare Anaemias: The ENERCA White Book" [16]. Some of the recommendations and benefits outlined in Table 3 are encompassed in this white book and together with the context of the EU directive (2011/24/EU) [15] , underscore how a centre can develop into a Centre of Excellence/Expertise or Reference Centre by developing and pooling together specialised knowledge and experience as well as establishing networks between centres of expertise. From these ideas and policies, when tailored to the needs and prevailing situation across any country or region of the world, patients with haemoglobin disorders, the healthcare specialists, and the healthcare systems at large could benefit significantly, as has been the case with rare diseases across the EU.

Table 3. *Value of Reference Centres for rare anaemias in the EU.*

Value
1. Providing patients and healthcare professionals access to experts and expertise throughout all EU Member States, regardless of the country of origin or practice, thereby reducing inequalities and maximising the cost-effective use of resources
2. Implementing epidemiological surveillance throughout the EU that gathers comparable data on patients affected by rare anaemias and launching preventive programmes for tackling rare anaemias
3. Fostering best practices for prevention, diagnosis and clinical management
4. Promoting the dissemination of knowledge, the sharing of expertise, supporting research, and increasing awareness of rare anaemias
5. Facilitating the transposition of the Directive 2011/24/EU of 9 March 2011 on the application of patients' rights in cross-border healthcare. The ERN between healthcare providers and Centres of Expertise is a main point of interest of the directive, especially for rare diseases. The networks will be a tool to 'improve the access to diagnosis and the provision of high-quality healthcare to all patients who have conditions requiring a particular concentration of resources or expertise and could also be focal points for medical training and research, information dissemination and evaluation, especially for rare diseases

Abbreviations: EU, European Union; ERN, European Reference Network.

5. THALASSAEMIA INTERNATIONAL FEDERATION COLLABORATING CENTERS

TIF, as a patient-driven umbrella organisation, has a constitutional mandate to continually identify ways and tools to enhance the quality of care provided to patients with haemoglobin disorders [17]. Since its establishment in 1986, TIF has placed particular emphasis on promoting work and efforts in this direction. In this context, it has initiated a new project in 2019, aimed at identifying and supporting treatment centres worldwide to improve their services and to standardise practices enabling provision of 'optimal' care as recommended and described in TIF's management guidelines authored by international experts and authorities in their fields. These centres will be known as 'TIF's Collaborating Centres', with the aim of gradually evolving into national and regional hubs that offer consultation, expert opinions, and continuing medical education for healthcare professionals. They will also facilitate patient engagement, participate in research and clinical trials, and regularly provide recommendations for improvements to relevant competent authorities in their countries.

The ultimate goal of TIF is to establish regional networks of these centres, which will significantly contribute to the accumulation of knowledge and the exchange of best practices. This initiative will greatly strengthen the advocacy potential of both healthcare professionals and the patient community for promoting and achieving further improvements. The key goals of the programmes are to:

- Provide scientific opinions and advice on key topics in clinical management including accurate diagnostic techniques, blood safety, correct iron monitoring and dealing with complications

through a multidisciplinary approach in order to achieve continuous improvement within the healthcare delivery system for haemoglobinopathies worldwide.

- Establish an international network (if and where possible) of Reference Centres.
- Improve patient and programme safety in all activities and initiatives.
- Facilitate all patients' access to expert management and contribute to the reduction of inequalities in the care that patients receive.
- Provide educational and training outlets for the centre's staff for stimulating the organisation's quality improvement efforts. Secondary centres will have the opportunity to send staff for training and continuing medical education to the TIF Collaborating Centres.
- Provide networking opportunities with other regional and international treating centres and benefit from staff training and teleconsultation to enable stakeholders to promote quality services from central level (government).
- Enhance community confidence in thalassaemia care in all affected countries.

The programme focuses on the application of specific quality standards as these apply to EU Reference Centres and are also complemented by prerequisites and principles already developed by the relevant organisations including: The Joint Commission International (JCI) 'JCI Survey Process Guide for Ambulatory Care' (4th Edition, 2019, [18]); EUCERD 'Quality Criteria for Centres of Expertise for Rare Diseases in Member States' (2011, [13]) and 'Recommendations on Rare Disease European Networks' (2013, [14]); Guidelines for Good Clinical Practice (GCP); American Institute of Health Quality (AIHQ); US Department of Health and Human Services, Health Resources and Service Administration Quality Improvement framework; UK National Health Survives (NHS) peer review of health services for people with haemoglobin disorders (2015 review); TIF guidelines for the management of thalassaemia (TDT, non-transfusion-dependent β-thalassaemia, α-thalassaemia (latest editions [19-21]); specific standards such as the International Collaboration for Transfusion Medicine (ICTMG) 'Red Blood Cell Specifications for Patients with Haemoglobinopathies: a Systematic Review and Guideline' (2018, [22]), ENERCA White Book [16]; the European Haemophilia Network (EUHANET) 'Guidelines for the Certification of Haemophilia Centres' (2013, [23]); and current literature reviews.

Table 4 summarises general prerequisites sought by TIF in assigning centres as TIF's Collaborating Centres. In addition: (i) there must be evidence of governance and more specifically, health system support, (ii) free access of patients to treatment modalities, (iii) the centres' administrative structure, working hours, and clinical space availability must also be taken into consideration, with the patient experience in mind, (iv) deficiencies and gaps must be promptly identified and corrected, (v) regular assessment of the experience and knowledge of professional staff, and (vi) the patient perceptions of the quality of the services and the relationship with the staff should be monitored regularly through professional tools and taken into account in quality assessment. TIF considers a number of these prerequisites mandatory, before a centre is considered for TIF's assessment as a collaborating one. Others may need to be developed within an agreed period, while some may be of lesser importance as they do not interfere to any extent with the capacity of the centre to act a TIF Collaborative Centre. Table 5 summarises TIF's detailed operational standards for haemoglobinopathy Reference Centres developed by experts following a survey sent to 142 centres treating haemoglobin disorders [24].

Table 4. *Summary of prerequisites for Thalassaemia International Federation's Collaborating Centres.*

Prerequisites
The criterion for recognising any centre as a Reference Centre is certainly the quality of services and its patient-centred care, and not just availability of various technical components necessary for β-thalassaemia (and other haemoglobin disorders) care. It includes following national or international evidence-based guidelines, which allow for good patient outcomes. A Reference Centre must, for example: **1.** Have the capacity to provide expert diagnosis of the disease as well as its long-term complications **2.** Have the capacity to provide expert case management, based on best practice guidelines including a multidisciplinary approach and psychosocial support. These requirements imply experienced healthcare personnel in adequate numbers to ensure continuity of care **3.** Ensure that health care professionals work in a structured environment with clearly defined roles and hierarchy **4.** Maintain a patient registry with the ability to report patient outcomes and other epidemiological information. Electronic information systems must be regarded as essential tools for the provision of quality services **5.** Have regular auditing of clinical and laboratory guidelines **6.** Serve a sufficient number of patients (at least 50 transfusion-dependent patients) to maintain staff experience (what is a sufficient number of patients is not clear, but a consensus should be reached) **7.** Provide patients with sufficient knowledge and information to promote partnership models and self-management support **8.** Have a significant contribution to research as evidenced by peer-reviewed journal publications **9.** Establish networks with: **a.** Secondary treatment centres to provide education and share knowledge and expertise as well as expert opinion on challenging cases **b.** Other Reference Centres nationally if available **10.** Establish networks/collaborations with other Reference Centres outside the country, regional and international, to share best practices **11.** Maintain close links with patient organisations and other community resources at national, regional, and international levels **12.** Make a major contribution to educational activities **13.** Provide evidence of the improvement of patients' survival, clinical outcomes, and quality of life

Table 5. *Thalassaemia International Federation's suggested operational standards for haemoglobinopathy Reference Centres. Modified with permission from [24].*

Reference Centres

1. Governance

- The existence of a hierarchical structure, ordained by law and policy. This should include a chief executive/managing director and a professional team which is coordinated and includes multidisciplinary services, recognising the complex pathology of haemoglobin disorders
- The administrative structure and staff organisation clearly describes the rules and regulations of the centre's services
- A clear definition of the centre's mission and the existence of policies and programmes to fulfil the mission
- Ensuring staff qualifications, experience, and continual education
- Staff includes the specialist practitioners who may serve the patients' needs even if their regular position is outside the centre
- Monitoring and evaluating the functions of the centre by the management, including staff performance and patient safety
- The existence of plans for quality improvement and advocacy to health authorities
- Connection with patient support associations, with patient representation on advisory bodies
- Taking into account all stakeholders views regarding matters of priority and focus on any quality improvement activity.
- All decisions are based on data, obtained through patient records and outcomes, as well as any new developments that have been noted through publications and trials
- A culture promoting ethical practices in all aspects of administration and clinical care, considering internationally accepted patients' rights.
- Paediatric to adult transition programme in place (depending on country and conditions)

2. Safety concerns

- Staff education on safety is programmed
- Patient identification is clear in individual records (electronic or paper based), of blood transfusions, and laboratory results
- There is effective patient communication and explanation of all interventions
- Haemovigilance and pharmacovigilance are practised, including drug safety alerts
- There are evidence-based hand hygiene guidelines
- There are measures to reduce accidents, such as falls in the centre. A secure environment is planned and regularly inspected. Hazardous material handling and disposal (such as needles), is part of the centre's daily procedures
- There are treatment rooms, and resuscitation equipment
- Fire safety and certification by the country's fire services is available. This includes regular testing of any devices required for fire control

- Cigarette and other smoking is forbidden on the premises
- Emergency procedures are in place in the event of power and water cuts or contamination. Monitoring water quality is performed regularly

3. Access to care

- The centre clearly serves benign haematology patients and does not include malignancies as they constitute a dangerous and vulnerable cohabitator
- Patient flow: there must be adequate numbers of patients of each diagnostic group, at least 50 thalassaemia patients and/or 50 sickle cell disease patients for the centre to be regarded as experienced
- Continuity of care is safeguarded by low staff turnover and the presence of experienced and qualified caregivers
- Clinical records with lifetime data are kept
- Multidisciplinary care is provided with a referral system where necessary, and collaboration with in-patient services
- Networking with secondary centres as well as with other centres of excellence, nationally or internationally is an added value. A twinning programme with an academic centre is also an additional advantage
- Any existing electronic health record must fulfil all the requirements of patient safety, including patient consent, confidentiality and anonymisation in data storage and sharing of data for research
- Barriers to patient access, including distance, language, cultural or religious barriers are considered and dealt with
- Respect for patient rights and time is a must in all cases
- Informed consent for all procedures is obtained

4. Partnership model

- Adequate information to patients/families about the disease and any treatment decisions, including possible side effects, is always provided
- Patients are given choices about their treatment
- Self-management is encouraged
- Special attention to patient adherence is given and the patient is supported appropriately
- Workshops for patients/families are held regularly, at least once a year

5. Guidelines and standards for clinical care

- Evidence-based national guidelines, put together by experts in the field or international guidelines (e.g., Thalassaemia International Federation) are used in the centre and adhered to
- Pain screening is performed, and a pain management system is in place
- Assessing the quality of laboratory and other technologies used to monitor patients is the responsibility of the clinical team which must alert the providers of any divergent or inaccurate results
- Infection control procedures are part of the clinical standards of the centre
- Availability of food during day care is necessary and the quality and nutritional value must be monitored

- Blood transfusion procedures and standards according to international directives are kept
- Any medical treatment, such as intravenous fluids and exchange transfusions, are provided according to standards that ensure patient safety
- Continual medical and other professional education are part of the centres long term programme
- Staff qualifications, skills, knowledge, and experience are defined and described along with the job description of each
- Staff/patient ratio is defined approximately as 1 doctor per 100 patients and 1 nurse per 50 patients

6. Quality improvement

- Having surveyed all aspects of the service, and noted all strengths and weaknesses, the survey team will present a report and make suggestions for quality improvement where necessary
- Quality improvement is a systematic approach to changes aiming to upgrade services and correct any deficiencies in the governance, structure, and functions of the service. Quality improvement includes better patient experience and outcomes, by changing provider behaviour
- The way in which change is introduced and implemented is a matter of concern and may require expert advice. In this process the following are considered:
  - External influences, such as governmental policies or interest, budgetary support, professional requirements
  - Understanding the issues involved at all levels, including why a problem exists
  - Setting goals and monitoring progress
  - Choosing the tools to bring about change. These could be skills development, computerisation, updating guidelines etc.
  - Full staff engagement is necessary. There often needs to be a multidisciplinary approach to change making
  - The patient's voice must be involved in all stages of quality improvement. Patient/families can also effectively monitor the effects and benefits of change since they experience the whole 'patient pathway'
  - Studying other centres experience in change making: have the changes been successful elsewhere?

7. Information management

- Patient records (paper or electronic) are kept with due consideration to confidentiality, security and accuracy of data
- The retention time of records in a haemoglobinopathy setting is lifelong, since the current clinical condition may be influenced by past events and disease control (such as iron levels)
- Standard diagnosis codes are kept (e.g., ICD10)
- E-health systems are assessed and tested prior to implementation, for quality and patient safety
- Protection against loss, unauthorised access or use is ensured
- Policies and procedures concerning record keeping are clearly directed to all the staff, through documents and training
- The patient should be clearly identified on each record
- Those authorised to have access to clinical records are clearly defined.

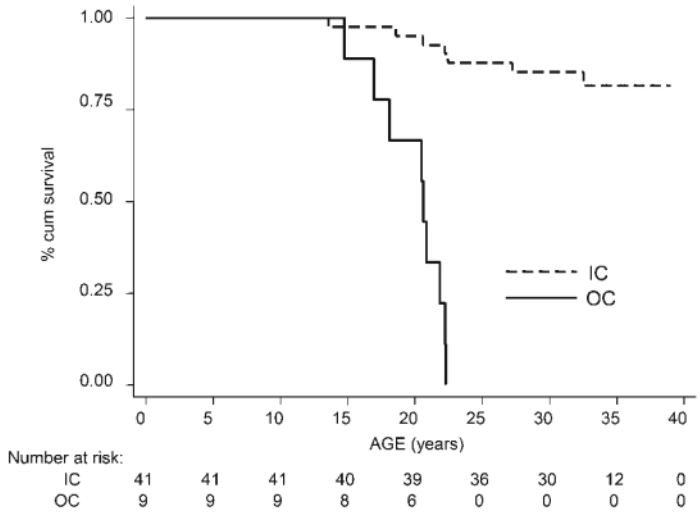
Through this programme, TIF may provide: i) on-site audit of the centre's performance by external reviewers with regards to the quality of the processes, outcomes, and structures involved in the care it provides to haemoglobinopathy patients, ii) technical support and recommendations for improvement to reach desired outcomes, and (iii) networking opportunities with other regional and international centres for the exchange of knowledge and expertise.

Successful centres are granted the status of TIF's Collaborating Centre for Haemoglobinopathies and provided with needs-based technical support and personalised recommendations for continuous improvement to reach the desired outcomes. This status is valid for a period of 5 years before the evaluation team is called back to the centre to ensure that quality of care is maintained and/or improved to the required standards.

This work of TIF is not meant to replace the value of quality assessment tools, including simple but valuable ones such as audit and peer review, that are already practiced in many countries, especially in the West. Moreover, it is certainly not meant to replace those of dedicated accrediting organisations which offer their work at a cost to assess and establish quality standards in the services provided by health institutions – hospitals, clinics or centres, public or private. Indeed, TIF strongly encourages competent authorities to adopt such a methodology where and when possible.

TIF, through its programme outlined above, mainly aims to initiate an effort towards raising awareness on the value of multidisciplinary care and Reference Centres in improving survival and quality of life of patients with haemoglobin disorders, as has already been documented in a few countries (Figure 1) [25]. It aims to offer a simpler methodology as a first step to support the upgrading of services provided by treating centres particularly, but not only, of the developing economies by introducing the practice of multidisciplinary care and by stronger acknowledgment of the value of pooling knowledge and experience, and sharing best practices through the existence of Reference Centres.

Figure 1. *Kaplan-Meier overall survival curves of patients referred to specialised centres (IC) versus patients referred to nonspecialised centres (OC). Log-rank p <0.0001; hazard ratio of OC versus IC adjusted for sex (Cox model): 18.1, 95% confidence interval: 4.7-69.0; p <0.001. Abbreviations: IC, specialised centre; OC, non-specialised centre. Reproduced with permission from [25].*



6. THALASSAEMIA INTERNATIONAL FEDERATION NETWORKS

In its efforts to enhance the TIF Collaborating Centres initiative, TIF has taken an additional step to advance care, knowledge, and advocacy for haemoglobin disorders across diverse regions by establishing regional haemoglobin disorders networks where prevalence of haemoglobin disorders is either high or the disorders are considered rare. These networks aim to create a robust platform connecting patient associations, healthcare professionals, researchers, and other key stakeholders across their regions to enhance care and awareness. By fostering collaboration and facilitating the exchange of expertise and resources, the networks seek to significantly improve the quality of care and support individuals with these disorders. The objectives of the different regional haemoglobin disorders networks include:

- **Enhancing collaboration:** by uniting patient associations and healthcare providers, the networks foster a collaborative approach to care that amplifies a shared voice for advocacy, ultimately strengthening policies and resources directed at managing haemoglobin disorders.
- **Raising awareness:** the networks prioritise awareness among both public and healthcare professionals. Through educational campaigns and informational resources, they seek to broaden understanding of haemoglobin disorders and increase recognition of the needs of affected individuals.

- **Improving standards of care and supporting the development of Reference Centres:** promoting access to cutting-edge treatments and comprehensive care, the network emphasises the adoption of best practices and the dissemination of advancements in medical research to improve patient outcomes and quality of life.
- **Policy advocacy:** working with government bodies and health organisations, the networks actively promote policies that support the needs of patients with haemoglobin disorders, ensuring that healthcare systems adequately address these requirements within national health frameworks.

Activities that the networks undertake are educational workshops and conferences, resource sharing such as research data and treatment guidelines, patient support programmes, and research collaborations.

Currently the following networks have been created and successfully operate: Arab Patient Associations' Forum, American Network for Haemoglobin Disorders (ANHD), Nordic Haemoglobinopathy Forum, ASEAN Thalassaemia Federation (ATF), and Indian Subcontinent Network for Haemoglobin Disorders.

7. HEALTHCARE COVERAGE AND COST OF CARE

β-Thalassaemia patients and their families around the globe are faced with difficult challenges in obtaining timely, up-to-date and adequate care (accessible, acceptable, affordable, safe, and effective) on a daily basis and have to navigate through various different healthcare systems along with a broad array of reimbursement methods. An ideal universal health coverage within which all people have access to a full range of the quality health services they need, when and where they need them, free or without financial hardship is currently the exception rather than the rule [26]. In particular, a full continuum of essential health services, from health promotion to prevention, treatment, rehabilitation, and palliative care is usually a luxury for β-thalassaemia patients [27]. Few countries offer free or shared universal health coverage for genetic disorders, while others have different methods of co-payment either related to national and/or private insurance-based plans or even point-of-service and out-of-pocket compensation schemes for the coverage of health expenses. Nevertheless, irrespective of payment structures and levels of coverage, multidisciplinary care has proven extremely useful for many different diseases and chronic health problems.

Healthcare services are becoming increasingly strained, and healthcare authorities worldwide need to invest in integrated care particularly in the case of chronic, complex diseases such as the haemoglobin disorders, to first and above all deliver higher quality services for the patients while at the same time containing costs. Evidence-based multidisciplinary care constitutes a cost-effective solution and a financially beneficial choice for national healthcare systems in the long run [28, 29]. Unfortunately, existing evidence of the cost-effectiveness of integrated care is limited for haemoglobin disorders. Future economic evaluation should target methodological issues to aid policy decisions with more robust evidence-based, reliable, nationwide data [30].

8. FINAL THOUGHTS AND VISION FOR THE FUTURE

Provision of multidisciplinary care and existence of Reference Centres constitute a modern clinical and cooperative approach for the management of complex diseases that require physiological, psychological, and social interventions with complementary expert consultation. Such care provided by teams of experts from different disciplines includes health professionals (primary care physicians, specialists, nurses, psychologists, physiotherapists, etc.), scientists, social workers, educators, and other health-related employees. The importance of coordination for the management of chronic diseases becomes even more important for hereditary disorders; particularly for the management of both children and adults with β-thalassaemia in order to achieve a much-needed holistic support [31].

It is hoped that the work of every country around the world towards promoting the United Nation's (UN) Sustainable Development Goals 2030 [32] and the work of the WHO on disease-specific but also many other relevant resolutions (EB118/5 on thalassaemia and other haemoglobinopathies [33] and A59/9 on sickle cell anaemia [34]), recommendations and programmes including blood and patient safety, will contribute towards achieving significant progress in the prevention and management of these disorders and will 'allow' them to further improve and introduce more specialised care, as described in this Chapter.

It is also hoped that the contribution of this updated 5th edition of TIF's 'Guidelines for the Management of Transfusion Dependent β-Thalassaemia (TDT)', and the work of TIF at large, greatly supported by the WHO, the UN and its Economic and Social Council (ECOSOC), the EU, a large group of medical and scientific bodies and experts including the Editors and Authors of this book, and very importantly by patients and families themselves, at national and international level, will contribute to the efforts of every country in providing a better future and ensuring more equity for patients with β-thalassaemia and other haemoglobin disorders.

The introduction and implementation of effective, multidisciplinary care programmes are essential to address the complex needs of patients with haemoglobin disorders, particularly TDT. Non-governmental organisations like TIF play a crucial role in complementing and supporting the efforts of governments and official health bodies. Through their expertise, advocacy, and coordination of resources, they significantly contribute to elevating the quality of services and optimising patient outcomes.

By fostering collaboration, advancing education, and driving patient-centred support, TIF and its initiatives, such as the TIF Collaborating Centres and TIF Regional Networks for Haemoglobin Disorders, have contributed to setting the foundation for a robust framework of care. These initiatives are not only improving care but also promoting advancements in research and the integration of innovative technologies, such as artificial intelligence, to streamline information sharing and improve decision-making for all stakeholders. As TIF's network grows and evolves, it will be instrumental in building a sustainable and far-reaching impact on care standards, patient empowerment, and overall awareness, contributing to a better future for individuals affected by β-thalassaemia and other haemoglobin disorders.

REFERENCES

1. Eleftheriou A, Angastiniotis M. Global Thalassaemia Review 2023. Nicosia, Cyprus: Thalassaemia International Federation, 2023.
2. Allen D, Gillen E, Rixson L. The Effectiveness of Integrated Care Pathways for Adults and Children in Health Care Settings: A Systematic Review. JBI Libr Syst Rev 2009;7(3):80-129.
3. Rocks S, Berntson D, Gil-Salmeron A, Kadu M, Ehrenberg N, Stein V, Tsiachristas A. Cost and effects of integrated care: a systematic literature review and meta-analysis. Eur J Health Econ 2020;21(8):1211-1221.
4. European Commission. European Reference Networks. Available at: https://health.ec.europa.eu/rare-diseases-and-european-reference-networks/european-reference-networks_en. Accessed: 15 Nov 2024.
5. Ruffo GB, Russo R, Casini T, Lombardini L, Orecchia V, Voi V, Origa R, Forni GL, Marchetti M, Gigante A, Garibotto G, Maggio A, De Franceschi L. Nephrological Complications in Hemoglobinopathies: SITE Good Practice. J Clin Med 2023;12(23).
6. Casale M, Baldini MI, Del Monte P, Gigante A, Grandone A, Origa R, Poggi M, Gadda F, Lai R, Marchetti M, Forni GL. Good Clinical Practice of the Italian Society of Thalassemia and Haemoglobinopathies (SITE) for the Management of Endocrine Complications in Patients with Haemoglobinopathies. J Clin Med 2022;11(7).
7. DeBaun MR, Telfair J. Transition and sickle cell disease. Pediatrics 2012;130(5):926-935.
8. Musallam K, Cappellini MD, Taher A. Challenges associated with prolonged survival of patients with thalassemia: transitioning from childhood to adulthood. Pediatrics 2008;121(5):e1426-1429.
9. GOT TRANSITION®. Six Core Elements of Health Care Transition™. Available at: https://www.gottransition.org/six-core-elements/. Accessed: 15 Nov 2024.
10. Chandra J, Sahi PK. Transition of Care for Patients with Thalassemia. Indian J Pediatr 2023;90(12):1227-1231.
11. Morse LA, Sawh RN. Transfer of Care for People with Severe Forms of Thalassemia: Learning from Past Experiences to Create a Transition Plan. J Pediatr Nurs 2021;61:378-386.
12. Sawh RN. The patient perspective: The struggles of living with thalassemia as an adult. Ann N Y Acad Sci 2023;1527(1):20-24.
13. EUCERD. Recommendations on quality criteria for centres of expertise for rare diseases in Member States. 24 October 2011. Available at: https://www.rd-action.eu/eucerd/EUCERD_Recommendations/eucerd-recommendations(1).pdf. Accessed: 16 Nov 2024.
14. EUCERD. REUCERD Recommendations on European Reference Networks for Rare Diseases. 31 January 2013. Available at: https://www.rd-action.eu/eucerd/EUCERD_Recommendations/ern_recos.pdf. Accessed: 16 Nov 2024.
15. European Parliment. Directive 2011/24/EU of the European Parliament and of the Council of 9 March 2011 on the application of patients' rights in cross-border healthcare. Official Journal of the European Union 9 March 2021. Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32011L0024. Accessed: 16 Nov 2024.
16. Vives Corrons J-L, del Mar Mañú Pereira M, Romeo-Casabona C, Nicolás P, Gulbis B, Eleftheriou A, Angastiniotis M, Aguilar-Martínez P, Bianchi P, Van Wijk R, Heimpel H, De la Salle B, Mosca A. Recommendations for centres of expertise in rare anaemias. The ENERCA white book. Thalassemia Rep 2014;4(3):4878.
17. Angastiniotis M, Eleftheriou A. Assessing services for haemoglobin disorders: a toolkit for service planning. In: Greene M, ed. Thalassemia: Causes, Treatment Options and Long-Term Health Outcomes (Recent Advances in Hemotology Research). UK ed: Nova Science Pub Inc, 2014.

18. Joint Commission International. JCI Survey Process Guide for Ambulatory Care, 4th Edition. 1 July 2019. Available at: https://store.jointcommissioninternational.org/jci-survey-process-guide-for-ambulatory-care-4th-edition/. Accessed: 16 Nov 2024.
19. Taher A, Musallam K, Cappellini MD. Guidelines for the Management of Non-Transfusion-Dependent β-Thalassaemia. 3rd ed. Nicosia, Cyprus: Thalassaemia International Federation, 2023.
20. Cappellini MD, Farmakis D, Porter J, Taher A. Guidelines for the management of transfusion dependent thalassaemia (TDT). 4th ed. Nicosia, Cyprus: Thalassaemia International Federation, 2021.
21. Amid A, Lal A, Coates TD, Fucharoen S. Guidelines for the management of α-thalassemia. Nicosia, Cyprus: Thalassaemia International Federation, 2023.
22. Compernolle V, Chou ST, Tanael S, Savage W, Howard J, Josephson CD, Odame I, Hogan C, Denomme G, Shehata N, International Collaboration for Transfusion Medicine G. Red blood cell specifications for patients with hemoglobinopathies: a systematic review and guideline. Transfusion 2018;58(6):1555-1566.
23. EUHANET. European guidelines for the certification of Haemophilia Centres. 07 June 2013. Available at: https://www.euhanet.org/docs/euhanet-european_guidelines_for_the_certification_of_haemophilia_centres_2013.pdf. Accessed: 16 Nov 2024.
24. Angastiniotis M, Eleftheriou A, Naveed M, Al Assaf A, Polynikis A, Soteriades ES, Farmakis D. TIF standards for haemoglobinopathy reference centres. Thalassemia Rep 2023;13:10-20.
25. Forni GL, Puntoni M, Boeri E, Terenzani L, Balocco M. The influence of treatment in specialized centers on survival of patients with thalassemia major. Am J Hematol 2009;84(5):317-318.
26. WHO. Universal health coverage. Available at: https://www.who.int/health-topics/universal-health-coverage#tab=tab_1. Accessed: 16 Nov 2024.
27. Yousuf R, Akter S, Wasek SM, Sinha S, Ahmad R, Haque M. Thalassemia: A Review of the Challenges to the Families and Caregivers. Cureus 2022;14(12):e32491.
28. Kansal A, Latour JM, See KC, Rai S, Cecconi M, Britto C, Conway Morris A, Dominic Savio R, Nadkarni VM, Rao BK, Mishra R. Correction to: Interventions to promote cost-effectiveness in adult intensive care units: consensus statement and considerations for best practice from a multidisciplinary and multinational eDelphi study. Crit Care 2024;28(1):121.
29. Shaw E, Nunns M, Spicer SG, Lawal H, Briscoe S, Melendez-Torres GJ, Garside R, Liabo K, Coon JT. What is the volume, quality and characteristics of evidence relating to the effectiveness and cost-effectiveness of multi-disciplinary occupational health interventions aiming to improve work-related outcomes for employed adults? An evidence and gap map of systematic reviews. Campbell Syst Rev 2024;20(2):e1412.
30. Aguilar Martinez P, Angastiniotis M, Eleftheriou A, Gulbis B, Manu Pereira Mdel M, Petrova-Benedict R, Corrons JL. Haemoglobinopathies in Europe: health & migration policy perspectives. Orphanet J Rare Dis 2014;9:97.
31. Huang X, Lu J, Huang R, Lu Q, Luo Y, Huang X, An L, Chen Y. Effects of multidisciplinary team continuous care model on psychological behavior and quality of life in children with beta-thalassemia major. Am J Transl Res 2024;16(3):905-915.
32. United Nations. Sustainable Development Goals. Available at: https://www.un.org/sustainabledevelopment/sustainable-development-goals/. Accessed: 16 Nov 2024.
33. WHO. Thalassaemia and other haemoglobinopathies. Report by the Secretariat. 11 May 2006. Available at: https://iris.who.int/bitstream/handle/10665/21519/B118_5-en.pdf?sequence=1&isAllowed=y. Accessed: 16 Nov 2024.
34. WHO. Sickle cell anaemia. Report by the Secretariat. 24 April 2006. Available at: https://apps.who.int/gb/ebwha/pdf_files/wha59/a59_9-en.pdf. Accessed: 16 Nov 2024.

14 HAEMATOPOIETIC CELL TRANSPLANTATION

Authors: **Valeria Maria Pinto[1], Massimiliano Gambella[1], Pietro Sodani[2], Lena Oevermann[3], Emanuele Angelucci[1]**

[1.] Hematology and Cellular Therapy Unit, IRCCS Ospedale Policlinico San Martino, Genoa, Italy

[2.] Unità Operativa Complessa Internal Medicine and Hematology, Azienda Ospedaliera Ospedali

[3.] Department of Pediatric Oncology and Hematology, Charité-Universitätsmedizin Berlin, Berlin, Germany

Competing interest: VMP reports consultant fees from Bluebird Bio; advisory board fees from Bluebird Bio, Bristol Myers Squibb and Vertex Pharmaceuticals; and speaker bureau fees from Novartis. EA reports being a Data Monitoring Committee chair for Vertex Pharmaceuticals and Bristol Myers Squibb; advisory board fees from Regeneron and Novartis; speaker bureau fees from Bristol Myers Squibb and Sanofi; and honoraria from Sanofi. LO reports consultancy fees from Pfizer and Vertex Pharmaceuticals; advisory boards from Chiesi, Vertex Pharmaceuticals and Novartis; and speakers fees from Pfizer and Novartis. The remaining authors have no competing interests to disclose. All competing interests are outside the present work.

1. INTRODUCTION

Allogeneic haematopoietic cell transplantation (HCT) has been the first therapeutic option for curing transfusion-dependent β-thalassaemia (TDT) and has dramatically changed the life prospect of these patients. Allogeneic HCT consists of the substitution of the diseased, ineffective erythropoiesis with an allogeneic, effective one. However, HCT replaces the entire haemopoietic system, not just the defective erythropoiesis, which is the fundamental difference from gene therapy (see Chapter 15). Up until the last decade, nearly 10,000 transplants have been performed in TDT worldwide with outstanding results [1]. In recent years, several factors including improved conditioning regimens, improved prevention of graft-versus-host disease (GvHD), more effective antibacterial, antiviral and antifungal treatment and, most importantly, significant improvement in the medical care of TDT have led to a substantial improvement in patient outcomes [2]. Nevertheless, HCT is not without risks and possible complications. This Chapter will provide an overview of the current state of the art of HCT in TDT.

2. TRANSPLANTATION FOR TRANSFUSION-DEPENDENT β-THALASSAEMIA

The story and initial experiences of HCT in TDT began in the early 1980s. In that decade, the influence of pre-transplant characteristics on outcome was analysed in a prospective consecutive cohort of 161 patients under 17 years of age [3, 4], leading to the development of a well-known scoring system. Although much time has passed since these data were acquired (before the oral chelation era), and the precise numerical classification is likely outdated, these results remain important because they provide proof of concept for how prolonged exposure to iron toxicity can cause oxidative damage to human tissues [5-7], which are consequently made more susceptible by transplantation and its associated toxicity [2].

2.1. Haematopoietic cell transplantation from HLA-matched family donors

In a large European Society for Blood and Marrow Transplantation (EBMT) survey of 1061 cases of matched sibling donor transplants performed between 2000 and 2010 in 132 centres in 28 countries with a median patient age of 7 years, long-term overall survival (OS) and thalassaemia-free survival (TFS) were 91% and 83%, respectively [8]. A statistically significant difference in OS was reported comparing children in the 10 to <14 years with those in the 14 to <18 years of age range (96% versus 82%). In the 82 adult (>18 years) patients, OS and TFS were 80% and 77%, respectively. These results were confirmed in a more recent analysis by the same author group [9].

Similar results have been reported in studies from the USA [10], China [11, 12], and India [13], although in the latter, the threshold for optimal results was lower, further highlighting the impact of continuous iron-related oxidative stress on tissues and organ susceptibility. Moreover, a recent analysis in a large cohort of TDT patients followed over 50 years at seven major centres in Italy has shown absence of deaths from HCT complications after the year 2010 demonstrating not only the improvement of the procedures but also the optimal patient's selection [14]. What is most important to emphasise is that these results represent real-world outcomes and do not stem from controlled trials with stringent inclusion and exclusion criteria.

Table 1 summarises recent data provided by the Haemoglobinopathies Working Party of EBMT [9].

Table 1. *Report from the EBMT registry on 2891 consecutive patients. Outcomes are illustrated by donor type. Modified with permission form [9].*

Donor	MSD	HLA matched related	HLA mismatched related	Unrelated donor 10/10 matched	Unrelated donor <10/10 matched
OS	91.8%	88.3%	85.3%	93.2%	81.4%
EFS	83.0%	79.5%	62.4%	85.7%	68.0%
Thalassaemia recurrence	8.8%	8.8%	22.9%	7.5%	13.4%
NRM	8.1%	11.6%	14.6%	6.7%	18.5%
Ac GvHD	6.6%	9.3%	3.1%	12.7%	14.2%
Cr GvHD	13.1%	15.9%	9.3%	15.0%	17.8%

Abbreviations: EBMT, European Society for Blood and Marrow Transplantation; OS, overall survival; EFS, event-free survival; NRM, non-relapse mortality; Ac GvHD, acute graft-versus-host disease; Cr GvHD, chronic graft-versus-host disease; MSD, matched sibling donor.

2.2. Haematopoietic cell transplantation from HLA-matched unrelated donors

A major obstacle to successful transplantation is the limited number of HLA-matched related donors within families. More than 60% of patients do not have a suitable sibling donor. Some of these patients could benefit from HCT from a matched unrelated donor (MUD).

Several studies involving a few hundred patients transplanted worldwide have shown that MUD can cure a large proportion of patients with TDT, provided that the donor is selected using high-

resolution molecular typing for both HLA class I and II molecules. The risk of rejection can be reduced by selecting unrelated donors who do not have non-permissive mismatches at the HLA-DPB1 locus in the host-versus-graft direction [15].

The 2018 EBMT study [9] clearly demonstrated that a fully matched unrelated donor (i.e.,10/10 matched) produces results that are almost identical to those obtained when the donor is an HLA identical sibling. Moreover, it showed even better outcomes when the donor was a fully matched unrelated donor compared to an HLA identical sibling, but this was likely due to a centre effect, as only expert centres had activated unrelated transplant programmes.

Sacchi and colleagues recently demonstrated that for Caucasians, the probability of finding a completely matched donor is around 60% [16]. A major limitation of this approach is that ethnic groups in which thalassaemia is prevalent are still largely under-represented in global donor registries.

Limited experience has been gained using haemopoietic cord blood unrelated cells, with poor results [17]. We do not recommend this approach outside a well-defined, controlled clinical trial.

2.3. Haploidentical haematopoietic cell transplantation

Haploidentical transplantation means that patient and donor share only one of the HLA haplotypes. This is similar to what happens between children and parents, where the probability of having an haploidentical sibling is 50%. It follows that, in principle, haploidentical HCT could potentially extend the use of this treatment option to almost all patients who lack a matched sibling donor or an HLA-identical unrelated donor. Several attempts have been made using complex *in vitro* manipulation that are difficult to be reproduced. The best results have been obtained with the use of TCRαβ^+/CD19$^+$-depleted grafts, which have been associated with significantly reduced graft failure [18].

A recently developed alternative platform for haploidentical HCT uses T-cell-replete grafts and post-transplant high-dose cyclophosphamide (PT-Cy) developed after the Leo Luznik experience with malignancies [19, 20]. An intensive preconditioning immuno-ablation followed by a myeloablative conditioning regimen and PT-Cy in the haploidentical setting for thalassaemia has shown promising results, with a 3-years OS and TFS of 96% in a consecutive series of 83 TDT patients (median age: 12 years; range: 1-28), with a minimum follow-up of 6 months (median: 15 months; range: 7-53) [21]. Notably, in this series of paediatric and adult patients, no graft failures were recorded.

2.4. Considerations for adults

Transplant experience in adults has so far been limited, with only a few hundred of patients undergoing HCT since the year 2000 [9]. In the EBMT analysis, only 9% of thalassaemia transplant recipients were over the age of 17 years (range: 18-45, with a median age of 22 years) [9]. As with children, prevention of organ toxicity from toxic iron species is likely to be a key predictor of good outcomes, although this has not been conclusively demonstrated in clinical trials. At the time of previous analyses, almost all adults were not regularly receiving chelation therapy and had already developed significant organ injury. Today, the clinical situation of adult patients is greatly improved. Based on the statement that age per se is not as important as the extension of tissue damage developed from years of exposure to tissue reactive iron species [2, 5, 6, 22-24], a controlled trial in well-chelated adult patients is warranted [25].

2.5. Mixed chimerism following haematopoietic cell transplantation for thalassaemia

Mixed haematopoietic chimerism (MC) is an interesting phenomenon that sometimes occurs after HCT for thalassaemia. This means that, in few patients in the years after transplant, two different populations (patient and donor haemopoietic stem cells) have coexisted with reciprocal immunologic tolerance. In this situation, the donor's effective erythropoiesis has a selective advantage over the patient's ineffective erythropoiesis. Despite a limited presence in the bone marrow environment down to 20%, the donor's erythropoiesis is able to produce a sufficient level of normal haemoglobin to achieve transfusion independence and clinically control the disease. In several experiences, and even in sickle cell disease, this phenomenon has been observed in approximately 10-11% of transplanted patients [26]. It is important to note that the above was an observation made during clinical studies following a fully myeloablative conditioning regimen. Following this biological evidence, all the attempts to induce a persistent mixed chimerism by a reduced intensity regimen failed and a myeloablative regimen is still considered mandatory in transplantation for TDT. Intentionally inducing persistent mixed chimerism is an important objective (potentially strongly reducing transplant-related toxicity and mortality), but it must be considered an experimental approach that should not be conducted outside of a controlled trial.

3. PRE-TRANSPLANT EVALUATION

Particular attention must be paid to an appropriate pre-transplant work-up. In addition to classical pre-HCT evaluations, this should include accurate iron studies based on magnetic resonance imaging (MRI) analysis to evaluate liver iron load (liver iron concentration [LIC]) and cardiac MRI T2* to evaluate cardiac iron load and function [22]. Assessment of the level of liver fibrosis by liver transient elastography or by liver biopsy should also be offered for all candidates for HCT.

Endocrine function (fasting blood glucose levels, thyroid function tests, growth-hormone-releasing hormone [GHRH] stimulation test) should be performed to evaluate iron-related damage to endocrine organs. Fertility assessment with sperm banking and ovarian tissue preservation should be offered. Detailed indications for accurate pre-transplant work-up have been published elsewhere [27].

4. CONDITIONING REGIMENS

Preparatory regimens for HCT must achieve two objectives: elimination of the disordered marrow and establishment of a tolerant environment that will allow the transplanted marrow to survive and prosper. There is considerable evidence and decades of experience supporting the use of busulfan and its derivatives for ablating marrow in patients undergoing HCT for the treatment of non-malignant conditions [28]. Busulfan is usually associated with a cytoreductive and immunosuppressive agent and, in recent years, fludarabine has gradually substituted cyclophosphamide [29].

Treosulfan-based conditioning has been introduced as a well-tolerated alternative to busulfan with myeloablative capacity with reduced extra bone marrow toxicity [29, 30]. Treosulfan appears to be particularly indicated in patients with a history of inadequate iron chelation and tissue/organ deterioration consequent to iron induced oxidative stress and in adult patients.

When the allogeneic graft starts to proliferate in the recipient, an immunological reaction against the recipient may occur. Several regimens for GvHD prophylaxis have been developed depending on donor characteristics but this topic is outside the scope of this Chapter.

5. SOURCE OF HAEMOPOIETIC CELLS

Because there is no need for an anti-malignancy effect, bone marrow is usually preferred over peripheral blood haematopoietic cells. However, the latter are used by several centres worldwide with excellent results [31]. Optimal results have also been obtained using cord blood haemopoietic cells from an HLA identical sibling [32].

6. FOLLOW UP AFTER HAEMATOPOIETIC CELL TRANSPLANTATION

Adequate post-transplant clinical follow up is of particular importance. Within the first year, careful monitoring of haematological and engraftment parameters, infectious complications, and GvHD is essential. Appropriate immunisation is necessary in the second year, if there is no GvHD. Due to the nature of the disease, transplanted thalassaemia patients require specific post-transplant studies in addition standard HCT follow up.

Long-term follow up is of particular importance with respect to monitoring the evolution of multi-system thalassaemia-related problems (iron overload, pubertal development, growth and endocrine deficiencies). A number of reports indicate that iron overload, chronic hepatitis, cardiac function, and endocrine deficiencies can be managed more easily after transplantation, sometimes permitting the healing of severely damaged organs [33-35].

Persistence of iron overload after transplant can continue producing tissue-reactive species, leading to progressive organ damage and even life-threatening organ failure, particularly when associated with other morbidities [36]. Therefore, it is mandatory to remove excess iron after transplantation by phlebotomy (venesection) [37, 38] or chelation therapy [39]. All iron removal treatments should be started only once the graft is stabilised and the patient is free from any immunosuppressive treatment or prophylaxis. Endocrine dysfunction and infertility require specific expertise and follow up after HCT.

Health-related quality of life is an important aspect to consider both in patients who underwent HCT or gene therapy. Data on long-term follow up should provide a better comprehension of the advantages and potential risks of the procedure and will be a useful tool in the treatment decision-making process.

7. COST AND COST-EFFECTIVENESS

Medical care for TDT is a complex, multidisciplinary and expensive process requiring dedicated and experienced units. An Italian study based on cost/benefit estimations from a societal perspective quantified tariffs, expenses, and net earnings in 2006 for thalassaemia patients. The mean costs were EUR 1,242/patient/month, of which 55.5% was attributed to iron chelation therapy and 33.2% to transfusions [40]. These data compare to the total overall median costs of HCT from a matched sibling donor, which are approximately 150,000 USD, translating to 1,900 USD per expected life year after HCT [41]. However, the cost of transplantation can vary significantly around the world.

When considering the very significant combined costs of lifelong blood transfusions, iron chelation, and management of complications for optimal TDT care (which clearly exceed the healthcare resources available in most non-industrialised countries), transplantation is certainly a cost-effective option.

8. COUNSELLING FOR TRANSPLANTATION

TDT patients are continuously followed by their primary care providers or specialised centres. It is good clinical practice for the initial discussion about HCT to start with their thalassaemia doctors, followed by a joint evaluation and discussion with transplant specialists. Several thousand transplants have been performed in recent years, but this activity has been restricted to a few selected centres, usually in constant collaboration with thalassaemia centres. Thalassaemia requires specific disease knowledge, expertise, and competence even in the context of transplantation. However, we continue to see centres that self-qualify as experts in thalassaemia for transplant and gene therapy without necessarily having the appropriate background.

9. THE EBMT HAEMOGLOBINOPATHY WORKING PARTY

Within the EBMT, a working party dedicated to haemoglobinopathies (TDT and sickle cell disease) has recently been established. The aim of this working party is to promote the progression and improvement of this curative procedure, enhance knowledge, and increase the dissemination and availability of transplantation worldwide (https://www.ebmt.org/research/working-parties). This goal can only be achieved through close collaboration with thalassaemia patients and physicians. Consequently, a strong connection and collaboration between the Thalassaemia International Federation (TIF) and the Haemoglobinopathy Working Party of the EBMT can enhance research and medical care for all TDT patients, with several objectives, the most important of which is defining the right therapy at the right time for each patient.

10. HAEMATOPOIETIC CELL TRANSPLANTATION AND GENE THERAPY

Despite several limitations, HCT has achieved outstanding results in clinical practice globally, with consistent long-term (decades) outcomes. HCT is an affordable, cost-effective treatment available in most countries. Experience with HCT has contributed to the development of novel gene manipulation techniques (see Chapter 15) in several aspects (patient selection, patient work-up, conditioning regimens, post-transplant care, etc.). Transplant results are also the benchmark to which gene therapy must be compared for its future development and widespread distribution.

KEY POINTS AND RECOMMENDATIONS

1. Haematopoietic cell transplantation (HCT) should be offered to transfusion-dependent β-thalassaemia (TDT) patients and their parents at an early age, before complications due to iron overload develop, if an HLA identical donor is available (**Grade B, Class I**).
2. Either bone marrow or cord blood from an HLA-identical sibling can be used (**Grade B, Class I**).
3. A matched unrelated donor can be used, provided that high compatibility criteria for both HLA class I and II loci are met (**Grade B, Class I**).
4. Haploidentical HCT shows promising results but should be considered only in experienced HCT centres in the context of well-designed clinical trials (**Grade B, Class IIb**).
5. Myeloablative conditioning regimens should always be used for standard transplantation (**Grade B, Class I**).
6. Special attention is required for adult patients (**Grade B, Class I**).
7. Post-transplant care should include all transplant and thalassaemia-related complications (**Grade B, Class I**).
8. Only thalassaemia-expert transplant centres should perform HCT, always in strict connection with thalassaemia refence centres (**Grade C, Class I**).
9. In TDT patients, HCT is cost-effective when compared to life-long supportive therapy (**Grade B, Class I**).

REFERENCES

1. Angelucci E. Hematopoietic stem cell transplantation in thalassemia. Hematology Am Soc Hematol Educ Program 2010;2010:456-462.
2. Angelucci E, Pilo F, Coates TD. Transplantation in thalassemia: Revisiting the Pesaro risk factors 25 years later. Am J Hematol 2017;92(5):411-413.
3. Lucarelli G, Galimberti M, Polchi P, Angelucci E, Baronciani D, Giardini C, Politi P, Durazzi SM, Muretto P, Albertini F. Bone marrow transplantation in patients with thalassemia. N Engl J Med 1990;322(7):417-421.
4. Lucarelli G, Galimberti M, Polchi P, Angelucci E, Baronciani D, Giardini C, Andreani M, Agostinelli F, Albertini F, Clift RA. Marrow transplantation in patients with thalassemia responsive to iron chelation therapy. N Engl J Med 1993;329(12):840-844.
5. Coates TD. Physiology and pathophysiology of iron in hemoglobin-associated diseases. Free Radic Biol Med 2014;72:23-40.
6. Coates TD. Iron overload in transfusion-dependent patients. Hematology Am Soc Hematol Educ Program 2019;2019(1):337-344.
7. Coates TD, Carson S, Wood JC, Berdoukas V. Management of iron overload in hemoglobinopathies: what is the appropriate target iron level? Ann N Y Acad Sci 2016;1368(1):95-106.
8. Baronciani D, Angelucci E, Potschger U, Gaziev J, Yesilipek A, Zecca M, Orofino MG, Giardini C, Al-Ahmari A, Marktel S, de la Fuente J, Ghavamzadeh A, Hussein AA, Targhetta C, Pilo F, Locatelli F, Dini G, Bader P, Peters C. Hemopoietic stem cell transplantation in thalassemia: a report from the European Society for Blood and Bone Marrow Transplantation Hemoglobinopathy Registry, 2000-2010. Bone Marrow Transplant 2016;51(4):536-541.
9. Baronciani D, Boumendil A, Dalissier A, Gaziev J, Ghavamzadeh A, La Fuente D, J., Zecca M, Al-Seraihy A, Kupesiz OA, Tbakhi A, Socié G, Kitra-Roussou V, Lankester A, Forni GL, Bader P, Angelucci E. Hematopoietic Cell Transplantation in Thalassemia and Sickle Cell Disease: Report from the European Society for Blood and Bone Marrow Transplantation Hemoglobinopathy Registry: 2000-2017 [abstract]. Blood 2018;132(Suppl 1):168.
10. Li C, Mathews V, Kim S, George B, Hebert K, Jiang H, Li C, Zhu Y, Keesler DA, Boelens JJ, Dvorak CC, Agarwal R, Auletta JJ, Goyal RK, Hanna R, Kasow K, Shenoy S, Smith AR, Walters MC, Eapen M. Related and unrelated donor transplantation for beta-thalassemia major: results of an international survey. Blood Adv 2019;3(17):2562-2570.
11. He Y, Jiang H, Li C, Zhu Y, Wu X, Liu S, Qu Y, Liao J, Feng X, Liu H, Peng Z, He L, Pu C, Gautam M, Leung W, Li C. Long-term results of the NF-08-TM protocol in stem cell transplant for patients with thalassemia major: A multi-center large-sample study. Am J Hematol 2020;95(11):E297-E299.
12. Li C, Wu X, Feng X, He Y, Liu H, Pei F, Liao J, He L, Shi L, Li N, Liu Q, Liu S, Chen G, Su Q, Ren Y, Wang Y, Tan W. A novel conditioning regimen improves outcomes in beta-thalassemia major patients using unrelated donor peripheral blood stem cell transplantation. Blood 2012;120(19):3875-3881.
13. Swaminathan VV, Uppuluri R, Patel S, Ravichandran N, Ramanan KM, Vaidhyanathan L, Ramakrishnan B, Jayakumar I, Raj R. Matched Family versus Alternative Donor Hematopoietic Stem Cell Transplantation for Patients with Thalassemia Major: Experience from a Tertiary Referral Center in South India. Biol Blood Marrow Transplant 2020;26(7):1326-1331.
14. Forni GL, Gianesin B, Musallam KM, Longo F, Rosso R, Lisi R, Gamberini MR, Pinto VM, Graziadei G, Vitucci A, Bonetti F, Musto P, Piga A, Cappellini MD, Borgna-Pignatti C, Webthal p. Overall and complication-free survival in a large cohort of patients with beta-thalassemia major followed over 50 years. Am J Hematol 2023;98(3):381-387.
15. Fleischhauer K, Locatelli F, Zecca M, Orofino MG, Giardini C, De Stefano P, Pession A, Iannone AM, Carcassi C, Zino E, La Nasa G. Graft rejection after unrelated donor hematopoietic stem cell transplantation for thalassemia is associated with nonpermissive HLA-DPB1 disparity in host-versus-graft direction. Blood 2006;107(7):2984-2992.

16. Sacchi N, Ciceri F, Bonifazi F, Algeri M, Gallina A, Pollichieni S, Raggio E, Hadj-Amar B, Lombardini L, Pupella S, Liumbruno G, Cardillo M. Availability of HLA-allele-matched unrelated donors and registry size: Estimation from haplotype frequency in the Italian population. Hum Immunol 2021;82(10):758-766.
17. Kamani NR, Walters MC, Carter S, Aquino V, Brochstein JA, Chaudhury S, Eapen M, Freed BM, Grimley M, Levine JE, Logan B, Moore T, Panepinto J, Parikh S, Pulsipher MA, Sande J, Schultz KR, Spellman S, Shenoy S. Unrelated donor cord blood transplantation for children with severe sickle cell disease: results of one cohort from the phase II study from the Blood and Marrow Transplant Clinical Trials Network (BMT CTN). Biol Blood Marrow Transplant 2012;18(8):1265-1272.
18. Gaziev J, Isgro A, Sodani P, Paciaroni K, De Angelis G, Marziali M, Ribersani M, Alfieri C, Lanti A, Galluccio T, Adorno G, Andreani M. Haploidentical HSCT for hemoglobinopathies: improved outcomes with TCRalpha-beta(+)/CD19(+)-depleted grafts. Blood Adv 2018;2(3):263-270.
19. Luznik L, Bolanos-Meade J, Zahurak M, Chen AR, Smith BD, Brodsky R, Huff CA, Borrello I, Matsui W, Powell JD, Kasamon Y, Goodman SN, Hess A, Levitsky HI, Ambinder RF, Jones RJ, Fuchs EJ. High-dose cyclophosphamide as single-agent, short-course prophylaxis of graft-versus-host disease. Blood 2010;115(16):3224-3230.
20. Raiola AM, Dominietto A, di Grazia C, Lamparelli T, Gualandi F, Ibatici A, Bregante S, Van Lint MT, Varaldo R, Ghiso A, Gobbi M, Carella AM, Signori A, Galaverna F, Bacigalupo A. Unmanipulated haploidentical transplants compared with other alternative donors and matched sibling grafts. Biol Blood Marrow Transplant 2014;20(10):1573-1579.
21. Anurathapan U, Hongeng S, Pakakasama S, Songdej D, Sirachainan N, Pongphitcha P, Chuansumrit A, Charoenkwan P, Jetsrisuparb A, Sanpakit K, Rujkijyanont P, Meekaewkunchorn A, Lektrakul Y, Iamsirirak P, Surapolchai P, Sirireung S, Sruamsiri R, Wahidiyat PA, Andersson BS. Hematopoietic Stem Cell Transplantation for Severe Thalassemia Patients from Haploidentical Donors Using a Novel Conditioning Regimen. Biol Blood Marrow Transplant 2020;26(6):1106-1112.
22. Angelucci E. How I manage iron overload in the hemopoietic cell transplantation setting. Blood 2024.
23. Pilo F, Angelucci E. A storm in the niche: Iron, oxidative stress and haemopoiesis. Blood Rev 2018;32(1):29-35.
24. Pilo F, Angelucci E. Iron Toxicity and Hemopoietic Cell Transplantation: Time to Change the Paradigm. Mediterr J Hematol Infect Dis 2019;11(1):e2019030.
25. Angelucci E, Matthes-Martin S, Baronciani D, Bernaudin F, Bonanomi S, Cappellini MD, Dalle JH, Di Bartolomeo P, de Heredia CD, Dickerhoff R, Giardini C, Gluckman E, Hussein AA, Kamani N, Minkov M, Locatelli F, Rocha V, Sedlacek P, Smiers F, Thuret I, Yaniv I, Cavazzana M, Peters C, Error EI, Parties EPW. Hematopoietic stem cell transplantation in thalassemia major and sickle cell disease: indications and management recommendations from an international expert panel. Haematologica 2014;99(5):811-820.
26. Lucarelli G, Andreani M, Angelucci E. The cure of thalassemia by bone marrow transplantation. Blood Rev 2002;16(2):81-85.
27. Angelucci E, Benz EJ, Jr. UpToDate. Hematopoietic stem cell transplantation and other curative therapies for transfusion-dependent thalassemia. 03 December 2024. Avilable at: https://www.uptodate.com/contents/hematopoietic-stem-cell-transplantation-and-other-curative-therapies-for-transfusion-dependent-thalassemia. Accessed: 26 Dec 2024.
28. Parkman R, Rappeport J, Geha R, Belli J, Cassady R, Levey R, Nathan DG, Rosen FS. Complete correction of the Wiskott-Aldrich syndrome by allogeneic bone-marrow transplantation. N Engl J Med 1978;298(17):921-927.
29. Luftinger R, Zubarovskaya N, Galimard JE, Cseh A, Salzer E, Locatelli F, Algeri M, Yesilipek A, de la Fuente J, Isgro A, Alseraihy A, Angelucci E, Smiers FJ, La La Nasa G, Zecca M, Fisgin T, Unal E, Kleinschmidt K, Peters C, Lankester A, Corbacioglu S, Ebmt Pediatric Diseases IEWP. Busulfan-fludarabine- or treosulfan-fludarabine-based myeloablative conditioning for children with thalassemia major. Ann Hematol 2022;101(3):655-665.
30. Mathews V, George B, Viswabandya A, Abraham A, Ahmed R, Ganapule A, Sindhuvi E, Lakshmi KM, Srivastava A. Improved clinical outcomes of high risk beta thalassemia major patients undergoing a HLA matched related

allogeneic stem cell transplant with a treosulfan based conditioning regimen and peripheral blood stem cell grafts. PLoS One 2013;8(4):e61637.
31. Baronciani D, Angelucci E, Agostinelli F, Mariotti E, Baldassarri M, Martinelli F, Lacerra G, Lucarelli G. Bone marrow transplantation in a thalassemia patient with congenital heart disease. Bone Marrow Transplant 1996;17(1):119-120.
32. Locatelli F, Kabbara N, Ruggeri A, Ghavamzadeh A, Roberts I, Li CK, Bernaudin F, Vermylen C, Dalle JH, Stein J, Wynn R, Cordonnier C, Pinto F, Angelucci E, Socie G, Gluckman E, Walters MC, Rocha V, Eurocord, European B, Marrow Transplantation g. Outcome of patients with hemoglobinopathies given either cord blood or bone marrow transplantation from an HLA-identical sibling. Blood 2013;122(6):1072-1078.
33. Mariotti E, Angelucci E, Agostini A, Baronciani D, Sgarbi E, Lucarelli G. Evaluation of cardiac status in iron-loaded thalassaemia patients following bone marrow transplantation: improvement in cardiac function during reduction in body iron burden. Br J Haematol 1998;103(4):916-921.
34. Muretto P, Angelucci E. Images in clinical medicine. Successful treatment of severe thalassemia. N Engl J Med 1999;341(2):92.
35. Muretto P, Angelucci E, Lucarelli G. Reversibility of cirrhosis in patients cured of thalassemia by bone marrow transplantation. Ann Intern Med 2002;136(9):667-672.
36. Angelucci E, Muretto P, Nicolucci A, Baronciani D, Erer B, Gaziev J, Ripalti M, Sodani P, Tomassoni S, Visani G, Lucarelli G. Effects of iron overload and hepatitis C virus positivity in determining progression of liver fibrosis in thalassemia following bone marrow transplantation. Blood 2002;100(1):17-21.
37. Angelucci E, Brittenham GM, McLaren CE, Ripalti M, Baronciani D, Giardini C, Galimberti M, Polchi P, Lucarelli G. Hepatic iron concentration and total body iron stores in thalassemia major. N Engl J Med 2000;343(5):327-331.
38. Angelucci E, Muretto P, Lucarelli G, Ripalti M, Baronciani D, Erer B, Galimberti M, Giardini C, Gaziev D, Polchi P. Phlebotomy to reduce iron overload in patients cured of thalassemia by bone marrow transplantation. Italian Cooperative Group for Phlebotomy Treatment of Transplanted Thalassemia Patients. Blood 1997;90(3):994-998.
39. Inati A, Kahale M, Sbeiti N, Cappellini MD, Taher AT, Koussa S, Nasr TA, Musallam KM, Abbas HA, Porter JB. One-year results from a prospective randomized trial comparing phlebotomy with deferasirox for the treatment of iron overload in pediatric patients with thalassemia major following curative stem cell transplantation. Pediatr Blood Cancer 2017;64(1):188-196.
40. Scalone L, Mantovani LG, Krol M, Rofail D, Ravera S, Bisconte MG, Borgna-Pignatti C, Borsellino Z, Cianciulli P, Gallisai D, Prossomariti L, Stefano I, Cappellini MD. Costs, quality of life, treatment satisfaction and compliance in patients with beta-thalassemia major undergoing iron chelation therapy: the ITHACA study. Curr Med Res Opin 2008;24(7):1905-1917.
41. Matthes-Martin S, Potschger U, Barr R, Martin M, Boztug H, Klingebiel T, Attarbaschi A, Eibler W, Mann G. Costs and cost-effectiveness of allogeneic stem cell transplantation in children are predictable. Biol Blood Marrow Transplant 2012;18(10):1533-1539.

15 GENE MANIPULATION

Authors: **Franco Locatelli[1,2], Mattia Algeri1,[3]**

[1.] Department of Pediatric Hematology/Oncology and Cell and Gene Therapy, IRCCS Bambino Gesu Children's Hospital, Rome, Italy

[2.] Catholic University of the Sacred Heart, Rome, Italy

[3.] Department of Health Sciences, Magna Graecia University, Catanzaro, Italy

Competing interest: FL reports participating in speaker's bureau with Amgen, Bristol Myers Squibb, Miltenyi Biotec, Neovii, Novartis, SOBI and Vertex Pharmaceuticals; and consultancy for Sanofi, Vertex Pharmaceuticals and Amgen; and honoraria, advisory board participation for Amgen. MA reports scientific advisory board and steering committee participation for Vertex Pharmaceuticals. All competing interests are outside the present work.

1. INTRODUCTION

The advent of gene therapy, driven by advancements in genome sequencing, a deeper understanding of haemoglobin's β-globin *(HBB)* gene cluster and its regulatory mechanisms, as well as innovations in vector engineering and gene-editing technologies, has unlocked new and transformative treatment possibilities for patients with transfusion-dependent β-thalassaemia (TDT) [1]. While allogeneic haematopoietic stem cell transplantation (HSCT) has been the only potentially curative option for over four decades, its optimal safety and efficacy in TDT are mainly observed in patients below the age of 14 years, who are transplanted from an HLA-matched sibling donor. In contrast, older patients and those reliant on alternative donors face greater toxicity risks and significant immune-related complications, resulting in less favourable outcomes [2]. Consequently, the potential for a definitive cure has historically been limited to a minority of thalassaemia patients. Many of the limitations associated with allogeneic HSCT can potentially be overcome through emerging therapies that involve the collection, selection, and ex vivo genetic modification of patient-derived haematopoietic stem cells (HSCs), followed by reinfusion after a myeloablative conditioning regimen. These gene therapy approaches, which include lentiviral transduction, genome editing, base editing, and other cutting-edge strategies, are designed to either promote normal β-globin synthesis or reactivate foetal γ-globin production. This can primarily be accomplished through two main strategies: 'gene addition', which involves the semi-random integration of a functional copy of the therapeutic gene into the cellular genome via viral vectors, or 'gene editing', which induces a disease-modifying effect through the use of site-specific nucleases to precisely target and alter specific genomic sequences. Such interventions effectively reduce the accumulation of excess, unbound α-globin chains, restoring the balance between α and non-α globin within red blood cells (RBCs). This correction ultimately improves erythropoiesis, extends the lifespan of RBCs, and results in a higher number of erythrocytes with increased haemoglobin that persist longer in the bloodstream.

In this Chapter, we will review the main gene addition therapy and gene editing strategies for TDT, with a particular emphasis on those that have progressed to clinical application, discussing the outcomes observed in these contexts.

2. GENE ADDITION TECHNIQUES

Gene addition therapy involves the ex vivo insertion of a lentiviral vector that carries the full regulatory elements and genes responsible for β-globin or γ-globin production into autologous haematopoietic stem and progenitor cells (HSPCs). The development of gene addition therapy for β-thalassaemia has a history spanning over 40 years [3]. Throughout this time, viral vectors have been continuously modified to enhance their safety and efficacy, addressing specific challenges associated with haemoglobinopathies, particularly the requirement for controlled expression of large structural proteins in specific cell types, such as erythrocytes [4]. Furthermore, recent technological advancements have enabled the large-scale production of viral vectors containing the β-globin gene and its regulatory elements, achieving high levels of purification and potency. These vectors are now capable of effectively transducing a significant number of 'non-proliferating' human stem cells, leading to clinically meaningful outcomes [5]. Four major viral vector systems have been developed for gene therapy applications: (1) adenoviral vectors, (2) adeno-associated viral (AAV) vectors, (3) retroviral vectors, including those derived from murine leukaemia virus (MLV), and (4) lentiviral vectors. Each vector system has distinct advantages and limitations for gene therapy applications [5].

Lentiviral vectors integrate directly into the chromatin of the host cell and are able to transduce both proliferating and quiescent cells, including CD34+ HSPCs, making them particularly valuable for gene therapy targeting genetic disorders such as haemoglobinopathies [1]. Lentiviral vector particles enter host cells by interacting with glycoproteins on their surface envelope, subsequently fusing with the host cell membrane. Once inside the cytoplasm, the viral RNA genome is reversely transcribed into complementary DNA (cDNA) via reverse transcriptase. The resulting DNA is then integrated into the host cell genome [1, 5].

2.1. Gene addition: clinical trial results

The first gene-addition therapy clinical trial was conducted in 2007 in Paris, using myeloablative conditioning with busulfan (14 mg/kg) and a self-inactivating lentiviral vector (SIN-LV, HPV569). This vector was flanked by the cHS4 insulator and encoded a β-globin gene harbouring a critical amino acid substitution derived from the γ-globin chain (T87Q) that strongly inhibits the polymerisation of sickle haemoglobin in patients with sickle cell disease (SCD) and also permits precise quantification of vector-derived therapeutic globin expression *in vivo* (HbA^{T87Q}). One patient with compound β^E/β^0 thalassaemia became transfusion-independent 12 months post-treatment, after a dominant clone with an integration site near the *HMGA2* gene emerged. This benign dominant clone contributed to one third of the patient's total haemoglobin, and then gradually diminished over time without triggering oncogenesis [6].

In subsequent studies, the HPV569 vector was optimised by removing the cHS4 insulator and replacing the 5′ LTR promoter with the cytomegalovirus promoter, resulting in the development of the BB305 vector. The product composed of CD34+ HPSCs transduced *ex vivo* with the replication-defective, self-inactivating BB305 lentiviral vector, which facilitates the integration of functional copies of the β^{AT87Q} gene into the patient's genome, has been named betibeglogene autotemcel (beti-cel). In two phase 1/2 clinical trials (HGB-204 [NCT01745120] and HGB-205 [NCT02151526]) involving adolescents and adults, 11 out of 14 patients with TDT and a non-β^0/β^0 genotype achieved transfusion independence (TI) following infusion of beti-

cel. However, in patients achieving TI, the weighted average haemoglobin levels after infusion were generally lower than normal levels, being in the lower part of the range values (as low as 9.1 g/dL) and the amount of haemoglobin deriving from the genetically corrected cells was around 6-6.5 g/dL. Furthermore, only 3 of the 9 patients with a β^0/β^0 genotype or two copies of the IVS1-110 mutation were able to completely discontinue transfusions. It was documented that haemoglobin levels were associated with the vector copy number (VCN) and the percentage of lentiviral vector-positive cells in beti-cel. Therefore, to further improve outcomes, the transduction process was refined to increase the VCN in the drug product, thereby raising the levels of gene therapy-derived HbA^{T87Q} [7].

In a subsequent phase 3 trial (HGB-207, Northstar-2 [NCT02906202]), beti-cel was manufactured using this improved process and, following a myeloablative conditioning regimen with pharmacokinetically adjusted busulfan, the transduced cell product was infused into patients with TDT and a non-β^0/β^0 genotype. In this trial, 20 out of 22 evaluable patients (91%) met the primary endpoint of TI for at least 12 months. The amount of haemoglobin deriving from the genetically corrected cells increased to a median value of 9 g/dL. Patients achieving TI also demonstrated improvements in erythropoiesis and a reduction in liver iron concentration. The safety profile was consistent with that expected for busulfan-based myeloablation. Regarding efficacy, the same study indicated that the level of haemoglobin produced by cells transduced with lentiviral vectors carrying the β-globin gene correlates with both the median VCN and, more significantly, with the percentage of transduced CD34+ HSPCs [8]. More recently, results of another phase 3 trial (HGB-212, Northstar-3 [NCT03207009]), investigating beti-cel in TDT patients with β^0/β^0, $\beta^0/\beta^{+IVS\text{-}I\text{-}110}$, or $\beta^{+IVS\text{-}I\text{-}110}/\beta^{+IVS\text{-}I\text{-}110}$ genotypes were reported. Of the 18 patients who received beti-cel, 13 (72%) were younger than 18 years and five (28%) were older than 18 years. Twelve (67%) patients had β^0/β^0 genotypes, three (17%) had $\beta^0/\beta^{+IVS\text{-}I\text{-}110}$, and three (17%) had $\beta^{+IVS\text{-}I\text{-}110}/\beta^{+IVS\text{-}I\text{-}110}$. As of Jan 30, 2023, all 18 patients were evaluable for TI, with 16 (89%) of 18 reaching and maintaining TI to last follow-up (median follow-up time: 47.9 months; range 23.8-59.0) [9]. TDT patients completing the core trials were eligible to enrol in the ongoing 13-year long-term follow-up study (for a total of 15 years), LTF-303 (NCT02633943) [10].

In 2019, beti-cel received conditional marketing authorisation from the European Medicines Agency (EMA) for use in Europe for the treatment of TDT. However, the developer voluntarily withdrew market authorisation in 2021, not due to concerns regarding safety or efficacy. In 2022, beti-cel was approved by the U.S. Food and Drug Administration (FDA).

In another published phase 1/2 safety study of a lentiviral gene-insertion trial conducted by the San Raffaele Telethon Institute for Gene Therapy (SR-TIGET, NCT02453477), three adults and six children with TDT received intra-bone administration of stem cells transduced with the GLOBE lentiviral vector after a myeloablative conditioning regimen including treosulfan and thiotepa. The treatment resulted in successful haematopoietic engraftment in all patients, without signs of clonal dominance. Three out of four children became independent of blood transfusions, while adult patients experienced only a reduction in their transfusion needs, without achieving TI [11].

In 2012, a phase 1 clinical trial (NCT01639690) was conducted at the Memorial Sloan Kettering Cancer Center (MSKCC) in New York using the TNS9.3.55 β-globin vector in four adult patients

with TDT. Partial myeloablation was applied, resulting in a median cumulative busulfan exposure of 39.8 to 59.7 mg*L/h. This regimen provided stable engraftment but resulted in low *in vivo* VCN in peripheral blood mononuclear cells (PBMCs), with a median VCN of 0.03 (range: 0.01–0.11) at the last follow-up. As a result, only two patients experienced a moderate reduction (35-37%) in transfusion requirements. These findings underscored the critical role of more intensive myeloablation and a minimum CD34+ cell transduction threshold for achieving clinically meaningful benefit in thalassaemia gene therapy with currently available globin vectors. Additionally, moderate clonal expansions were observed near cancer-related gene integrations, suggesting non-erythroid activity of globin vectors in stem/progenitor cells and highlighting the need for careful monitoring of patients treated with globin vectors [12].

3. GENE EDITING TECHNIQUES

The field of genome editing has rapidly advanced due to technological progress, notably with the development of the safer, more effective, and cost-efficient clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) system. The most commonly used genome editing tools are zinc finger nucleases (ZFNs), transcription-activator like effector nucleases (TALENs), and the CRISPR-Cas9 system. Although each system has inherent limitations, the CRISPR/Cas9 system offers significant advantages due to its simplicity, cost-effectiveness, and general efficiency [13].

Genome editing relies on the creation of a double-strand break (DSB) in a sequence-specific manner followed by repair by either non-homologous end joining (NHEJ) or homology-directed repair (HDR) in the presence of a donor template [14, 15]. Direct correction of pathogenic β-globin gene mutations through genome editing is challenging as it requires HDR, which demonstrates significantly lower efficiency as compared to NHEJ, particularly in CD34+ HSPC [1]. By contrast, NHEJ is highly efficient and can be leveraged to modify the genomic sequence through small insertions/deletions (INDELs), resulting in interrupted expression of the targeted gene or by changing the sequence motif required for protein binding [15]. In the context of haemoglobinopathies, genetic disruption strategies relying on the NHEJ are increasingly employed to promote compensatory foetal haemoglobin (HbF) expression, which can ameliorate the clinical severity of these disorders. Indeed, a naturally occurring, benign genetic condition termed hereditary persistence of HbF (HPFH), characterised by pancellular elevation of HbF levels in postnatal RBCs, significantly mitigate the disease phenotype of co-inherited haemoglobinopathies [16-18]. Similarly, neonates and infants with TDT or SCD are typically asymptomatic in the first months of life, while HbF levels are still high, and start experiencing initial disease manifestations only when HbF declines [19]. Globin expression is controlled by a developmentally regulated mechanism known as globin switching and includes the interaction of transcriptional factors with the upstream enhancer cluster and gene promoters [16, 17, 19]. Details regarding the process of globin switching and the regulation of HbF are discussed in Chapter 1. Genome editing approaches aimed at restoring HbF synthesis in adult RBCs primarily focus on introducing naturally occurring mutations associated to elevated HbF levels, disrupting HbF repressors, and modulating epigenetic regulators that control HbF expression [20].

3.1. Gene editing: clinical trial results

One of the main targets of gene-disruption strategies is represented by the transcription factor BCL11A, which acts as key HbF repressor protein binding to a regulatory region around the β-globin gene cluster [21]. Genetic variation in the expression of BCL11A and persistent HbF production have been shown to reduce the clinical severity of β-thalassaemia [22, 23].

Preclinical studies have shown that gene editing approaches that remove the repressive function of the BCL11A protein will lead to increases in HbF and subsequently amelioration of TDT and SCD symptoms [24]. Since BCL11A plays key roles outside the erythroid lineage, such as in the development and function of haematopoietic stem cells and B-lymphocytes, clinical translation of a BCL11A-targeting therapy requires lineage specificity to selectively reduce the BCL11A protein in erythrocytes [25]. Exagamaglogene autotemcel (exa-cel; formerly CTX-001) is a cell therapy that is specifically designed to reactivate HbF through *ex vivo* CRISPR-Cas9 editing in autologous CD34+ HSPCs. This approach targets a critical binding site of the transcription factor GATA1 in the non-coding erythroid lineage-specific enhancer region of BCL11A, located on chromosome 2. Repair of these double-stranded DNA breaks by the cell using NHEJ produces INDELs, disrupting GATA1 binding, and in turn, selectively lowering BCL11A transcription only in erythroid cells [26].

In 2021, Frangoul and colleagues reported on preclinical investigations evaluating the precision of CRISPR-Cas9 editing and preliminary clinical findings from the first two patients treated with exa-cel, one diagnosed with TDT and the other with SCD [26]. The gene editing frequency at the erythroid-specific enhancer of BCL11A in CD34+ HSPCs from ten healthy donors demonstrated high rates of allelic editing (mean 80%) across all CD34+ cell subpopulations, which persisted longitudinally. In immunocompromised murine models, engraftment of CD34+ HSCs was comparable between control and single-guide RNA (sgRNA) CRISPR-Cas9-edited cells. Computational analyses utilising sequence similarity, combined with experimental, high-depth next-generation sequencing (e.g., GUIDE-seq) revealed no detectable off-target effects in edited stem cells from four healthy donors. Functionally, the engraftment potential of edited versus unedited HSCs was equivalent, indicating that CRISPR-Cas9 editing did not adversely affect haematopoietic stem cell functionality. Furthermore, differentiation of edited cells into the erythroid lineage showed increased mean HbF levels compared to unedited controls [26]. From a clinical perspective, after myeloablative conditioning with busulfan followed by exa-cel infusion, both patients achieved successful engraftment of neutrophils and platelets. Increases in total haemoglobin and HbF with pancellular distribution were observed, along with high allelic editing rates in bone marrow and peripheral blood. Notably, after over a year of follow-up, the TDT patient remained free of transfusion requirements, and the SCD patient experienced no vaso-occlusive crises (VOCs) [26].

Exa-cel was then comprehensively evaluated in two phase 3 clinical trials: CLIMB THAL-111 (NCT03655678) in TDT [27] and CLIMB SCD-121 (NCT03745287) in SCD [28]. As of May 2024, 56 TDT participants (mean age of all participants: 21.2 years, range: 12-35), including 35 (62.5%) with severe genotypes (β^0/β^0, β^0/β^0-like), have received exa-cel within the CLIMB THAL-111 trial. The primary efficacy endpoint was TI, defined as proportion of participants maintaining a weighted average haemoglobin ≥9 g/dL without RBC transfusion for ≥12 consecutive months (TI12). Of the 52 participants evaluable for the primary endpoint, 49 achieved TI12 (94.2%;

95%CI: 84.1-98.8), without differences between adolescents and adults. Participants achieving TI12 stopped transfusions at a mean of 1.1 months after exa-cel infusion and remained free from transfusions for up to 5 years (mean 32.4 months, range: 14.3-60.8). Among the remaining 3 patients, two additional subjects became transfusion independent at later time points in the long-term follow up roll-over study, CLIMB-131 (NCT04208529). The mean total haemoglobin was maintained at normal or near normal levels of 12 g/dL from month 5 onward and the mean HbF was 11 g/dL from month 5 onward with pancellular distribution (95% RBCs expressing HbF). The proportion of edited *BCL11A* alleles was stable after infusion in bone marrow CD34+ cells and stable from month 2 onward in peripheral blood nucleated cells. Mean serum ferritin decreased to below baseline by month 12, with 26/56 (46.4%) of participants stopping iron removal therapy [29].

A closely similar approach was employed by a Chinese group to treat two paediatric patients with TDT (7 and 8 years old) who achieved TI and normal haemoglobin levels at 18 months follow-up [30].

The erythroid enhancer region of the *BCL11A* gene has been targeted ex vivo also using the ZFN technology. The product consisting of autologous HSPCs edited with this approach, termed ST-400, has been evaluated in a phase 1/2 trial (THALES, NCT03432364) in five patients with TDT. All participants exhibited only a temporary increase in HbF levels, without any significant or sustained clinical improvement, indicative of suboptimal transduction efficiency in long-term, bone marrow-repopulating HSPCs [31].

Some HPFH variants affects the BCL11A binding motifs in the γ-globin promoter, indicating that disruption of the same motifs by genome-editing-mediated NHEJ could induce HbF therapeutically without ablating the expression of the erythroid repressor [32-34]. Approaches targeting *HGB1* and *HGB2* promoter region are primarily being explored for the treatment of SCD, although they may also theoretically demonstrate efficacy in TDT [35]. The phase 1/2 EdiTHAL study (NCT05444894) uses the autologous HSPCs drug product (renizgamglogene autogedtemcel, reni-cel; formerly EDIT-301) generated by using CRISPR-Cas12a to disrupt the BCL11A binding motif in the γ-globin (*HBG1* and *HBG2*) promoter region. Similar to the CRISPR-Cas9 system, CRISPR-Cas12a is a programmable single RNA-guide endonuclease system. While the Cas12a and Cas9 proteins have functional similarities, there are also substantial differences between these proteins. The Cas12a protein contains a single nuclease domain, unlike the dual nuclease domains of Cas9, and possesses intrinsic RNA processing activity, allowing for multigene editing of RNA transcripts. In addition, whereas CRISPR-Cas9 editing results in blunt DNA ends, Cas12a editing leads to the generation of staggered DNA ends. As of February 2024, 7 patients with TDT had been dosed with reni-cel and all achieved neutrophil and platelet engraftment. Following reni-cel infusion, total haemoglobin, driven by HbF production, remained above the TI threshold of 9.0 g/dL, and increased to 12.8 g/dL by month 6 (n = 3), allowing all 7 subjects to discontinue RBC transfusions for a range of 1.2-9.9 months [36].

The need to rely on the NHEJ pathway to repair the double-stranded breaks induced by gene editing systems such as CRISPR-Cas9, TALEN, and ZFN has led to the development of alternative gene editing techniques that can generate precise point mutations without double-stranded DNA breaks. Base editing is an emerging gene editing approach where nucleotide changes can be introduced at specific genomic locations, generating a DNA point substitution without using

cellular repair pathways [37]. Two types of base editors (BEs) have been developed: cystine base editors which can facilitate the conversion of C:G base pairs to T:A base pairs, while adenine base editors can facilitate the conversion of A:T base pairs to G:C base pairs [38, 39]. These BEs can promote HbF synthesis by introducing point mutations in the γ-globin promoter that disrupt BCL11A binding motifs or create novel binding sites for transcriptional activators. Preliminary data suggest that the formation of new binding motifs, which cannot be easily achieved through Cas9-mediated NHEJ, may result in more robust HbF induction compared to the disruption of repressor motifs or direct targeting of the BCL11A erythroid enhancer [40]. The recently initiated phase 1/2 BEACON (NCT05456880) clinical trial is investigating autologous CD34+ HSPCs edited *ex vivo* to introduce naturally occurring A-to-G substitutions in *HBG1/2* promoters (BEAM-101), thereby disrupting BCL11A repressor binding sites in patients with SCD. Initial results are promising, and this approach may be adaptable for the treatment of TDT as well [41].

Furthermore, adenine BEs have been recently employed in a pre-clinical setting to directly correct the (IVS1-110 [G>A]) mutation, which is one is one of the most common genetic defects responsible for TDT in the Middle East and Mediterranean area [42].

Induction of HbF expression can also be achieved with strategies based on lentiviral vectors. These include (1) the transduction with a lentiviral vector containing a modified γ-globin^{G16D} gene (ARU-1801) [43], (2) upregulation of γ-globin expression via the targeted depletion of BCL11A using shRNA delivered by a lentiviral vector [44], and (c) induction of γ-globin expression by forced chromatin looping mediated by a Zink-Finger/Ldb1-fusion protein expressed from a lentiviral vector [45]. While these methodologies have been mainly investigated for SCD, their potential application in the treatment of TDT patients is theoretically feasible. Despite this evidence of feasibility, further development of one of these strategies, ARU-1801, has been recently interrupted by the manufacturer.

A summary of key clinical trials of gene addition and editing techniques is provided in Table 1.

Table 1. *Gene addition and gene editing trials for transfusion-dependent β-thalassaemia. Most relevant studies for single product and indication are reported.*

Drug prduct (trial identifier)	Mechanism of action	Phase (status)	Study population	Conditioning	Efficacy outcomes	Safety outcomes
TNS9.3.55 *(NCT01639690)*	TNS9.3.55 LVV encoding the normal human β-globin gene	Phase 1 *(Active, not recruiting)*	• TDT • ≥18 years of age • Age range:18-39 years	Non-myeloablative busulfan	• No achievement of TI • Moderate reduction (35-37%) in transfusion requirements in 2/4 patients	• No SAEs or unexpected safety issues related to TNS9.3.55
GLOBE lentiviral vector *(NCT02453477)*	GLOBE LVV encoding the normal human β-globin gene	Phase 1/2 *(Active, not recruiting)*	• TDT • ≥3 and < 65 years of age • 3 children (4-13 years), 6 adults (31-35 years)	Treosulfan-Thiotepa	• Reduction in transfusion requirements in 3/3 adults, without TI • Achievement of TI in 3/4 evaluable children	• Safety profile consistent with myeloablative conditioning, no deaths • No AEs related to GLOBE • No evidence of clonal expansion
Beti-cel *(HGB-207, Northstar-2, NCT02906202)*	BB305 LVV encoding the β^{AT87Q} globin gene	Phase 3 (*Completed*)	• TDT • non-β^0/β^0 genotype • ≤50 years of age • Median age: 15 years (range 4-34), 61% <18 years	Myeloablative, PK-adjusted busulfan	• TI in 91% (n=20) of evaluable participants (n=22)	• Safety consistent with busulfan myeloablation • No deaths and no malignancies • No evidence of insertional oncogenesis
Beti-cel *(HGB-212, Northstar-3, NCT03207009)*	BB305 LVV encoding the β^{AT87Q} globin gene	Phase 3 *(Completed)*	• TDT • β^0/β^0, $\beta^0/\beta^{+IVS-I-110}$, or $\beta^{+IVS-I-110}/\beta^{+IVS-I-110}$ genotypes • ≤50 years of age • Median age: 12.5 years (range 4-33), 72% <18 years	Myeloablative, PK-adjusted busulfan	• TI in 89% (n=16) of evaluable participants (n=18)	• Safety consistent with busulfan myeloablation • No serious adverse events considered to be related to beti-cel • No deaths
ST-400 *(NCT03432364)*	ZFN-based disruption of *BCL11A* erythroid enhancer (enhanced HbF synthesis)	Phase 1/2 *(Completed)*	• TDT • ≥18 and ≤40 years of age • 5 patients, age range:18-36 years	Myeloablative, PK-adjusted busulfan	• Only temporary increase in HbF • No achievement of TI • No significant or sustained clinical improvement	• 1 SAE (hypersensitivity reaction considered to be DMSO-related) • Safety consistent with myeloablation • No clonal expansion

Table 1. *Continued*

Drug prduct (trial identifier)	Mechanism of action	Phase (status)	Study population	Conditioning	Efficacy outcomes	Safety outcomes
Exa-cel *(CLIMB THAL-111, NCT03655678)*	CRISPR/Cas9-based disruption of *BCL11A* erythroid enhancer *(enhanced HbF synthesis)*	Phase 1/2/3 *(Active, not recruiting)*	• TDT • 62.5% with severe genotypes (β^0/β^0, β^0/β^0-like) • ≥12 and ≤35 years of age	Myeloablative, PK-adjusted busulfan	• TI in 49/52 evaluable participants in the CLIMB THAL-111 study • 2/3 remaining subjects achieved TI in the long-term CLIMB-131 study	• Safety consistent with myeloablation • Most AEs/SAEs within the first 6 months after exa-cel infusion • 2 SAEs (1 HLH/ARDS, 1 delayed engraftment) related to exa-cel (and busulfan) that resolved • No deaths, malignancies, or discontinuations due to AEs
- *(NCT04211480)*	CRISPR/Cas9-based disruption of *BCL11A* erythroid enhancer (enhanced HbF synthesis)	Phase 1/2 *(Completed)*	• TDT • ≥5 and ≤15 years of age • 2 patients, 7 and 8 years	Myeloablative, PK-adjusted busulfan	• Achievement of TI in 2/2 TDT children, with normal haemoglobin • levels	• No deaths • No notable AEs on exploratory analysis of single-cell transcriptome and indel patterns in edited PBMCs
Reni-cel *(EdiTHAL, NCT05444894)*	AsCas12a-based editing of the γ-globin gene *(HBG1/2)* promoters (enhanced HbF synthesis)	Phase 1/2 *(Active, recruiting)*	• TDT • ≥18 and ≤35 years of age • 7 patients, age range:18-24 years	Myeloablative, PK-adjusted busulfan	• Achievement of TI in all 7 TDT patients, driven by HbF synthesis	• Safety consistent with myeloablation

Abbreviations: TDT, transfusion-dependent β-thalassaemia; beti-cel, betibeglogene autotemcel; exa-cel, exagamaglogene autotemcel, reni-cel, renizgamglogene autogedtemcel; LVV, lentiviral vector; ZFN, zinc finger nucleases; CRISPR-Cas9, clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9; HbF, foetal haemoglobin; PK, pharmacokinetics; TI, transfusion independence; AEs/SAEs, adverse events/severe adverse events; DMSO, dimethyl sulfoxide; HLH, haemophagocytic lymphohistiocytosis; ARDS, acute respiratory distress syndrome; PBMCs, peripheral blood mononuclear cells.

4. THE JOURNEY OF PATIENTS RECEIVING GENE ADDITION AND GENE EDITING PRODUCTS

Ex vivo gene addition therapy or gene editing for TDT is essentially an autologous HSCT-based procedure, in which patient-derived HSPCs need to be collected and manufactured before the infusion [46]. Autologous HSPCs in TDT are mobilised with granulocyte colony-stimulating factor (G-CSF) plus plerixafor, harvested by apheresis, CD34+ cell-enriched by immunomagnetic separation, and genetically modified *ex vivo*. The process of harvesting and manufacturing the patient's cells before re-infusion can take several months, especially if multiple collection cycles are needed. This is a relevant difference in comparison to allogeneic HSCT, in which, once a suitable donor is identified, the transplantation process can proceed relatively quickly. In TDT patients, the combination of G-CSF and plerixafor is highly effective and the majority of subjects only require one cycle of mobilisation and apheresis to generate a sufficient quantity of gene-modified cells (generally >3 x10^6 CD34+ cells/kg) [8, 27].

Once the product has been manufactured and has completed all necessary quality control assessments, the patient undergoes myeloablative conditioning, followed by the infusion of the gene-modified HSPCs, and remains hospitalised until sustained haematopoietic recovery is achieved.

For the procedure to be successful, the infused gene-modified HSPCs must be introduced into an environment which favours their survival and long-term expansion. Unlike other conditions where gene therapy has demonstrated significant success, such as severe combined immunodeficiency (SCID), corrected cells in haemoglobinopathies have only a modest proliferative advantage over cells harbouring the uncorrected genetic defect [46]. As a consequence, in order to achieve durable engraftment and not to dilute the therapeutic effect, it is crucial to implement a carefully tailored myeloablative conditioning regimen.

Differently from allogeneic transplantation, gene therapy does not require immune ablation involving serotherapy or lympholytic cytotoxic agents. In clinical trials, myeloablative conditioning with either busulfan alone or the combination of treosulfan and thiotepa has been employed [8, 11, 12, 27]. However, only protocols using myeloablative busulfan have consistently achieved TI. An earlier trial utilising non-myeloablative busulfan failed to produce successful outcomes, primarily due to relatively low transduction efficiency of gene-modified cells [12]. In this perspective, it is absolutely mandatory to ensure therapeutic drug monitoring of busulfan to achieve adequate systemic exposure to the alkylating agent, aiming to avoid on one side underexposure, which may result in insufficient myeloablation, and on the other overexposure, which increases the risk of toxicity. Indeed, the myeloablative conditioning regimen is the main factor responsible for the acute toxicities associated with gene therapy, which typically include cytopaenia, mucositis, bacterial infections, and bleeding. The most significant complication observed in largest clinical trials reported so far is represented by the occurrence of hepatic vaso-occlusive disease (VOD), which has been reported in 10-13% of cases after myeloablative busulfan and always resolved with appropriate therapy [8, 27]. In addition, myeloablative chemo-conditioning may also result in long-term sequelae, most importantly, infertility. Although additional long-term concerns include the potential risk of treatment-induced malignancies, a recent systematic literature review suggests that the incidence of busulfan-related secondary malignancies is low, and likely to be substantially

less than 1% in paediatric transplant recipients, especially those receiving busulfan monotherapy for non-malignant conditions other than SCD [47].

In order to avoid the toxicities of chemotherapy, alternative ways of myeloablation are under investigation. These employ the depletion of CD45+ or CD117+ precursors with toxic immune conjugates and hold the promise to achieve successful myeloablation with minimal systemic toxicity [48, 49]. However, the potential effectiveness of these types of immune conjugates-mediated conditioning within the hyperexpanded erythroid marrow peculiar of TDT remains a topic of debate.

In summary, in comparison to allogeneic HSCT, the main advantages of gene therapy for the treatment of thalassaemia are the following:

- Donor independence: gene therapy is based on autologous HSCs sourced directly from the patient. This eliminates the need for an HLA-matched donor and circumvents the limitations and risks associated with donor availability/compatibility.
- Absence/reduction of immune-mediated complications: the use of the patient's own genetically modified HSCs abrogates the risk of graft-versus-host disease (GvHD) and graft rejection, which are major contributors for morbidity and mortality in allogeneic HSCT.
- Lower toxicity: the safety profile observed in clinical trials is generally consistent with single agent busulfan myeloablation in the context of an autologous HSCT procedure.

Thanks to these characteristics, the procedures of gene addition and gene editing result in a more manageable safety profile and, thus, offer broad eligibility for a wider range of patients, allowing the access to curative treatment to subjects who may not be candidates for allogeneic HSCT due to the lack of suitable donors or increased risk factors related to age or comorbidities. Nonetheless, the potential risk for treatment-related toxicity cannot be entirely neglected. Consequently, the administration of gene therapy in TDT candidates with severe pre-existing organ damage, should be approached with caution until substantial real-world data and clinical experience are accrued to better inform patient selection and management strategies.

5. GENOTOXICITY RISKS OF GENE THERAPY

All gene therapy strategies have a theoretical risk of genotoxicity. The integration of lentiviral vector into the genome is not random, exhibiting a preference for transcriptionally active regions, which raises concerns about insertional mutagenesis with lentiviral vectors [50]. To address this risk, third-generation lentiviral vectors, such as those used in the beti-cel gene addition, have been specifically designed to be replication-incompetent and self-inactivating in order to mitigate the potential risk of insertional oncogenesis. A recent meta-analysis of gene therapy trials involving HSPCs for monogenic disorders, encompassing 406 treated patients, demonstrated an improved safety profile for those receiving lentiviral vector-transduced HSPCs as compared to γ-retroviral vector [51].

To date, no cases of insertional oncogenesis have been reported in patients with TDT treated with beti-cel. However, two patients with SCD who received lovotibeglogene autotemcel, an earlier version of the lentiviral vector and manufacturing process used in beti-cel, were diagnosed with acute myeloid leukaemia (AML) at 3 years and 5.5 years post-infusion. In both cases, comprehensive studies did not identify insertional oncogenesis as the cause, although leukaemic blast cells in one patient showed BB305 lentivector insertion. This integration occurred near the *VAMP4* gene, which

has never been implicated in leukaemogenesis/oncogenesis [52, 53]. These findings underscore the importance of long-term surveillance of patients treated with the BB305 lentiviral vector to further elucidate insertional patterns and the associated risks of lentiviral vector transduction.

Genome editing presents several theoretical advantages over lentiviral-mediated gene addition; however, it may result in potentially genotoxic, nonspecific ('off-target') DNA alterations. The development of high-fidelity Cas9 nucleases has been instrumental in reducing the incidence of such off-target events [54]. Thus, comprehensive genome-wide off-target analyses and evaluations for potential genomic rearrangements are essential to validate the safety of gene editing approaches for haemoglobinopathies. Researchers can employ *in silico* prediction tools to model potential off-target events based on the sequence of a given guide RNA (gRNA). Additionally, next-generation sequencing (NGS) assays, such as GUIDE-seq, DISCOVER-seq, and ChIP-seq can be utilised to comprehensively survey the genome for occurrences of off-target editing, thereby confirming the precision of the gene editing process [55]. HSCs are notably susceptible to DSBs, particularly when multiple on-target or simultaneous on-target and off-target events occur [56]. Unintended off-target DSBs, frequently found in genomic regions with partial homology to the sgRNA, may disrupt normal gene function [57]. Despite the precision of these nucleases, the creation of DSBs at both on- and off-target loci can result in extensive chromosomal deletions, structural rearrangements, aneuploidy, or chromothripsis, often accompanied by TP53 pathway activation [58, 59]. Within the phase 3 exa-cel trials, the potential for off-target editing was investigated by nominating candidate off-target sites through a computational homology search and GUIDE-seq (a method that identifies double-strand breaks). Each nominated off-target locus was then tested with the use of high-coverage hybrid-capture sequencing (which identifies insertions and deletions). The CRISPR-Cas9 editing process was precise, with no evidence of off-target editing on the basis of preclinical evaluations in samples from six patients (three patients with TDT and three patients with SCD) and no evidence of chromosomal abnormalities [60]. Despite these results, the limited number of samples, particularly patient-derived samples, analysed and the inherent limitations of detection of hybrid-capture sequencing and GUIDE-seq warrant additional investigation.

That said, it is crucial to acknowledge that the risk of translocations in CRISPR-Cas9 editing applications is mitigated by the high precision of genome editing and the minimisation of off-target editing events. Translocations require the concurrent generation of edits at two distinct genomic sites; thus, in the absence of off-target modifications, conditions that would promote translocation formation are not present. Additionally, chromothripsis has only been demonstrated in cell line models where tumour suppressor pathways have been disrupted, and it has never been observed in primary HSPCs [61].

Compared to Cas9 nucleases, BEs significantly reduce the incidence of DSBs, thereby decreasing certain genotoxic risks. Nevertheless, current base editing technology is still limited by the potential for random mutagenesis and off-target effects in both DNA and RNA due to the non-specific activity of nucleobase deaminase enzymes [62].

6. FINANCIAL HURDLES IN ADOPTION

The results achieved by gene therapy certainly offer new curative opportunities to patients affected with TDT, as heralded by the conditional approval of two different advanced therapy medicinal products (ATMPs) for this specific indication. Nevertheless, the issue of costs, even in high-income countries, may still represent a major obstacle in providing broad access to such treatments [63].

In this regard, the history of beti-cel in Europe represents a paradigmatic example of how cost issues may hamper the widespread adoption of innovative therapies. After receiving conditional marketing approval in Europe, bluebird bio, the manufacturer of Zynteglo (commercial name for beti-cel), proposed a price of 1.65 million Euro (1.9 million USD), which was based on potential savings from 50 years of hypothetical thalassaemia-free life. Although bluebird bio suggested to adopt a payment method diluted over five years and contingent on sustained clinical effectiveness, the product's price remained prohibitively high for public and private payers in many high-income countries and totally unaffordable for low- to middle-income countries. Despite conditional marketing approval, no European country has agreed on payment or reimbursement terms, leaving patients unable to access this treatment. This, along with delayed decisions from other major European Union (EU) countries, led bluebird bio to cease operations in Europe and reorganise in the USA, as announced in August 2021, with complete withdrawal of beti-cel's marketing authorisation from both the EU and the United Kingdom in 2022. Beti-cel is now approved in the USA for paediatric and adult patients with TDT since 2022.

Starting from November 2023, Casgevy (commercial name for exa-cel) has secured regulatory approval for the treatment of TDT in patients aged 12 years and older from authorities in the United Kingdom, USA, Europe, Bahrain, and Saudi Arabia. In the United Kingdom, exa-cel is priced at 1.65 million GBP per patient, although Vertex Pharmaceuticals, the manufacturer, has negotiated a confidential discounted rate for the NHS [64]. In the USA, the cost is set at 2.2 million USD per treatment [65]. Details regarding negotiations with other European regulatory bodies remain limited. However, the anticipated costs are likely to place considerable financial burden on European, universalistic, healthcare systems. Regardless of the outcome of the negotiations in individual countries, a global and dynamic framework for pricing agreements for this new category of treatments is urgently needed. Such a framework should promote transparent, evidence-based discussions that incorporate the therapeutic efficacy claims of pharmaceutical companies with validation by comprehensive, long-term real-world data. It is crucial for global health stakeholders to collaboratively establish standards that encompass drug pricing, research and development investments, production expenses, and the cost-benefit analysis associated with reduced dependency on prolonged supportive care [66]. This comprehensive approach would help mitigate reimbursement complexities and promote the seamless integration of novel therapies into clinical settings. Additional price mitigation may derive from future technological advancements, increased competition, and scalable production, but these improvements are not anticipated in the near term. Establishing sustainable, equitable pricing models is now essential to ensure the accessibility and adoption of groundbreaking gene therapies.

KEY POINTS AND RECOMMENDATIONS

1. The outcomes achieved with gene therapy approaches in experimental studies, along with the initial regulatory approval of these treatments, are beginning to reshape the landscape of potentially curative options for transfusion-dependent β-thalassaemia (TDT), redefining particularly the role of allogeneic transplantation.

2. In this regard, it is critical to emphasise that paediatric patients up to the age of 14 years undergoing allogeneic haematopoietic stem cell transplantation (HSCT) from HLA-identical sibling donors demonstrate excellent clinical outcomes (see Chapter 14). Within this framework, allogeneic HSCT remains the preferred curative intervention and warrants thorough consideration, particularly in regions outside the USA where no gene therapy is yet commercially accessible for patients below 12 years (**Grade B, Class I**).

3. Recent registry data further suggest that, in highly specialised transplantation centres, outcomes of allogeneic HSCT using fully HLA-matched (10/10) unrelated volunteers are comparable to those achieved with HLA-identical sibling donors. Nonetheless, the decision to pursue HSCT from unrelated registry donors in paediatric patients with TDT should be meticulously discussed with the families (**Grade B, Class IIa**). This discussion should encompass the potentially higher risk of immune-mediated complications associated with unrelated donors, along with the anticipated broader availability of gene addition and gene editing strategies in the near future.

4. For patients aged 14 years and older or for those who do not have an HLA-identical family donor, gene therapy instead constitutes an optimal therapeutic option (**Grade C, Class IIa**):

   - Findings from the betibeglogene autotemcel (beti-cel) and exagamaglogene autotemcel (exa-cel) registrational studies did not reveal any specific clinical characteristics associated with an improved safety and efficacy profile.
   - Results were comparable between adolescent and adult subjects, with no observed differences in outcomes based on iron overload status, genotype, transfusion burden, or other factors.
   - Consequently, there are no absolute indicators, within the studied population, to determine which patients should be prioritised for treatment with the either one of the two products.
   - In addition, the unique challenges posed by autologous gene therapy approaches require new frameworks that cannot be directly extrapolated from the experiences and knowledge gained through allogeneic transplantation (Table 2).

Table 2. *Key variables influencing outcomes: comparison between allogeneic haematopoietic stem cell transplantation and autologous gene therapy.*

Variables	Allogeneic HSCT	Autologous gene therapy
Age	• Younger patients had better outcomes than older patients, with an age threshold of around 14 years or younger determined to be optimal	• Majority of treated patients are adolescents and adults (few children enrolled in the beti-cel trial) • The upper age limit for enrolment in clinical trials arbitrarily set at 35-50 years BUT the upper age limit of treated patients is around 35 years in most studies • No differences in outcomes between adolescents and adults in the largest cohorts reported so far • The number of treated patients is still limited
Type of donor	• Data from large cohort studies have confirmed that an HLA-identical family donor is the donor profile that guarantees the best outcome in terms of overall and disease-free survival • Allogeneic HSCT from 10/10 HLA-matched unrelated donors offers excellent outcomes in paediatric patients when performed in highly specialised centres	• Not applicable (autologous HSPCs are employed) • Drug product specific features? - VCN and transduction efficiency in gene-addition therapy have been variably associated with efficacy outcomes - Needs to be further investigated in genome editing trials
Disease severity and iron overload	• Pesaro Criteria provided the proof of concept that prolonged exposure to iron toxicity in TDT is a relevant cause of oxidative damage to human tissues and increases the risk of transplant-related complications and toxicity - Limits: mostly qualitative, not validated in adults, in autologous setting, and in recent years • No other disease-specific features have been consistently associated with outcomes	• No available criteria for patient selection or stratification • No reported differences in main safety and efficacy outcomes according to genotype, transfusion burden, or iron overload status

Abbreviations: HSCT, haematopoietic stem cell transplantation; beti-cel, betibeglogene autotemcel; HSPCs, haematopoietic stem and progenitor cells; VCN, vector copy number; TDT, transfusion-dependent β-thalassaemia.

5. Beti-cel is currently available only in the USA, where it is FDA-approved for paediatric and adult subjects with TDT, irrespective of patient's age and genotype. Exa-cel has been recently approved by regulatory agencies in Europe (EMA), North America (FDA), United Kingdom, Bahrain and Saudi Arabia for TDT patients aged 12 years and above, without an upper age limit. Despite the absence of age limitations, during the decision-making process, it is advisable to conduct an initial selection based on the primary inclusion/exclusion criteria used in the Northstar and CLIMB THAL-111 studies, which supported the submission for regulatory approvals. At least in the initial phases, it is recommended not to consider the treatment of patients who do not meet the eligibility characteristics defined by the registrational studies (**Grade C, Class IIb**):

   - With respect to patient's age, the safety and efficacy of beti-cel was studied in clinical trials that enrolled patients between the ages of 4 and 35 years old, while the exa-cel study was conducted on patients aged 12 to 35 years. Within this age range, the ideal candidate is a patient who exhibits the following characteristics **(Grade C, Class IIa)**:
     - Significant transfusion history (at least 100 mL/kg or at least 10 units/year of packed red blood cells [pRBCs]).
     - Adequate control of iron overload (liver iron concentration [LIC] ≤7 mg/g dry liver weight and cardiac T2* >20 ms).
     - Normal ventricular function and respiratory function tests.
     - Absence of significant hepatosplenomegaly.
     - Absence of gallstone disease.
     - Potential for effective fertility preservation.
     - High level of personal motivation.
     - This type of patient profile is associated with the highest likelihood of success and the lowest risk of treatment-related toxicity. Moreover, this category of patients is most likely to benefit from such an intervention due to the following factors:
       - A greater number of transfusion-free years if treatment is successful, contributing to a longer life expectancy.
       - Lower risk of exacerbation or chronicity of prior damage related to the underlying disease.
       - High probability of successful mobilisation and collection of haematopoietic stem cells (reducing the need for additional cycles beyond the initial one).
       - Decreased risk of delayed engraftment and related complications.
       - Minimisation of treatment-related infertility risk, positively impacting post-treatment quality of life.
   - Patients presenting with at least one of the characteristics listed below should also be considered for treatment, ideally within a short timeframe (24 months), provided that the treatment is administered in centres with proven expertise in managing TDT patients using allogeneic HSCT or gene therapy approaches (**Grade C, Class IIb**):
     - Age between 35 and 45 years.

- Moderate-to-severe iron overload (LIC >7 and <15 mg/g dry liver weight and cardiac T2* <20 and >15 ms).
- Rare erythrocyte phenotypes or a history of alloimmunisation, which could lead to foreseeable difficulties in the identification and long-term availability of suitable RBC units (provided that transfusion support can be ensured during treatment phases).
- Proven intolerance to iron chelating drugs (due to allergic reactions or excessive side effects) that would likely result in a rapidly progressive worsening of iron overload.

REFERENCES

1. Magrin E, Miccio A, Cavazzana M. Lentiviral and genome-editing strategies for the treatment of beta-hemoglobinopathies. Blood 2019;134(15):1203-1213.
2. Algeri M, Lodi M, Locatelli F. Hematopoietic Stem Cell Transplantation in Thalassemia. Hematol Oncol Clin North Am 2023;37(2):413-432.
3. Cline MJ. Perspectives for gene therapy: inserting new genetic information into mammalian cells by physical techniques and viral vectors. Pharmacol Ther 1985;29(1):69-92.
4. Bulcha JT, Wang Y, Ma H, Tai PWL, Gao G. Viral vector platforms within the gene therapy landscape. Signal Transduct Target Ther 2021;6(1):53.
5. Ferrari G, Thrasher AJ, Aiuti A. Gene therapy using haematopoietic stem and progenitor cells. Nat Rev Genet 2021;22(4):216-234.
6. Cavazzana-Calvo M, Payen E, Negre O, Wang G, Hehir K, Fusil F, Down J, Denaro M, Brady T, Westerman K, Cavallesco R, Gillet-Legrand B, Caccavelli L, Sgarra R, Maouche-Chretien L, Bernaudin F, Girot R, Dorazio R, Mulder GJ, Polack A, Bank A, Soulier J, Larghero J, Kabbara N, Dalle B, Gourmel B, Socie G, Chretien S, Cartier N, Aubourg P, Fischer A, Cornetta K, Galacteros F, Beuzard Y, Gluckman E, Bushman F, Hacein-Bey-Abina S, Leboulch P. Transfusion independence and HMGA2 activation after gene therapy of human beta-thalassaemia. Nature 2010;467(7313):318-322.
7. Thompson AA, Walters MC, Kwiatkowski J, Rasko JEJ, Ribeil JA, Hongeng S, Magrin E, Schiller GJ, Payen E, Semeraro M, Moshous D, Lefrere F, Puy H, Bourget P, Magnani A, Caccavelli L, Diana JS, Suarez F, Monpoux F, Brousse V, Poirot C, Brouzes C, Meritet JF, Pondarre C, Beuzard Y, Chretien S, Lefebvre T, Teachey DT, Anurathapan U, Ho PJ, von Kalle C, Kletzel M, Vichinsky E, Soni S, Veres G, Negre O, Ross RW, Davidson D, Petrusich A, Sandler L, Asmal M, Hermine O, De Montalembert M, Hacein-Bey-Abina S, Blanche S, Leboulch P, Cavazzana M. Gene Therapy in Patients with Transfusion-Dependent beta-Thalassemia. N Engl J Med 2018;378(16):1479-1493.
8. Locatelli F, Thompson AA, Kwiatkowski JL, Porter JB, Thrasher AJ, Hongeng S, Sauer MG, Thuret I, Lal A, Algeri M, Schneiderman J, Olson TS, Carpenter B, Amrolia PJ, Anurathapan U, Schambach A, Chabannon C, Schmidt M, Labik I, Elliot H, Guo R, Asmal M, Colvin RA, Walters MC. Betibeglogene Autotemcel Gene Therapy for Non-beta(0)/beta(0) Genotype beta-Thalassemia. N Engl J Med 2022;386(5):415-427.
9. Kwiatkowski JL, Walters MC, Hongeng S, Yannaki E, Kulozik AE, Kunz JB, Sauer MG, Thrasher AJ, Thuret I, Lal A, Tao G, Ali S, Thakar HL, Elliot H, Lodaya A, Lee J, Colvin RA, Locatelli F, Thompson AA. Betibeglogene autotemcel gene therapy in patients with transfusion-dependent, severe genotype beta-thalassaemia (HGB-212): a non-randomised, multicentre, single-arm, open-label, single-dose, phase 3 trial. Lancet 2024;404(10468):2175-2186.
10. Magrin E, Semeraro M, Hebert N, Joseph L, Magnani A, Chalumeau A, Gabrion A, Roudaut C, Marouene J, Lefrere F, Diana JS, Denis A, Neven B, Funck-Brentano I, Negre O, Renolleau S, Brousse V, Kiger L, Touzot F, Poirot C, Bourget P, El Nemer W, Blanche S, Treluyer JM, Asmal M, Walls C, Beuzard Y, Schmidt M, Hacein-Bey-Abina S, Asnafi V, Guichard I, Poiree M, Monpoux F, Touraine P, Brouzes C, de Montalembert M, Payen E, Six E, Ribeil JA, Miccio A, Bartolucci P, Leboulch P, Cavazzana M. Long-term outcomes of lentiviral gene therapy for the beta-hemoglobinopathies: the HGB-205 trial. Nat Med 2022;28(1):81-88.
11. Marktel S, Scaramuzza S, Cicalese MP, Giglio F, Galimberti S, Lidonnici MR, Calbi V, Assanelli A, Bernardo ME, Rossi C, Calabria A, Milani R, Gattillo S, Benedicenti F, Spinozzi G, Aprile A, Bergami A, Casiraghi M, Consiglieri G, Masera N, D'Angelo E, Mirra N, Origa R, Tartaglione I, Perrotta S, Winter R, Coppola M, Viarengo G, Santoleri L, Graziadei G, Gabaldo M, Valsecchi MG, Montini E, Naldini L, Cappellini MD, Ciceri F, Aiuti A, Ferrari G. Intrabone hematopoietic stem cell gene therapy for adult and pediatric patients affected by transfusion-dependent ss-thalassemia. Nat Med 2019;25(2):234-241.

12. Boulad F, Maggio A, Wang X, Moi P, Acuto S, Kogel F, Takpradit C, Prockop S, Mansilla-Soto J, Cabriolu A, Odak A, Qu J, Thummar K, Du F, Shen L, Raso S, Barone R, Di Maggio R, Pitrolo L, Giambona A, Mingoia M, Everett JK, Hokama P, Roche AM, Cantu VA, Adhikari H, Reddy S, Bouhassira E, Mohandas N, Bushman FD, Riviere I, Sadelain M. Lentiviral globin gene therapy with reduced-intensity conditioning in adults with beta-thalassemia: a phase 1 trial. Nat Med 2022;28(1):63-70.
13. Locatelli F, Cavazzana M, Frangoul H, Fuente J, Algeri M, Meisel R. Autologous gene therapy for hemoglobinopathies: From bench to patient's bedside. Mol Ther 2024;32(5):1202-1218.
14. Demirci S, Leonard A, Tisdale JF. Genome editing strategies for fetal hemoglobin induction in beta-hemoglobinopathies. Hum Mol Genet 2020;29(R1):R100-R106.
15. Canver MC, Orkin SH. Customizing the genome as therapy for the beta-hemoglobinopathies. Blood 2016;127(21):2536-2545.
16. Steinberg MH. Fetal hemoglobin in sickle cell anemia. Blood 2020;136(21):2392-2400.
17. Khandros E, Blobel GA. Elevating fetal hemoglobin: recently discovered regulators and mechanisms. Blood 2024;144(8):845-852.
18. Musallam KM, Sankaran VG, Cappellini MD, Duca L, Nathan DG, Taher AT. Fetal hemoglobin levels and morbidity in untransfused patients with beta-thalassemia intermedia. Blood 2012;119(2):364-367.
19. Sankaran VG, Orkin SH. The switch from fetal to adult hemoglobin. Cold Spring Harb Perspect Med 2013;3(1):a011643.
20. Demirci S, Leonard A, Haro-Mora JJ, Uchida N, Tisdale JF. CRISPR/Cas9 for Sickle Cell Disease: Applications, Future Possibilities, and Challenges. Adv Exp Med Biol 2019;1144:37-52.
21. Sankaran VG, Menne TF, Xu J, Akie TE, Lettre G, Van Handel B, Mikkola HK, Hirschhorn JN, Cantor AB, Orkin SH. Human fetal hemoglobin expression is regulated by the developmental stage-specific repressor BCL11A. Science 2008;322(5909):1839-1842.
22. Uda M, Galanello R, Sanna S, Lettre G, Sankaran VG, Chen W, Usala G, Busonero F, Maschio A, Albai G, Piras MG, Sestu N, Lai S, Dei M, Mulas A, Crisponi L, Naitza S, Asunis I, Deiana M, Nagaraja R, Perseu L, Satta S, Cipollina MD, Sollaino C, Moi P, Hirschhorn JN, Orkin SH, Abecasis GR, Schlessinger D, Cao A. Genome-wide association study shows BCL11A associated with persistent fetal hemoglobin and amelioration of the phenotype of beta-thalassemia. Proc Natl Acad Sci U S A 2008;105(5):1620-1625.
23. Galanello R, Sanna S, Perseu L, Sollaino MC, Satta S, Lai ME, Barella S, Uda M, Usala G, Abecasis GR, Cao A. Amelioration of Sardinian beta0 thalassemia by genetic modifiers. Blood 2009;114(18):3935-3937.
24. Hoban MD, Orkin SH, Bauer DE. Genetic treatment of a molecular disorder: gene therapy approaches to sickle cell disease. Blood 2016;127(7):839-848.
25. Bauer DE, Kamran SC, Lessard S, Xu J, Fujiwara Y, Lin C, Shao Z, Canver MC, Smith EC, Pinello L, Sabo PJ, Vierstra J, Voit RA, Yuan GC, Porteus MH, Stamatoyannopoulos JA, Lettre G, Orkin SH. An erythroid enhancer of BCL11A subject to genetic variation determines fetal hemoglobin level. Science 2013;342(6155):253-257.
26. Frangoul H, Altshuler D, Cappellini MD, Chen YS, Domm J, Eustace BK, Foell J, de la Fuente J, Grupp S, Handgretinger R, Ho TW, Kattamis A, Kernytsky A, Lekstrom-Himes J, Li AM, Locatelli F, Mapara MY, de Montalembert M, Rondelli D, Sharma A, Sheth S, Soni S, Steinberg MH, Wall D, Yen A, Corbacioglu S. CRISPR-Cas9 Gene Editing for Sickle Cell Disease and beta-Thalassemia. N Engl J Med 2021;384(3):252-260.
27. Locatelli F, Lang P, Wall D, Meisel R, Corbacioglu S, Li AM, de la Fuente J, Shah AJ, Carpenter B, Kwiatkowski JL, Mapara M, Liem RI, Cappellini MD, Algeri M, Kattamis A, Sheth S, Grupp S, Handgretinger R, Kohli P, Shi D, Ross L, Bobruff Y, Simard C, Zhang L, Morrow PK, Hobbs WE, Frangoul H, Group CT-S. Exagamglogene Autotemcel for Transfusion-Dependent beta-Thalassemia. N Engl J Med 2024;390(18):1663-1676.
28. Frangoul H, Locatelli F, Sharma A, Bhatia M, Mapara M, Molinari L, Wall D, Liem RI, Telfer P, Shah AJ, Cavazzana M, Corbacioglu S, Rondelli D, Meisel R, Dedeken L, Lobitz S, de Montalembert M, Steinberg MH, Walters MC,

Eckrich MJ, Imren S, Bower L, Simard C, Zhou W, Xuan F, Morrow PK, Hobbs WE, Grupp SA, Group CS-S. Exagamglogene Autotemcel for Severe Sickle Cell Disease. N Engl J Med 2024;390(18):1649-1662.

29. Locatelli F, Lang P, Meisel R, Wall D, Corbacioglu S, Li AM, de la Fuente J, Shah AJ, Carpenter B, Kwiatkowski JL, Mapara MY, Liem RI, Cappellini MD, Algeri M, Kattamis A, Sheth S, Grupp SA, Merkeley HL, Kuo KHM, Rupprecht J, Kohli P, Xu G, Ross L, Bobruff Y, Tong B, Hobbs W, Frangoul H. Durable Clinical Benefits with Exagamglogene Autotemcel for Transfusion-Dependent β-Thalassemia [abstract]. Blood 2024;144(Suppl 1):512.
30. Fu B, Liao J, Chen S, Li W, Wang Q, Hu J, Yang F, Hsiao S, Jiang Y, Wang L, Chen F, Zhang Y, Wang X, Li D, Liu M, Wu Y. CRISPR-Cas9-mediated gene editing of the BCL11A enhancer for pediatric beta(0)/beta(0) transfusion-dependent beta-thalassemia. Nat Med 2022;28(8):1573-1580.
31. Walters MC, Smith AR, Schiller GJ, Esrick EB, Williams DA, Gogoleva T, Rouy D, Cockroft BM, Vercellotti GM. Updated Results of a Phase 1/2 Clinical Study of Zinc Finger Nuclease-Mediated Editing of BCL11A in Autologous Hematopoietic Stem Cells for Transfusion-Dependent Beta Thalassemia [abstract]. Blood 2021;138(Suppl 1):3974.
32. Ye L, Wang J, Tan Y, Beyer AI, Xie F, Muench MO, Kan YW. Genome editing using CRISPR-Cas9 to create the HPFH genotype in HSPCs: An approach for treating sickle cell disease and beta-thalassemia. Proc Natl Acad Sci U S A 2016;113(38):10661-10665.
33. Traxler EA, Yao Y, Wang YD, Woodard KJ, Kurita R, Nakamura Y, Hughes JR, Hardison RC, Blobel GA, Li C, Weiss MJ. A genome-editing strategy to treat beta-hemoglobinopathies that recapitulates a mutation associated with a benign genetic condition. Nat Med 2016;22(9):987-990.
34. Metais JY, Doerfler PA, Mayuranathan T, Bauer DE, Fowler SC, Hsieh MM, Katta V, Keriwala S, Lazzarotto CR, Luk K, Neel MD, Perry SS, Peters ST, Porter SN, Ryu BY, Sharma A, Shea D, Tisdale JF, Uchida N, Wolfe SA, Woodard KJ, Wu Y, Yao Y, Zeng J, Pruett-Miller S, Tsai SQ, Weiss MJ. Genome editing of HBG1 and HBG2 to induce fetal hemoglobin. Blood Adv 2019;3(21):3379-3392.
35. Sharma A, Boelens JJ, Cancio M, Hankins JS, Bhad P, Azizy M, Lewandowski A, Zhao X, Chitnis S, Peddinti R, Zheng Y, Kapoor N, Ciceri F, Maclachlan T, Yang Y, Liu Y, Yuan J, Naumann U, Yu VWC, Stevenson SC, De Vita S, LaBelle JL. CRISPR-Cas9 Editing of the HBG1 and HBG2 Promoters to Treat Sickle Cell Disease. N Engl J Med 2023;389(9):820-832.
36. Franghoul H, Hanna R, Walters M, Chang K, Jaskolka M, Kim K, Mei B, Olubunmi A, Thompson A. Reni-cel, the first AsCas12a gene-edited cell therapy, shows promising preliminary results in key clinical outcomes in transfusion-dependent beta-thalassemia patients treated in the EDITHAL trial [abstract]. Hemasphere 2024;8(S1):2727-2728.
37. Rees HA, Liu DR. Base editing: precision chemistry on the genome and transcriptome of living cells. Nat Rev Genet 2018;19(12):770-788.
38. Gaudelli NM, Komor AC, Rees HA, Packer MS, Badran AH, Bryson DI, Liu DR. Programmable base editing of A*T to G*C in genomic DNA without DNA cleavage. Nature 2017;551(7681):464-471.
39. Komor AC, Kim YB, Packer MS, Zuris JA, Liu DR. Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. Nature 2016;533(7603):420-424.
40. Christakopoulos GE, Telange R, Yen J, Weiss MJ. Gene Therapy and Gene Editing for beta-Thalassemia. Hematol Oncol Clin North Am 2023;37(2):433-447.
41. Gupta AO, Sharma A, Frangoul H, Dalal J, Kanter J, Alavi A, DiPersio J, Eapen M, Jaroscak JJ, Ayala E, Ziga ED, Rifkin-Zenenberg S, Minella AC, Chen G, Chen Y, Chockalingam PS, Lin L, Joseney-Antoine M, Ianniello L, Gardner B, Hartigan AJ, Ciaramella G, Goyal S, Simon A, Thompson A, Heeney MM. Initial results from the BEACON clinical study: a phase 1/2 study evaluating the safety and efficacy of a single dose of autologous CD34+ base edited hematopoietic stem cells (BEAM-101) in patients with sickle cell disease with severe vaso-occlusive crises [abstract]. Blood 2024;144(Suppl 1):513.

42. Hardouin G, Antoniou P, Martinucci P, Felix T, Manceau S, Joseph L, Masson C, Scaramuzza S, Ferrari G, Cavazzana M, Miccio A. Adenine base editor-mediated correction of the common and severe IVS1-110 (G>A) beta-thalassemia mutation. Blood 2023;141(10):1169-1179.
43. Grimley M, Asnani M, Shrestha A, Felker S, Lutzko C, Arumugam PI, Witting S, Knight-Madden J, Niss O, Quinn CT, Lo C, Little CR, Dong A, Malik P. Safety and Efficacy of ARU-1801 in Patients with Sickle Cell Disease: Early Results from the Phase 1/2 Momentum Study of a Modified Gamma Globin Gene Therapy and Reduced Intensity Conditioning [abstract]. Blood 2021;138(Suppl 1):3970.
44. Esrick EB, Lehmann LE, Biffi A, Achebe M, Brendel C, Ciuculescu MF, Daley H, MacKinnon B, Morris E, Federico A, Abriss D, Boardman K, Khelladi R, Shaw K, Negre H, Negre O, Nikiforow S, Ritz J, Pai SY, London WB, Dansereau C, Heeney MM, Armant M, Manis JP, Williams DA. Post-Transcriptional Genetic Silencing of BCL11A to Treat Sickle Cell Disease. N Engl J Med 2021;384(3):205-215.
45. Breda L, Motta I, Lourenco S, Gemmo C, Deng W, Rupon JW, Abdulmalik OY, Manwani D, Blobel GA, Rivella S. Forced chromatin looping raises fetal hemoglobin in adult sickle cells to higher levels than pharmacologic inducers. Blood 2016;128(8):1139-1143.
46. Kunz JB, Kulozik AE. Gene Therapy of the Hemoglobinopathies. Hemasphere 2020;4(5):e479.
47. Long-Boyle JR, Kohn DB, Shah AJ, Spencer SM, Sevilla J, Booth C, Lopez Lorenzo JL, Nicoletti E, Shah A, Reatz M, Matos J, Schwartz JD. Busulfan and subsequent malignancy: An evidence-based risk assessment. Pediatr Blood Cancer 2024;71(1):e30738.
48. Palchaudhuri R, Saez B, Hoggatt J, Schajnovitz A, Sykes DB, Tate TA, Czechowicz A, Kfoury Y, Ruchika F, Rossi DJ, Verdine GL, Mansour MK, Scadden DT. Non-genotoxic conditioning for hematopoietic stem cell transplantation using a hematopoietic-cell-specific internalizing immunotoxin. Nat Biotechnol 2016;34(7):738-745.
49. Uchida N, Stasula U, Demirci S, Germino-Watnick P, Hinds M, Le A, Chu R, Berg A, Liu X, Su L, Wu X, Krouse AE, Linde NS, Bonifacino A, Hong SG, Dunbar CE, Lanieri L, Bhat A, Palchaudhuri R, Bennet B, Hoban M, Bertelsen K, Olson LM, Donahue RE, Tisdale JF. Fertility-preserving myeloablative conditioning using single-dose CD117 antibody-drug conjugate in a rhesus gene therapy model. Nat Commun 2023;14(1):6291.
50. Cavazza A, Moiani A, Mavilio F. Mechanisms of retroviral integration and mutagenesis. Hum Gene Ther 2013;24(2):119-131.
51. Tucci F, Galimberti S, Naldini L, Valsecchi MG, Aiuti A. A systematic review and meta-analysis of gene therapy with hematopoietic stem and progenitor cells for monogenic disorders. Nat Commun 2022;13(1):1315.
52. Goyal S, Tisdale J, Schmidt M, Kanter J, Jaroscak J, Whitney D, Bitter H, Gregory PD, Parsons G, Foos M, Yeri A, Gioia M, Voytek SB, Miller A, Lynch J, Colvin RA, Bonner M. Acute Myeloid Leukemia Case after Gene Therapy for Sickle Cell Disease. N Engl J Med 2022;386(2):138-147.
53. Hsieh MM, Bonner M, Pierciey FJ, Uchida N, Rottman J, Demopoulos L, Schmidt M, Kanter J, Walters MC, Thompson AA, Asmal M, Tisdale JF. Myelodysplastic syndrome unrelated to lentiviral vector in a patient treated with gene therapy for sickle cell disease. Blood Adv 2020;4(9):2058-2063.
54. Vakulskas CA, Dever DP, Rettig GR, Turk R, Jacobi AM, Collingwood MA, Bode NM, McNeill MS, Yan S, Camarena J, Lee CM, Park SH, Wiebking V, Bak RO, Gomez-Ospina N, Pavel-Dinu M, Sun W, Bao G, Porteus MH, Behlke MA. A high-fidelity Cas9 mutant delivered as a ribonucleoprotein complex enables efficient gene editing in human hematopoietic stem and progenitor cells. Nat Med 2018;24(8):1216-1224.
55. Tao J, Bauer DE, Chiarle R. Assessing and advancing the safety of CRISPR-Cas tools: from DNA to RNA editing. Nat Commun 2023;14(1):212.
56. Milyavsky M, Gan OI, Trottier M, Komosa M, Tabach O, Notta F, Lechman E, Hermans KG, Eppert K, Konovalova Z, Ornatsky O, Domany E, Meyn MS, Dick JE. A distinctive DNA damage response in human hematopoietic stem cells reveals an apoptosis-independent role for p53 in self-renewal. Cell Stem Cell 2010;7(2):186-197.
57. Cromer MK, Vaidyanathan S, Ryan DE, Curry B, Lucas AB, Camarena J, Kaushik M, Hay SR, Martin RM, Steinfeld I, Bak RO, Dever DP, Hendel A, Bruhn L, Porteus MH. Global Transcriptional Response to CRISPR/Cas9-AAV6-

Based Genome Editing in CD34(+) Hematopoietic Stem and Progenitor Cells. Mol Ther 2018;26(10):2431-2442.
58. Enache OM, Rendo V, Abdusamad M, Lam D, Davison D, Pal S, Currimjee N, Hess J, Pantel S, Nag A, Thorner AR, Doench JG, Vazquez F, Beroukhim R, Golub TR, Ben-David U. Cas9 activates the p53 pathway and selects for p53-inactivating mutations. Nat Genet 2020;52(7):662-668.
59. Kosicki M, Tomberg K, Bradley A. Repair of double-strand breaks induced by CRISPR-Cas9 leads to large deletions and complex rearrangements. Nat Biotechnol 2018;36(8):765-771.
60. Yen A, Zappala Z, Fine RS, Majarian TD, Sripakdeevong P, Altshuler D. Specificity of CRISPR-Cas9 Editing in Exagamglogene Autotemcel. N Engl J Med 2024;390(18):1723-1725.
61. Leibowitz ML, Papathanasiou S, Doerfler PA, Blaine LJ, Sun L, Yao Y, Zhang CZ, Weiss MJ, Pellman D. Chromothripsis as an on-target consequence of CRISPR-Cas9 genome editing. Nat Genet 2021;53(6):895-905.
62. Slesarenko YS, Lavrov AV, Smirnikhina SA. Off-target effects of base editors: what we know and how we can reduce it. Curr Genet 2022;68(1):39-48.
63. Thuret I, Ruggeri A, Angelucci E, Chabannon C. Hurdles to the Adoption of Gene Therapy as a Curative Option for Transfusion-Dependent Thalassemia. Stem Cells Transl Med 2022;11(4):407-414.
64. NICE. Exagamglogene autotemcel for treating transfusion-dependent beta-thalassaemia in people 12 years and over. Technology appraisal guidance. Reference number: TA100311 September 2024. Available at: https://www.nice.org.uk/guidance/ta1003. Accessed: 16 Dec 2024.
65. United States Securities and Exchange Comission. FORM 8-K. Vertex Pharmaceuticals Incorporated. 08 December 2023. Available at: https://www.sec.gov/ix?doc=/Archives/edgar/data/0000875320/000087532023000054/vrtx-20231208.htm. Accessed: 16 Dec 2024.
66. Quach D, Jiao B, Basu A, Bender MA, Hankins J, Ramsey S, Devine B. A landscape analysis and discussion of value of gene therapies for sickle cell disease. Expert Rev Pharmacoecon Outcomes Res 2022;22(6):891-911.

16 NOVEL DISEASE-MODIFYING AGENTS

Authors: Khaled M. Musallam[1,2,3], Sujit Sheth[3], Maria Domenica Cappellini[4], Kevin H. M. Kuo[5], Stefano Rivella[6], Aurelio Maggio[7], Vip Viprakasit[8], Ali T. Taher[9]

[1.] Center for Research on Rare Blood Disorders (CR-RBD), Burjeel Medical City, Abu Dhabi, United Arab Emirates

[2.] Department of Public Health & Epidemiology, Khalifa University, Abu Dhabi, United Arab Emirates

[3.] Division of Hematology/Oncology, Department of Pediatrics, Weill Cornell Medicine, New York, NY, USA

[4.] Unit of Medicine and Metabolic Disease, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

[5.] Division of Hematology, Department of Medicine, University of Toronto, Toronto, ON, Canada

[6.] Division of Hematology, Department of Pediatrics; Raymond G. Perelman Center for Cellular and Molecular Therapeutics; Penn Center for Musculoskeletal Disorders, The Children's Hospital of Philadelphia; RNA Gene Therapeutics Group, Cell and Molecular Biology Affinity Group, Penn Institute for RNA Innovation, Penn Institute for Regenerative Medicine, University of Pennsylvania, Philadelphia, PA, USA

[7.] Campus of Haematology Franco and Piera Cutino, UOC Ematologia e Malattie Rare, Centro ERN, Centro HUB for Thalassemia and Haemoglobinipathy, AOOR Villa Sofia-V. Cervello, Palermo, Italy

[8.] Department of Pediatrics & Thalassemia Center, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand

[9.] Department of Internal Medicine, American University of Beirut Medical Center, Beirut, Lebanon

Competing interest: KMM reports consultancy fees from Novartis, Bristol Myers Squibb (Celgene Corp), Agios Pharmaceuticals, CRISPR Therapeutics, Vifor Pharma, Novo Nordisk and Pharmacosmos; and research funding from Agios Pharmaceuticals and Pharmacosmos. SS reports consultancy fees from Agios Pharmaceuticals, Bristol Myers Squibb and Novo Nordisk; being a member of a clinical trial steering committee for Vertex Pharmaceuticals; and research funding (for clinical trials) from Agios, Bristol Myers Squibb, Novo Nordisk and Regeneron. MDC reports consultancy fees from Novartis, Bristol Myers Squibb (Celgene Corp), Vifor Pharma and Vertex Pharmaceuticals; and research funding from Novartis, Bristol Myers Squibb (Celgene Corp), La Jolla Pharmaceutical Company, Roche, Protagonist Therapeutics and CRISPR Therapeutics. KHMK reports consultancy fees from Agios Pharmaceuticals, Alexion Pharmaceuticals, Bristol Myers Squibb, Forma Therapeutics, Pfizer, Novo Nordisk and Vertex Pharmaceuticals; honoraria from Bristol Myers Squibb; membership on an advisory committee for Agios Pharmaceuticals and Bioverativ/Sanofi/Sangamo; and research funding from Agios Pharmaceuticals and Pfizer. SR reports being a member of the scientific advisory board for Ionis Pharmaceuticals; has acted as a consultant for Disc Medicine, Protagonist Therapeutics, La Jolla Pharmaceutical Company, Meira GTx, GSK, Bristol Myers Squibb, Incyte, Cambridge Healthcare Res, Celgene Corporation, Catenion, First Manhattan Co., FORMA Therapeutics, Ghost Tree Capital, Keros Therapeutics, Noble insight, Sanofi Aventis U.S., Slingshot Insight, Spexis AG, Techspert.io, BVF Partners L.P., Rallybio LLC, venBio Select LLC, ExpertConnect LLC and LifeSci Capital; and is a co-inventor for patents US8058061 B2 C12N 20111115 and US7541179 B2 C12N 20090602. VV reports consultancy fees from Bristol Myers Squibb (Celgene Corp). AM reports being a member of advisory boards for Novartis, Bristol Meyers Squibb, Bluebird Bio and Vertex Pharmaceuticals; and research funding and travel support from Agios Pharmaceuticals. VV reports consultancy fees from Bristol Myers Squibb (Celgene Corp). ATT reports consultancy fees from Novo Nordisk, Bristol Myers Squibb (Celgene Corp), Agios Pharmaceuticals, Pharmacosmos and Roche; and research funding from Novo Nordisk, Bristol Myers Squibb (Celgene Corp), Agios Pharmaceuticals, Pharmacosmos and Roche. All competing interests are outside the present work.

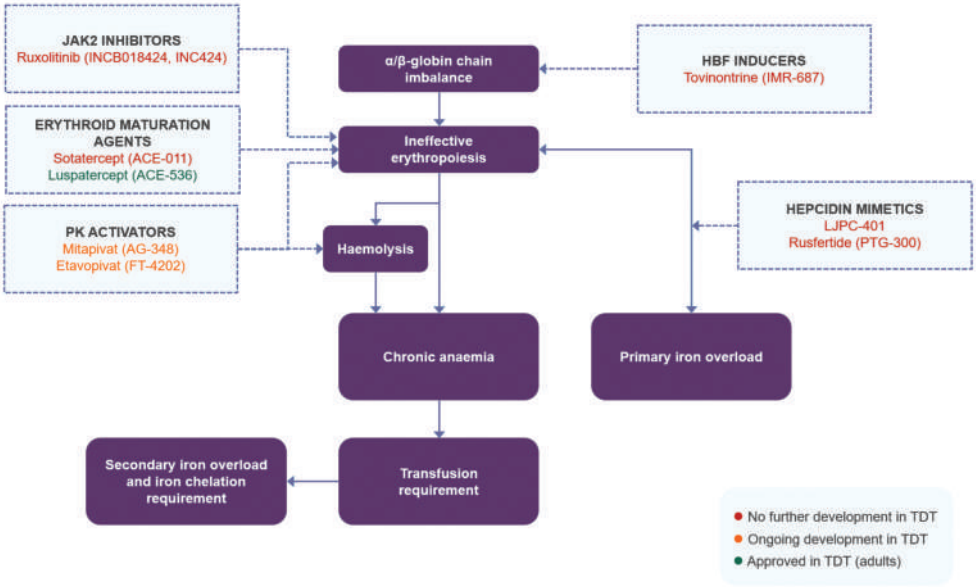
1. INTRODUCTION

In patients with transfusion-dependent β-thalassaemia (TDT), adequate transfusion and iron chelation therapy have surely translated to improved patient survival over the years [1]. However, several challenges hinder optimal patient care, especially in resource-limited settings. In many parts of the world, pretransfusion haemoglobin levels have to be maintained at suboptimal levels due to blood shortage [2, 3], which puts patients at increased risk of anaemia-related morbidity and mortality [4]. Even when blood transfusions are adequate, they come with a considerably added burden of frequent hospital visits, diminished quality of life, and the need for regular iron monitoring and lifelong chelation therapy, which comes with its own challenges of limited availability, poor compliance, or side effects [5-7]

The past few decades witnessed several efforts to evaluate agents and approaches that can reduce the need for transfusion therapy (and thus secondary iron overload and the need for chelation) or abolish it altogether. Therapies with a curative intent include haematopoietic stem cell transplantation (see Chapter 14) and gene manipulation techniques (see Chapter 15). Despite commendable success with most of these approaches, wide application may be limited by donor availability, prohibitive costs and reimbursement challenges, or the need for specialised expertise which may not be available in many parts of the world. The search for disease-modifying agents has thus continued. Several existing drugs have been repurposed for use in TDT patients, especially those with the ability to induce γ-globin gene expression, and consequently foetal haemoglobin (HbF) synthesis. These include hydroxyurea, thalidomide and derivatives, short-chain fatty acids, erythropoietin and derivatives, and DNA methylation inhibitors (e.g., 5-azacytidine and decitabine), sirolimus, or their combinations [8-36]. Although an indication of erythroid response and clinical benefit was evident in some large studies and meta-analyses (especially for hydroxyurea) [9, 10], data were generated mostly in small, non-randomised clinical trials, or larger trials restricted to single country experiences (primarily China, India, Iran, and Pakistan) without dedicated/full development programmes. Responses have also often been restricted to patients with specific molecular profiles or diminished over long-term therapy [11, 12, 37]. Thus, no formal treatment recommendations for any of these agents can be made at this stage. However, efforts to repurpose existing drugs are always met with encouragement as they may support treatment options in low/middle-income countries with limited resources.

With the persisting unmet need, several new disease-modifying agents and programmes have been initiated in the past few years and these will be featured in this Chapter (Figure 1).

Figure 1. *Novel disease-modifying therapies that have been recently developed or approved in transfusion-dependent β-thalassaemia. Abbreviations: JAK2, janus kinase 2; PK, pyruvate kinase; HbF, foetal haemoglobin.*



2. PROGRAMMES HALTED DURING EARLY-PHASE DEVELOPMENT

2.1. Tovinontrine (IMR-687)

Tovinontrine (IMR-687) is a potent, specific, and highly selective small molecule inhibitor of phosphodiesterase (PDE) 9, which mediates cellular signalling pathways by degrading cyclic guanosine monophosphate (cGMP) to its inactive or monophosphate form. By inhibiting PDE9, it increases intracellular cGMP levels and stimulates the production of HbF [38-40]. Forte (NCT04411082) was a phase 2, randomised, double-blind, placebo-controlled trial aiming to evaluate the safety, tolerability, pharmacokinetics, and pharmacodynamics of tovinontrine administered orally once daily for 36 weeks in 120 adult subjects with β-thalassaemia (TDT and non-transfusion-dependent β-thalassaemia [NTDT]). Secondary objectives in TDT patients included reduction in transfusion burden, iron loading rate, iron chelation dose requirements, and serum ferritin levels. Interim results showed sub-optimal clinical benefit and the trial and programme were terminated [41].

2.2. Ruxolitinib (INCB018424, INC424)

Janus Kinase 2 (JAK2) is another signalling molecule that regulates proliferation, differentiation, and survival of erythroid progenitors. Studies in TDT and NTDT mouse models indicated that a short treatment with a JAK2 inhibitor can ameliorate ineffective erythropoiesis and decrease spleen size [42, 43]. Moreover, *in vitro* studies indicated that the JAK2 inhibitor ruxolitinib can induce higher γ-globin gene expression compared with hydroxyurea [44]. A single-arm, phase 2a study (NCT02049450) to evaluate the efficacy and safety of oral ruxolitinib (INCB018424, INC424) administered at a starting dose of 10 mg twice daily among 30 adults with TDT and splenomegaly showed only a slight increase in pretransfusion haemoglobin level and a trend towards transfusion

reduction following 30 weeks of treatment. Reduction in spleen size was the only clinical benefit observed. Levels of HbF were not analysed in this trial. Adverse events included upper respiratory tract infection, nausea, upper abdominal pain, anaemia, diarrhoea, and weight increase [45]. Based on these results no further development was undertaken.

2.3. Hepcidin mimetics

Owing to the observed bidirectional relationship between ineffective erythropoiesis and hepcidin dysregulation in β-thalassaemia, the prospect of achieving an erythroid response through increasing hepcidin levels has been evaluated. In β-thalassaemia mouse models, moderate transgenic hepcidin expression not only decreased iron loading in the liver but also resulted in prolonged red blood cell life span and increased haemoglobin levels [46] In pre-clinical studies, synthetic long-acting hepcidin analogs (minihepcidins) were able to ameliorate ineffective erythropoiesis, anaemia, and splenomegaly [47-49].

A synthetic human hepcidin given as a subcutaneous injection, LJPC-401 [50] was evaluated in a phase 2, multicentre, randomised, open-label study (NCT03381833) in adult patients with TDT with the aim of improving cardiac iron overload among other haematologic benefits. The trial was prematurely terminated due to lack of efficacy on interim analysis [51]. Another injectable hepcidin mimetic, rusfertide (PTG-300), was also evaluated in TRANSCEND (NCT03802201), phase 2, open-label, single-arm, dose-escalation study including adults with NTDT and TDT, with the aim of reducing transfusion burden in patients with TDT. Preliminary data indicated that it may be too early to determine if rusfertide has clinically meaningful activity in patients with TDT, and no further development plans were announced [52].

3. APPROVED AGENTS

3.1. Luspatercept (ACE-536)

3.1.1. Mechanism of action

Luspatercept (ACE-536) is a recombinant fusion protein made of a modified extracellular domain of the human activin receptor type IIB fused to the Fc domain of human IgG1. These in turn bind to select transforming growth factor β superfamily ligands, block SMAD2/3 signalling, and enhance erythroid maturation during late-stage erythropoiesis [53-55]. In β-thalassaemia mouse models, its effects were shown to be mediated by restoring nuclear levels of the transcription factor GATA-1 in erythroid precursors [55], reduction of α-globin chain aggregation and haemolysis, increasing erythrocyte life span, and improving iron overload [54]. It also improved reduced bone mineral density and splenomegaly [53].

3.1.2. Development programme and trials

It is worth noting that the development of luspatercept was preceded by another ligand-trap fusion protein containing the modified extracellular domain of activin receptor type IIA, sotatercept (ACE-011). Although sotatercept was evaluated up to a phase 2 trial in β-thalassaemia adults with positive findings [56], the decision was not to advance sotatercept to phase 3 trials in this patient population, but to only progress with the luspatercept development programme instead.

Following a phase 1 trial indicating safety and tolerability in healthy volunteers [57], luspatercept was evaluated in a multicentre, open-label, dose-ranging phase 2 study (NCT01749540) in adults with β-thalassaemia, confirming its safety and effectiveness in reducing transfusion requirement in TDT and improving haemoglobin levels in NTDT [58], with benefits maintained throughout a 5-year extension study (NCT02268409) [59].

BELIEVE (NCT02604433) was a randomised, double-blind, placebo-controlled phase 3 trial including 336 adults (≥18 years) with TDT [60]. Patients had to have received 6-20 packed red blood cell units in the 24 weeks prior to randomisation with no ≥35-day transfusion-free period during that time. Patients were randomised in a 2:1 ratio to receive luspatercept (subcutaneous injection every three weeks) at a dose of 1.0 mg/kg with titration up to 1.25 mg/kg or placebo for ≥48 weeks. Patients who completed the double-blind period could continue or cross-over to luspatercept in an open label extension for up to 5 years. As soon as a long-term follow up (LTFU) study (NCT04064060) opened, patients were rolled over. Final data analyses for the BELIEVE trial included all patient follow up until they joined the LTFU study.

During the core 48-week treatment period, a significantly greater percentage of patients receiving luspatercept achieved the primary endpoint of a ≥33% reduction in transfusion burden from baseline during weeks 13-24 compared with placebo (21.4% vs 4.5%). Secondary endpoint responses of a ≥33% reduction from weeks 37-48, and ≥50% reduction from weeks 13-24 and weeks 37-48 were also significantly higher in luspatercept than placebo [60]. Analyses of 'rolling intervals' were also conducted, since fixed-period results may not fully reflect observation periods in clinical practice. A significantly greater proportion of luspatercept-treated patients achieved ≥33% and ≥50% reduction during any rolling 12-week or 24-week interval (70.5% vs 29.5% had ≥33% reduction during any 12 weeks). Most common adverse events observed at a higher rate in luspatercept vs placebo included transient bone pain, arthralgia, dizziness, hypertension, and hyperuricemia [60].

Luspatercept responders were more likely to show improvements in quality of life, which was overall maintained during the core treatment period [61], while improvements in iron overload indices were only modest and primarily attributed to iron redistribution due to enhanced erythropoiesis [60, 62].

On long-term follow up, luspatercept-treated patients from the BELIEVE trial continued to experience reductions in transfusion burden in both the open-label extension and LTFU study [63, 64], with no new safety concerns emerging [63, 65]. Data from long-term follow up also started showing considerable improvements in iron overload indices and decreasing trends of overall iron chelation use and dosing [63, 66-68]. A summary of BELIEVE trial key data is provided in Table 1 [69].

Table 1. *Summary of the BELIEVE trial in adults with transfusion-dependent β-thalassaemia. Modified with permission from [69].*

Feature	Details
Design	• Randomised (2:1), double-blind, placebo-controlled phase 3 trial • Countries: Australia, Bulgaria, Canada, France, Greece, Italy, Israel, Lebanon, Malaysia, Taiwan, Thailand, Turkey, Tunisia, United Kingdom, and USA
Population	• 336 adults with TDT (6-20 RBC units per 24 weeks before randomisation, and no ≥35-day transfusion-free period) • β-Thalassaemia and haemoglobin E/β-thalassaemia (concomitant α-globin deletion, mutation, or duplication were allowed)

Duration	• 48 weeks double-blind treatment • Up to 5 years open-label treatment (with cross-over) • 3 years post-treatment follow up • Roll-over to LTFU study up to 5 years
Intervention	• Luspatercept 1 mg/kg subcutaneously every 3 weeks (with up titration to 1.25 mg/kg) (n = 224) • Placebo (n = 112)
Erythroid response	• Primary endpoint (percentage of patients who had a reduction in the transfusion burden of ≥33% from baseline during weeks 13-24 plus a reduction of at least 2 red-cell units over this 12-week interval): 21.4% luspatercept vs 4.5% placebo (p <0.0001) • Primary endpoint in favour of luspatercept across all subgroups • Reduction of ≥33% during weeks 37-48: 19.6% luspatercept vs 3.6% placebo • Reduction of ≥50% during weeks 13-24: 7.6% luspatercept vs 1.8% placebo; during weeks 37-48: 10.3% luspatercept vs 0.9% placebo • Reduction of ≥33% during any 12 weeks: 70.5% luspatercept vs 29.5% placebo; during any 24 weeks: 41.1% luspatercept vs 2.7% placebo • Reduction of ≥50% during any 12 weeks: 40.2% luspatercept vs 6.3% placebo; during any 24 weeks: 16.5% luspatercept vs 2.9% placebo • Non-responders to luspatercept treatment in weeks 13-24 achieved greater transfusion reduction than placebo upon continuing therapy through week 48 • Longer-term data: 77.2% had ≥33% reduction during any 12 weeks after a median treatment duration of 153.6 weeks and 80.4% had ≥33% reduction during any 12 weeks after a median treatment duration of 229.1 weeks
Patient reported outcomes	• No significant changes in quality of life measures between luspatercept and placebo, but luspatercept responders were more likely to show improvements
Iron overload	• No clinically meaningful changes in iron parameters or iron chelator dosing during the 48-week treatment period • Longer-term data: increasing proportion of patients switching to safer iron overload thresholds and decreasing trends of overall iron chelation use and dosing
Adverse events	• Transient bone pain, arthralgia, dizziness, hypertension, and hyperuricemia were more common with luspatercept than placebo • Higher number of thromboembolic events in luspatercept compared with placebo, in patients with pre-existing risk factors • Longer-term data: no additional safety concerns

Abbreviations: TDT, transfusion-dependent β-thalassaemia; RBC, red blood cell; Hb, haemoglobin; LTFU, long-term follow up.

Two safety concerns which have been raised with luspatercept treatment based on higher rates observed in treated patients compared with placebo are thromboembolic events (primarily in TDT, BELIEVE trial) and worsening extramedullary haematopoietic masses (primarily in NTDT, BEYOND trial). However, these mainly occurred in patients with pre-existing risk factors for these complications, which are naturally common in patient with β-thalassaemia due to ineffective erythropoiesis and hypercoagulability [60, 70]. Details of luspatercept's dosing and monitoring recommendations are provided in the product's prescribing information and reviewed recently [69, 71-73].

Based on data from the BELIEVE trial, luspatercept was approved in the USA (2019) and Europe (2020) for the treatment of anaemia in adult patients with TDT. Luspatercept is also now approved in Europe (but not the USA) for the treatment of anaemia in adults with NTDT based on data from the BEYOND trial (NCT03342404) [70]. Clinical trials in paediatric patients (NCT04143724) [74] and in α-thalassaemia (NCT05664737) [75] are ongoing.

3.1.3. Real-world evidence

Data on luspatercept use in real-world settings are now available from few observational studies from Greece [76, 77], Italy [78-80], USA [81], Turkey [82], and Malaysia [83]. Collectively, these data resonate with findings from the BELIEVE trial and reflect erythroid responses (improvement in pretransfusion haemoglobin level, reduction in red blood cell units transfused, increase in transfusion intervals), improvement in iron indices and quality of life. Some new predictors of response were suggested including baseline HbF levels [78]. Some studies also showed that higher baseline transfusion burden patients (compared with BELIEVE) can be successfully treated, patients who had transitioned from NTDT to TDT may achieve transfusion independence, and patients who had difficulty receiving transfusions due to alloimmunisation can be effectively treated with luspatercept. High discontinuation rates, however, alluded to the need for more informed patient selection and treatment optimisation.

3.1.4. Practical insights

Although response with luspatercept was observed across all evaluated patient subgroups in BELIEVE, some subgroups showed different magnitudes or timing of response [60]. Patients who have a non-β^0/β^0 genotype, who are splenectomised, and who have low-moderate transfusion burden may show earlier response, higher magnitude of response, or require lower doses [60, 73, 76, 84]. Further data on predictors of response, ideally including easy-to-measure clinical data or biomarkers, may be imperative for clinicians to prioritise treatment for patients who are most likely to respond; especially in a resource-limited setting. Some guidance based on expert opinion has been recently published [85], and suggests the following group of patients to be assigned priority:

- Patients receiving moderate transfusion regimens (≤4 packed red blood cell units/month)
- Patients with non-β^0/β^0 genotype
- Splenectomised patients
- Patients unable to sustain transfusion regimen for target haemoglobin level
- Patients with progressive iron overload (nonadherence, poor tolerance/response to iron chelation therapy)

According to luspatercept's product label, response (defined as ≥33% reduction in transfusion burden in the European Medicines Agency label and as any reduction in the US Food and Drug Administration label) should be evaluated after two or more doses for potential dose escalation, while treatment discontinuation is recommended if no reduction in transfusion burden is noted

following three doses at the maximum dose (15 weeks from initiation of therapy) [71, 72]. However, delayed response has been observed in the BELIEVE trial, even in patients who did not achieve the primary endpoint of ≥33% reduction during weeks 13-24. Responses of ≥20% reduction in transfusion burden may also be considered clinically meaningful [60, 86, 87]. In some countries where pretransfusion haemoglobin level is maintained at levels below those recommended in these guidelines, improvements in pretransfusion haemoglobin with the same transfusion regimen may also be regarded as 'clinical benefit' [85].

4. ONGOING CLINICAL DEVELOPMENT PROGRAMMES

4.1. Mitapivat (AG-348)

Preclinical studies on pyruvate kinase (PK)-deficient mice have indicated that the metabolic disturbance in PK deficiency alters not only the survival of red blood cells but also the maturation of erythroid progenitors, resulting in ineffective erythropoiesis.

Mitapivat (AG-348) is a first-in-class oral, small-molecule, allosteric activator of the red blood cell-specific form of PK which has shown efficacy and safety and received approval in the USA and Europe for the treatment of haemolytic anaemia in adult patients with PK deficiency [88-91]. In thalassaemia mouse models, it reduced markers of ineffective erythropoiesis and haemolysis, and improved red blood cell survival, anaemia, transfusion burden, and indices of iron overload [92-94]. In an open-label, multicentre, phase 2 study of mitapivat in 20 adults with NTDT (median age 44 years, 50% identifying as Asian; 15 with β-thalassaemia and 5 with α-thalassaemia) and a haemoglobin level ≤10 g/dL, 16 (80%) patients had a response defined as a haemoglobin increase by ≥1.0 g/dL (5/5 in α-thalassaemia and 11/15 in β-thalassaemia). Favourable changes in markers of erythropoiesis and haemolysis were also noted. The most common treatment-emergent adverse events were initial insomnia (50%), dizziness (30%), and headache (25%) [95]. Mitapivat (100 mg orally, twice daily) is currently being evaluated in two phase 3, double-blind, randomised, placebo-controlled, multicentre trials in TDT (ENERGIZE-T, NCT04770779) and NTDT (ENERGIZE, NCT04770753) including both β-thalassaemia and α-thalassaemia. Data from the core follow up period in both trials were announced to positive in 2024, for all primary and key secondary endpoints [96-98].

In ENERGIZE-T, 258 adults with α- or β-TDT from 19 countries were randomised 2:1 to mitapivat 100 mg (n = 171) or placebo (n = 87) twice daily for 48 weeks. Transfusion dependence was defined as 6-20 red blood cell units transfused and a ≤6-week transfusion-free period during the 24-week period before randomisation. Randomisation was stratified by thalassaemia genotype (β^0/β^0 and non-β^0/β^0, including haemoglobin E/β-thalassaemia and α-thalassaemia/haemoglobin H disease) and geographic region. A total of 155 (90.6%) and 83 (95.4%) patients in the mitapivat and placebo arms, respectively, completed the 48-week double-blind period. The mean age was 35.5 years and 44.2% had a β^0/β^0 genotype. The primary end point of a transfusion reduction response defined as ≥50% reduction in transfused red blood cell units and a reduction of ≥2 units in any consecutive 12-week period through Week 48 compared with baseline was achieved in 30.4% of patients in the mitapivat arm vs 12.6% in the placebo arm (p = 0.0003), and response was observed in all pre-defined subgroups, at different effect estimates. Statistically significant reductions in transfusion burden for mitapivat vs placebo were also demonstrated for all other secondary definitions of transfusion response (14.6% vs 1.1% for ≥33% reduction and 7.6% vs 1.1% for ≥50% reduction during Weeks 13 to 48 vs baseline). Treatment-emergent adverse events occurring in ≥10% of

patients on mitapivat were headache, upper respiratory tract infection, initial insomnia, diarrhoea, and fatigue. Serious adverse events were reported in 11.0% and 15.3% of patients on mitapivat and placebo, respectively; and 2.3% and 1.2%, respectively, were considered treatment-related [98]. Patients who completed the core follow up period could cross-over or continue mitapivat therapy for five additional years.

Regulatory filing for mitapivat's for patients with α- and β-TDT (and NTDT) is currently underway, and if successful, this will offer a new oral treatment options for patients with TDT.

4.2. Etavopivat (FT-4202)

Etavopivat (FT-4202) is another PK activator currently being developed for patients with TDT [99]. GLADIOLUS (NCT04987489) is a phase 2, open-label trial evaluating the safety and efficacy of etavopivat in patients with thalassaemia (TDT and NTDT, α and β) or sickle cell disease [100]. The primary endpoint for TDT patients is the proportion of patients with ≥20% reduction in red blood cell transfusions over a continuous 12-week treatment period versus baseline red blood cell transfusion history. Data from this trial are awaited.

5. FUTURE OUTLOOK

Despite multiple failures in recent development programmes, we are fortunate to witness some trials delivering on their endpoints and agents finding their way to the market. Such advances in science and clinical development can only be truly successful if matched with pragmatic access programmes that ensure novel therapies are available to all patients worldwide, considering that most patients with TDT live in resource-limited countries. Application of clinical trial data in the real-world setting may not be as straight forward as expected, and efforts to design educational programmes that can support best practices are essential. The practicalities of altering patients' transfusion programmes and the impact of transfusion reduction on patients' lifestyles and quality of life should be further studied. More importantly, the impact of long-term therapy on iron overload and clinical morbidities should be more thoroughly evaluated in a chronic disease such as thalassaemia. The availability of several new treatment options would require robust evidence to support patient selection through head-to-head comparisons and combinatorial studies.

KEY POINTS AND RECOMMENDATIONS

1. Luspatercept is recommended in transfusion-dependent β-thalassaemia (TDT) adults (≥18 years) to achieve transfusion burden reduction (**Grade B, Class I**).
2. The following patient subgroups may be prioritised for luspatercept treatment (**Grade C, Class IIb**):
   - Patients receiving moderate transfusion regimens (≤4 packed red blood cell units/month).
   - Patients with non-β^0/β^0 genotype.
   - Splenectomised patients.
   - Patients unable to sustain transfusion regimen for target haemoglobin level.
   - Patients with progressive iron overload (nonadherence, poor tolerance/response to iron chelation therapy).
3. Luspatercept treatment dosing should follow local prescribing information; otherwise, the below guidance should be followed (**Grade B, Class I**):
   - Starting dose of 1 mg/kg subcutaneously every 3 weeks.
   - Dose increase to 1.25 mg/kg if patient has no reduction in transfusion burden after at least 2 consecutive doses (6 weeks) of 1 mg/kg.
     - Per US Food and Drug Administration (FDA) product label. In the European Medicine Agency (EMA) label, <33% transfusion burden reduction is required.
   - Treatment discontinuation if patient has no reduction in transfusion burden after 3 consecutive doses (9 weeks) of 1.25 mg/kg (minimum overall treatment duration of at least 15 weeks).
   - Treatment interruption if pre-dose haemoglobin is ≥11.5 g/dL in the absence of transfusions. Treatment can be restarted when haemoglobin is ≤11 g/dL.
   - Dose decrease if increase in haemoglobin is >2 g/dL within 3 weeks and in the absence of transfusions (decrease 1.25 mg to 1.0 mg, 1 mg to 0.8 mg, 0.8 mg to 0.6 mg, interrupt if 0.6 mg).
     - Luspatercept 0.6 mg/kg dose is approved for TDT patients by the US FDA label only; the EMA label states that the lowest dose should be 0.8 mg/kg.
4. Luspatercept treatment adverse event monitoring and management should follow local prescribing information; otherwise, the below guidance should be followed (**Grade B, Class I**):
   - Mild adverse events are generally manageable with over-the-counter analgesics / medications.
   - For patients who experience persistent high-grade adverse events:
     - Treatment may be interrupted until the adverse event resolves.
     - Dosage may be modified.
     - Treatment may be discontinued.
     - Individual patient assessment and close monitoring are essential.
   - Treatment should be discontinued for grade 3 or 4 hypersensitivity reactions.

- Thromboembolic events: considering the higher number of thromboembolic events observed in luspatercept-treated vs placebo patients in the BELIEVE trial, patients should be monitored for signs and symptoms of thromboembolic events and treatment instituted promptly. Thromboembolic risk assessment and prophylaxis in high-risk patients are advised in patients with β-thalassaemia (especially splenectomised adults) irrespective of luspatercept therapy.
- Hypertension: patients treated with luspatercept had an average increase in systolic and diastolic blood pressure of 5 mmHg from baseline:
  - Treatment must be started only if the blood pressure is adequately controlled.
  - Blood pressure should be monitored before each luspatercept administration.
  - Luspatercept dose may require adjustment or may be delayed, and patients should be treated for hypertension.
- Extramedullary haematopoiesis (EMH): cases of EMH were documented during luspatercept therapy across β-thalassaemia trials. Patients should be monitored at initiation and during treatment with luspatercept for signs and symptoms of EMH masses (paraspinal localisation being the most concerning), especially in non-transfusion-dependent or sub-optimally treated transfusion-dependent patients who are naturally at higher risk of EMH.

5. Application of transfusion reduction during luspatercept treatment (i.e., reduction of the number of transfused units vs increasing the transfusion visit interval) and any alternate definitions of transfusion reduction response (e.g., ≥20%) should follow the physician's discretion (**Grade C, Class IIb**).

6. Patients receiving luspatercept should continue to be monitored and managed for iron overload, with necessary adjustments based on changes in the rate of iron intake (**Grade C, Class IIb**).

7. The off-label clinical use and repurposing of drugs with a disease-modifying effect based on available literature in TDT should follow institutional policies and ethical standards (**Grade C, Class IIb**).

REFERENCES

1. Forni GL, Gianesin B, Musallam KM, Longo F, Rosso R, Lisi R, Gamberini MR, Pinto VM, Graziadei G, Vitucci A, Bonetti F, Musto P, Piga A, Cappellini MD, Borgna-Pignatti C, Webthal p. Overall and complication-free survival in a large cohort of patients with beta-thalassemia major followed over 50 years. Am J Hematol 2023;98(3):381-387.
2. El-Beshlawy A, Dewedar H, Hindawi S, Alkindi S, Tantawy AA, Yassin MA, Taher AT. Management of transfusion-dependent beta-thalassemia (TDT): Expert insights and practical overview from the Middle East. Blood Rev 2024;63:101138.
3. Viprakasit V, Gattermann N, Lee JW, Porter JB, Taher AT, Habr D, Martin N, Domokos G, Cappellini MD. Geographical variations in current clinical practice on transfusions and iron chelation therapy across various transfusion-dependent anaemias. Blood Transfus 2013;11(1):108-122.
4. Musallam KM, Barella S, Origa R, Ferrero GB, Lisi R, Pasanisi A, Longo F, Gianesin B, Forni GL. Pretransfusion hemoglobin level and mortality in adults with transfusion-dependent beta-thalassemia. Blood 2024;143(10):930-932.
5. Tang CH, Furnback W, Wang BCM, Tang J, Tang D, Lu MY, Huang VW, Musallam KM. Relationship between transfusion burden, healthcare resource utilization, and complications in patients with beta-thalassemia in Taiwan: A real-world analysis. Transfusion 2021;61(10):2906-2917.
6. Betts M, Flight PA, Paramore LC, Tian L, Milenkovic D, Sheth S. Systematic Literature Review of the Burden of Disease and Treatment for Transfusion-dependent beta-Thalassemia. Clin Ther 2020;42(2):322-337 e322.
7. Weiss M, Parisi Jun M, Sheth S. Clinical and economic burden of regularly transfused adult patients with beta-thalassemia in the United States: A retrospective cohort study using payer claims. Am J Hematol 2019;94(5):E129-E132.
8. Prosdocimi M, Zuccato C, Cosenza LC, Borgatti M, Lampronti I, Finotti A, Gambari R. A Rational Approach to Drug Repositioning in beta-thalassemia: Induction of Fetal Hemoglobin by Established Drugs. Wellcome Open Res 2022;7:150.
9. Algiraigri AH, Wright NAM, Paolucci EO, Kassam A. Hydroxyurea for lifelong transfusion-dependent beta-thalassemia: A meta-analysis. Pediatr Hematol Oncol 2017;34(8):435-448.
10. Hatamleh MI, Chenna VSH, Contractor H, Krishna Mohan GV, Tirumandyam G, Dammas N, Khan MW, Hirani S. Efficacy of Hydroxyurea in Transfusion-Dependent Major beta-Thalassemia Patients: A Meta-Analysis. Cureus 2023;15(4):e38135.
11. Musallam KM, Taher AT, Cappellini MD, Sankaran VG. Clinical experience with fetal hemoglobin induction therapy in patients with beta-thalassemia. Blood 2013;121(12):2199-2212; quiz 2372.
12. Musallam KM, Bou-Fakhredin R, Cappellini MD, Taher AT. 2021 update on clinical trials in beta-thalassemia. Am J Hematol 2021;96(11):1518-1531.
13. Kalantri SA, Ray R, Chattopadhyay A, Bhattacharjee S, Biswas A, Bhattacharyya M. Efficacy of decitabine as hemoglobin F inducer in HbE/beta-thalassemia. Ann Hematol 2018;97(9):1689-1694.
14. Jha K, Ray R, Bhattacharyya M. Decitabine vs hydroxyurea on hemoglobin F induction in patients of E beta thalassemia. Indian J Hematol Blood Transfus 2019;35(1):S52.
15. Zuccato C, Cosenza LC, Zurlo M, Gasparello J, Papi C, D'Aversa E, Breveglieri G, Lampronti I, Finotti A, Borgatti M, Scapoli C, Stievano A, Fortini M, Ramazzotti E, Marchetti N, Prosdocimi M, Gamberini MR, Gambari R. Expression of gamma-globin genes in beta-thalassemia patients treated with sirolimus: results from a pilot clinical trial (Sirthalaclin). Ther Adv Hematol 2022;13:20406207221100648.
16. Chen J, Zhu W, Cai N, Bu S, Li J, Huang L. Thalidomide induces haematologic responses in patients with beta-thalassaemia. Eur J Haematol 2017;99(5):437-441.
17. Shah S, Sheth R, Shah K, Patel K. Safety and effectiveness of thalidomide and hydroxyurea combination in beta-thalassaemia intermedia and major: a retrospective pilot study. Br J Haematol 2020;188(3):e18-e21.

18. Nag A, Radhakrishnan VS, Kumar J, Bhave S, Mishra DK, Nair R, Chandy M. Thalidomide in Patients with Transfusion-Dependent E-Beta Thalassemia Refractory to Hydroxyurea: A Single-Center Experience. Indian J Hematol Blood Transfus 2020;36(2):399-402.
19. Bhurani D, Kapoor J, Yadav N, Khushoo V, Agrawal N, Ahmed R, Arora JS, Mehta P. Experience with combination of hydroxyurea and low-dose thalidomide in transfusion-dependent beta thalassemia patients. Ann Hematol 2021;100(6):1417-1427.
20. Jain M, Chakrabarti P, Dolai TK, Ghosh P, Mandal PK, Baul SN, De R. Comparison of efficacy and safety of thalidomide vs hydroxyurea in patients with Hb E-beta thalassemia - a pilot study from a tertiary care Centre of India. Blood Cells Mol Dis 2021;88:102544.
21. Yang K, Wu Y, Zhou Y, Long B, Lu Q, Zhou T, Wang L, Geng Z, Yin X. Thalidomide for Patients with beta-Thalassemia: A Multicenter Experience. Mediterr J Hematol Infect Dis 2020;12(1):e2020021.
22. Yang K, Wu Y, Ma Y, Xiao J, Zhou Y, Yin X. The association of HBG2, BCL11A, and HBS1L-MYB polymorphisms to thalidomide response in Chinese beta-thalassemia patients. Blood Cells Mol Dis 2020;84:102442.
23. Ali Z, Ismail M, Rehman IU, Rani GF, Ali M, Khan MTM. Long-term clinical efficacy and safety of thalidomide in patients with transfusion-dependent beta-thalassemia: results from Thal-Thalido study. Sci Rep 2023;13(1):13592.
24. Yang WJ, Shi LD, Liang Y, Liang LM, Zhang H, Wang L, Zhou Q. Comparison of Efficacy and Safety Outcomes of Different Doses Schedules of Thalidomide for Treating Moderate-to-Severe beta-Thalassemia Patients. Ther Clin Risk Manag 2024;20:799-809.
25. Ju W, Din G, Huang J, Zheng M, Wang X, Liu L, Wang L, Xuan S, Xiao W, Chen A. Efficacy and safety of thalidomide in patients with beta-thalassemia intermedia and major. Medicine (Baltimore) 2024;103(43):e40328.
26. Bhattacharjee S, Ghosh S, Shaw J, Bhattacharjee S, Bhattacharyya M. Thalidomide and Hydroxyurea in Transfusion-Dependent Thalassemia: Efficacy, Safety Profile and Impact on Quality of Life. Hemoglobin 2024;48(3):161-168.
27. Zhu W, He Y, Huang M, Fu S, Liu Z, Wang X, Li Z, Li X, Chen J, Li Y. Long-Term Follow-Up of Patients Undergoing Thalidomide Therapy for Transfusion-Dependent beta-Thalassaemia: A Single-Center Experience. Int J Gen Med 2024;17:1729-1738.
28. Pinto VM, Romano N, Balocco M, Carrara P, Lamagna M, Quintino S, Castaldi A, Forni GL. Reduction of extramedullary erythropoiesis and amelioration of anemia in a beta-thalassemia patient treated with thalidomide. Am J Hematol 2024;99(3):463-464.
29. Yang K, Liu X, Peng W, Hua F, Li L, Chen K, Zhang J, Luo S, Li W, Ding Y, Chen J, Xiao J. Effects of Thalidomide on Erythropoiesis and Iron Homeostasis in Transfusion-Dependent beta-Thalassemia. Mediterr J Hematol Infect Dis 2024;16(1):e2024001.
30. Jian X, Liu X, Peng W, Li L, Hua F, Chen K, Zhang J, Luo S, Yang K, Wu Y. Long-term efficacy and safety of thalidomide treatment in children with beta -thalassemia major. Pediatr Blood Cancer 2023:e30391.
31. Garg A, Patel K, Shah K, Trivedi D, Raj A, Yadav R, Shah S. Safety and Efficacy of Thalidomide and Hydroxyurea Combination in Beta Thalassemia Patients. Indian J Hematol Blood Transfus 2023;39(1):85-89.
32. Bhattacharjee U, Khadwal A, Shafiq N, Lad D, Sharma P, Das R, Shukla P, Jain A, Prakash G, Malhotra P. A Phase 2 Randomized Controlled Trial of Single-Agent Hydroxyurea Versus Thalidomide Among Adult Transfusion Dependent beta Thalassemia Patients. Indian J Hematol Blood Transfus 2023;39(2):266-275.
33. Ansari SH, Ansari I, Wasim M, Sattar A, Khawaja S, Zohaib M, Hussain Z, Adil SO, Ansari AH, Ansari UH, Farooq F, Masqati NU. Evaluation of the combination therapy of hydroxyurea and thalidomide in beta-thalassemia. Blood Adv 2022;6(24):6162-6168.
34. Lu Y, Wei Z, Yang G, Lai Y, Liu R. Investigating the Efficacy and Safety of Thalidomide for Treating Patients With ss-Thalassemia: A Meta-Analysis. Front Pharmacol 2021;12:814302.

35. Chen JM, Zhu WJ, Liu J, Wang GZ, Chen XQ, Tan Y, Xu WW, Qu LW, Li JY, Yang HJ, Huang L, Cai N, Wang WD, Huang K, Xu JQ, Li GH, He S, Luo TY, Huang Y, Liu SH, Wu WQ, Lu QY, Zhou MG, Chen SY, Li RL, Hu ML, Huang Y, Wei JH, Li JM, Chen SJ, Zhou GB. Safety and efficacy of thalidomide in patients with transfusion-dependent beta-thalassemia: a randomized clinical trial. Signal Transduct Target Ther 2021;6(1):405.
36. Chandra J, Parakh N, Sidharth, Singh N, Sharma S, Goel M, Pemde H. Efficacy and Safety of Thalidomide in Patients With Transfusion-Dependent Thalassemia. Indian Pediatr 2021;58(7):611-616.
37. Mancuso A, Maggio A, Renda D, Di Marzo R, Rigano P. Treatment with hydroxycarbamide for intermedia thalassaemia: decrease of efficacy in some patients during long-term follow up. Br J Haematol 2006;133(1):105-106.
38. McArthur JG, Svenstrup N, Chen C, Fricot A, Carvalho C, Nguyen J, Nguyen P, Parachikova A, Abdulla F, Vercellotti GM, Hermine O, Edwards D, Ribeil JA, Belcher JD, Maciel TT. A novel, highly potent and selective phosphodiesterase-9 inhibitor for the treatment of sickle cell disease. Haematologica 2020;105(3):623-631.
39. Andemariam B, Bronte L, Gordeuk V, Howard J, Kanter J, Eleftheriou P, Pancham S, Hagar R, Clarke L, Blyden G, Lugthart S, Mant T, Fok H, Barroso F, Mason J, Yen J, Attie K, Lufkin J, Ballal R. The safety, pharmacokinetics & pharmacodynamic effects of IMR-687, a highly selective PDE9 inhibitor, in adults with sickle cell disease: phase-2a placebo-controlled & open-label extension studies [abstract]. Hemasphere 2021;5(S2):90.
40. Maciel TT, Carvalho C, Rignault R, Allali S, Andemariam B, OCain JI, Ballal R. PDE9 Inhibition By IMR-687 Improves Markers of Beta-Thalassemia in the Hbb th1/th1 Experimental Mouse Model [abstract]. Blood 2021;138(Suppl 1):945.
41. GLOBE NEWSWIRE. Imara Announces Results of Interim Analyses of Tovinontrine (IMR-687) Phase 2b Clinical Trials in Sickle Cell Disease and Beta-Thalassemia. 05 April 2022. Available at: https://www.globenewswire.com/news-release/2022/04/05/2416438/0/en/Imara-Announces-Results-of-Interim-Analyses-of-Tovinontrine-IMR-687-Phase-2b-Clinical-Trials-in-Sickle-Cell-Disease-and-Beta-Thalassemia.html. Accessed: 14 Dec 2024.
42. Libani IV, Guy EC, Melchiori L, Schiro R, Ramos P, Breda L, Scholzen T, Chadburn A, Liu Y, Kernbach M, Baron-Luhr B, Porotto M, de Sousa M, Rachmilewitz EA, Hood JD, Cappellini MD, Giardina PJ, Grady RW, Gerdes J, Rivella S. Decreased differentiation of erythroid cells exacerbates ineffective erythropoiesis in beta-thalassemia. Blood 2008;112(3):875-885.
43. Casu C, Presti VL, Oikonomidou PR, Melchiori L, Abdulmalik O, Ramos P, Rivella S. Short-term administration of JAK2 inhibitors reduces splenomegaly in mouse models of beta-thalassemia intermedia and major. Haematologica 2018;103(2):e46-e49.
44. Pecoraro A, Troia A, Maggio A, Di Marzo R. Efficacy of Ruxolitinib as Inducer of Fetal Hemoglobin in Primary Erythroid Cultures from Sickle Cell and Beta-Thalassemia Patients. Thalassemia Rep 2019;9(1):8101.
45. Taher AT, Karakas Z, Cassinerio E, Siritanaratkul N, Kattamis A, Maggio A, Rivella S, Hollaender N, Mahuzier B, Gadbaw B, Aydinok Y. Efficacy and safety of ruxolitinib in regularly transfused patients with thalassemia: results from a phase 2a study. Blood 2018;131(2):263-265.
46. Gardenghi S, Ramos P, Marongiu MF, Melchiori L, Breda L, Guy E, Muirhead K, Rao N, Roy CN, Andrews NC, Nemeth E, Follenzi A, An X, Mohandas N, Ginzburg Y, Rachmilewitz EA, Giardina PJ, Grady RW, Rivella S. Hepcidin as a therapeutic tool to limit iron overload and improve anemia in beta-thalassemic mice. J Clin Invest 2010;120(12):4466-4477.
47. Preza GC, Ruchala P, Pinon R, Ramos E, Qiao B, Peralta MA, Sharma S, Waring A, Ganz T, Nemeth E. Minihepcidins are rationally designed small peptides that mimic hepcidin activity in mice and may be useful for the treatment of iron overload. J Clin Invest 2011;121(12):4880-4888.
48. Casu C, Oikonomidou PR, Chen H, Nandi V, Ginzburg Y, Prasad P, Fleming RE, Shah YM, Valore EV, Nemeth E, Ganz T, MacDonald B, Rivella S. Minihepcidin peptides as disease modifiers in mice affected by beta-thalassemia and polycythemia vera. Blood 2016;128(2):265-276.

49. Casu C, Chessa R, Liu A, Gupta R, Drakesmith H, Fleming R, Ginzburg YZ, MacDonald B, Rivella S. Minihepcidins improve ineffective erythropoiesis and splenomegaly in a new mouse model of adult beta-thalassemia major. Haematologica 2020;105(7):1835-1844.
50. Porter J, Kowdley K, Taher A, Vacirca J, Shen Z, Chen S, Chawla L, Tidmarsh G. Effect of LJPC-401 (synthetic human hepcidin) on Iron Parameters in Healthy Adults [abstract]. Blood 2018;132(Suppl 1):2336.
51. Ithanet. La Jolla discontinued LJPC-401 (iron overload drug) in β-thalassaemia patients. 04 December 2019. Available at: https://www.ithanet.eu/latest-information/news/2986-la-jolla-discontinued-ljpc-401-iron-overload-drug-in-thalassemia-patients. Accessed: 14 Dec 2024.
52. Lal A, Voskaridou E, Flevari P, Taher A, Chew LP, Valone F, Gupta S, Viprakasit V. A hepcidin mimetic, PTG-300, demonstrates pharmacodynamic effects indicating reduced iron availability in transfusion-dependent beta-thalassemia subjects [abtsract]. Hemasphere 2020;4(S1):110.
53. Suragani RN, Cadena SM, Cawley SM, Sako D, Mitchell D, Li R, Davies MV, Alexander MJ, Devine M, Loveday KS, Underwood KW, Grinberg AV, Quisel JD, Chopra R, Pearsall RS, Seehra J, Kumar R. Transforming growth factor-beta superfamily ligand trap ACE-536 corrects anemia by promoting late-stage erythropoiesis. Nat Med 2014;20(4):408-414.
54. Suragani RN, Cawley SM, Li R, Wallner S, Alexander MJ, Mulivor AW, Gardenghi S, Rivella S, Grinberg AV, Pearsall RS. Modified activin receptor IIB ligand trap mitigates ineffective erythropoiesis and disease complications in murine β-thalassemia. Blood 2014;123(25):3864-3872.
55. Martinez PA, Li R, Ramanathan HN, Bhasin M, Pearsall RS, Kumar R, Suragani R. Smad2/3-pathway ligand trap luspatercept enhances erythroid differentiation in murine beta-thalassaemia by increasing GATA-1 availability. J Cell Mol Med 2020;24(11):6162-6177.
56. Cappellini MD, Porter J, Origa R, Forni GL, Voskaridou E, Galacteros F, Taher AT, Arlet JB, Ribeil JA, Garbowski M, Graziadei G, Brouzes C, Semeraro M, Laadem A, Miteva D, Zou J, Sung V, Zinger T, Attie KM, Hermine O. Sotatercept, a novel transforming growth factor beta ligand trap, improves anemia in beta-thalassemia: a phase II, open-label, dose-finding study. Haematologica 2019;104(3):477-484.
57. Attie KM, Allison MJ, McClure T, Boyd IE, Wilson DM, Pearsall AE, Sherman ML. A phase 1 study of ACE-536, a regulator of erythroid differentiation, in healthy volunteers. Am J Hematol 2014;89(7):766-770.
58. Piga A, Perrotta S, Gamberini MR, Voskaridou E, Melpignano A, Filosa A, Caruso V, Pietrangelo A, Longo F, Tartaglione I. Luspatercept improves hemoglobin levels and blood transfusion requirements in a study of patients with β-thalassemia. Blood 2019;133(12):1279-1289.
59. Piga A, Longo F, Gamberini MR, Voskaridou E, Ricchi P, Caruso V, Pietrangelo A, Zhang X, Shetty JK, Attie KM, Tartaglione I. Long-term safety and erythroid response with luspatercept treatment in patients with beta-thalassemia. Ther Adv Hematol 2022;13:20406207221134404.
60. Cappellini MD, Viprakasit V, Taher AT, Georgiev P, Kuo KHM, Coates T, Voskaridou E, Liew HK, Pazgal-Kobrowski I, Forni GL, Perrotta S, Khelif A, Lal A, Kattamis A, Vlachaki E, Origa R, Aydinok Y, Bejaoui M, Ho PJ, Chew LP, Bee PC, Lim SM, Lu MY, Tantiworawit A, Ganeva P, Gercheva L, Shah F, Neufeld EJ, Thompson A, Laadem A, Shetty JK, Zou J, Zhang J, Miteva D, Zinger T, Linde PG, Sherman ML, Hermine O, Porter J, Piga A, Investigators B. A Phase 3 Trial of Luspatercept in Patients with Transfusion-Dependent beta-Thalassemia. N Engl J Med 2020;382(13):1219-1231.
61. Cappellini MD, Taher AT, Piga A, Shah F, Voskaridou E, Viprakasit V, Porter JB, Hermine O, Neufeld EJ, Thompson AA, Tang D, Yucel A, Lord-Bessen J, Yu P, Guo S, Shetty JK, Miteva D, Zinger T, Backstrom JT, Oliva EN. Health-related quality of life in patients with beta-thalassemia: Data from the phase 3 BELIEVE trial of luspatercept. Eur J Haematol 2023;111(1):113-124.
62. Garbowski MW, Ugidos M, Risueno A, Shetty JK, Schwickart M, Hermine O, Porter JB, Thakurta A, Vodala S. Luspatercept stimulates erythropoiesis, increases iron utilization, and redistributes body iron in transfusion-dependent thalassemia. Am J Hematol 2024;99(2):182-192.

63. Cappellini MD, Taher AT, Viprakasit V, Georgiev P, Kuo KHM, Holot N, Vilmont V, Medlin LF, Kuo W-L, Lai Y, Bueno LM, Sheth S. Efficacy and Safety of Luspatercept in Patients Enrolled in the BELIEVE Trial: Data from the Phase 3b Long-Term Rollover Study [abstract]. Blood 2023;142(Suppl 1):3849–3850.
64. Cappellini MD, Taher AT, Porter JB, Kuo KHM, Coates TD, Voskaridou E, Forni GL, Perrotta S, Khelif A, Lal A, Kattamis A, Piga A, Hermine O, Holot N, Lersch F, Shetty JK, Vodala S, Zhang J, Miteva D, Viprakasit V. Longer-term analysis of efficacy of luspatercept versus placebo in patients with transfusion-dependent beta-thalassemia enrolled in the BELIEVE study [abstract]. Hemasphere 2022;6(S3):171-172.
65. Viprakasit V, Cappellini MD, Porter JB, Kuo KHM, Coates TD, Voskaridou E, Pinto VM, Tartaglione I, Khelif A, Lal A, Kattamis A, Piga A, Hermine O, Holot N, Lersch F, Shetty JK, Zhang J, Bueno L, Giuseppi AC, Miteva D, Taher AT. Long-term safety results of the BELIEVE study of luspatercept in adults with beta-thalassemia [abstract]. Hemasphere 2022;6(S3):1399-1400.
66. Hermine O, Cappellini MD, Taher AT, Coates TD, Viprakasit V, Voskaridou E, Lal A, Liew HK, Perrotta S, Khelif A, Kattamis A, Shetty JK, Zhang G, Tian Y, Miteva D, Zinger T, Tang D, Backstrom JT, Porter JB. Longitudinal effect of luspatercept treatment on iron overload and iron chelation therapy (ICT) in adult patients (pts) with β-thalassemia in the BELIEVE trial [abstract]. Blood 2020;136(Suppl 1):47-48.
67. Hermine O, Cappellini MD, Taher AT, Coates TD, Viprakasit V, Kattamis A, Shetty JK, Weisskopf MB, Holot N, Vodala S, Kuo W-L, Porter JB. Effect of Luspatercept on Red Blood Cell (RBC) Transfusion Burden, Iron Chelation Therapy (ICT), and Iron Overload in Adults with Transfusion-Dependent β-Thalassemia (TDT) from the BELIEVE Trial: A Long-Term Analysis [abstract]. Blood 2022;140(Suppl 1):8215–8217.
68. Porter JB, Cappellini MD, Kuo KHM, Dimopoulou M, Reverte M, Medlin LF, Sharma S, Wang K, Zhang J, Taher AT. Improvement of Iron Overload Parameters in Patients with Transfusion-Dependent β-Thalassemia Treated with Luspatercept: Data from the Phase 3b Long-Term Rollover Study Following the BELIEVE Trial [abstract]. Blood 2024;144(Suppl 1):2475.
69. Musallam KM, Taher AT. Luspatercept: a treatment for ineffective erythropoiesis in thalassemia. Hematology Am Soc Hematol Educ Program 2024;2024(1):419-425.
70. Taher AT, Cappellini MD, Kattamis A, Voskaridou E, Perrotta S, Piga AG, Filosa A, Porter JB, Coates TD, Forni GL, Thompson AA, Tartaglione I, Musallam KM, Backstrom JT, Esposito O, Giuseppi AC, Kuo WL, Miteva D, Lord-Bessen J, Yucel A, Zinger T, Shetty JK, Viprakasit V, Investigators B. Luspatercept for the treatment of anaemia in non-transfusion-dependent beta-thalassaemia (BEYOND): a phase 2, randomised, double-blind, multicentre, placebo-controlled trial. Lancet Haematol 2022;9(10):e733-e744.
71. Celgene Corporation. Reblozyl® (Reblozyl-aamt) for injections, for subcutaneous use. US Prescribing Information. 2020. Available at: https://www.accessdata.fda.gov/drugsatfda_docs/label/2020/761136orig2lbl.pdf.
72. Reblozyl (Reblozyl) Summary of Product Characteristics. Dublin, Ireland: Bristol Myers Squibb Pharma EIGG; June 2020. Available at: https://www.accessdata.fda.gov/drugsatfda_docs/label/2020/761136orig2lbl.pdf.
73. Sheth S, Taher AT, Coates TD, Kattamis A, Cappellini MD. Management of luspatercept therapy in patients with transfusion-dependent beta-thalassaemia. Br J Haematol 2023;201(5):824-831.
74. Kattamis A, Musallam KM, Perrotta S, Aydinok Y, Ferrero GB, Coates TD, Grabowska O, Bluemmert I, Simcock M, Bueno LM, Viprakasit V. Data from the Dose-Finding Cohorts: A Phase 2a Study of Luspatercept in Pediatric Patients With Beta-Thalassemia. Characterizing Patterns of Transfusion Burden (TB) Reduction in Patients (PTS) with Transfusion-Dependent (TD) Beta-Thalassemia Treated With Luspatercept in the BELIEVE Trial [abstract]. Hemasphere 2024;8(S1):2804-2805.
75. Viprakasit V, Lai Y, Forni GL, Lu M-Y, Yang L, Shi W, Medlin LF, Lersch F, Shetty JK, Bueno LM, Wei R, Kattamis A. Trial in progress: a phase 2, double-blind, randomized, placebo-controlled, multicenter study to evaluate the efficacy and safety of luspatercept to treat anemia in adults with alpha-thalassemia. Hemasphere 2023;7(S3):e19231b19232.

76. Delaporta P, Kourakli A, Delicou S, Diamantidis MD, Kalkana C, Vlachaki E, Eftychiadis E, Lafiatis I, Pantelidou D, Symeonidis A, Lazaris V, Xydaki A, Manafas A, Evliati L, Klonizakis F, Manika I, Papadopoulou D, Chatzieleftheriou M, Toutoudaki K, Kyriakopoulou D, Kattamis A. Real-world data on the use of luspatercept in Greek patients with transfusion dependent thalassemia [abstract]. Blood 2022;140(Suppl 1):8205-8207.
77. Chatzidavid S, Flevari P, Bartzi V, Kopsaftopoulou SA, Syriopoulou S, Anastasiadis G, Dimopoulou M. Improvement of anemia and transfusion independency (TI) achieved with luspatercept in β-thalassemic patients who become transfusion dependent (TD) late in adulthood and/or present alloimmunization and delayed hemolytic transfusion reactions (DHTRs) [abstract]. ASCAT 2024:6448359.
78. Panzieri DL, Consonni D, Scaramellini N, Ausenda G, Granata F, Caponio N, Duca L, Leoni S, Elli S, Ferraresi M, Bolis V, Curcio C, Irrera MA, Maira D, Graziadei G, Cassinerio E, Cappellini MD, Bou-Fakhredin R, Brancaleoni V, Motta I. Real-world efficacy and safety of luspatercept and predictive factors of response in patients with transfusion-dependent beta-thalassemia. Am J Hematol 2024;99(12):2395-2398.
79. Roccotelli D, Grande D, Cicco G, Palma A, Longo MC, Albano F, Vitucci A, Musto P. Real-life experience with luspatercept in transfusion-dependent beta-thalassemia. Ann Hematol 2023;102(10):2965-2967.
80. Gianesin B, Rosso R, Lisi R, Zappu A, Motta I, Longo F, Pinto V, Sanna PMG, De Franceschi L, Ruffo GB, DI Maggio R, Bulla A, Di Giorgio MA, Denotti AR, Panzieri DL, Culcasi M, Quintino S, Marchisio A, Mazzi F, Miciotto F, Barone R, Di Raimondo F, Ferraresi M, Marchetti B, Origa R. Luspatercept in the Treatment of Beta Thalassemia in Italy: Lights and Shadows in Clinical Practice [abstract]. Blood 2023;142(Suppl 1):2475.
81. Sheth S, Glassberg MB, Lee J, Ip Q, Liu JH, Bueno LM. Real-World Characteristics, Treatment Utilization, and Transfusion Burden in Patients with β-Thalassemia Initiating Luspatercept: A US Cohort Study [abstract]. Blood 2023;142(Suppl 1):2476.
82. Anutgan B, Karabilgin S, Kantar O, Kavruk T, Aydinok Y. Exploring the real-life efficacy and safety of luspatercept with a mixed pattern study [abstract]. Hemasphere 2024;8(S1):2812.
83. Khaiteri R, Lau T, Veena S. Retrospective analysis of the response of luspatercept among a small cohort of transfusion dependent beta thalassemia patients in Hospital Ampang [abstract]. TIF International Congress Abstract Book 2023:20.
84. Sheth S, Hermine O, Taher AT, Kuo KHM, Porter JB, Piga AG, Coates TD, Kattamis A, Medlin LF, Kuo W-L, Holot N, Cappellini MD. Effect of luspatercept in β-thalassemia patients with β0/β0 genotype: a subgroup analysis of the BELIEVE study [abstract]. Blood 2022;140(Suppl 1):1946-1948.
85. Musallam KM, Sheth S, Cappellini MD, Kattamis A, Kuo KHM, Taher AT. Luspatercept for transfusion-dependent beta-thalassemia: time to get real. Ther Adv Hematol 2023;14:20406207231195594.
86. Piga A, Cappellini MD, Porter JB, Hermine O, Kuo KHM, Coates TD, Kattamis A, Shah F, Khelif A, Aydinok Y, Zhang G, Shetty JK, Miteva D, Zinger T, Bueno L, Yucel A, Tang D, Taher AT. Benefit of continuing therapy with luspatercept in patients with beta-thalassemia who do not achieve ≥33% reduction in red blood cell (rbc) transfusion burden (tb) in weeks 13–24 in the BELIEVE trial [abstract]. Hemasphere 2021;5(S2):631.
87. Kuo KHM, Kattamis A, Taher AT, Motta I, Cappellini MD, Musallam KM, Bueno LM, Perin M, Medlin LF, Martin-Regueira P, Lai Y, Ugidos M, Sheth S. Characterizing Patterns of Transfusion Burden (TB) Reduction in Patients (PTS) with Transfusion-Dependent (TD) Beta-Thalassemia Treated With Luspatercept in the BELIEVE Trial [abstract]. Hemasphere 2023;8(S1):2816-2817.
88. Grace RF, Rose C, Layton DM, Galacteros F, Barcellini W, Morton DH, van Beers EJ, Yaish H, Ravindranath Y, Kuo KHM, Sheth S, Kwiatkowski JL, Barbier AJ, Bodie S, Silver B, Hua L, Kung C, Hawkins P, Jouvin MH, Bowden C, Glader B. Safety and Efficacy of Mitapivat in Pyruvate Kinase Deficiency. N Engl J Med 2019;381(10):933-944.
89. Yang H, Merica E, Chen Y, Cohen M, Goldwater R, Kosinski PA, Kung C, Yuan ZJ, Silverman L, Goldwasser M, Silver BA, Agresta S, Barbier AJ. Phase 1 Single- and Multiple-Ascending-Dose Randomized Studies of the Safety, Pharmacokinetics, and Pharmacodynamics of AG-348, a First-in-Class Allosteric Activator of Pyruvate Kinase R, in Healthy Volunteers. Clin Pharmacol Drug Dev 2019;8(2):246-259.

90. Al-Samkari H, Galacteros F, Glenthoj A, Rothman JA, Andres O, Grace RF, Morado-Arias M, Layton DM, Onodera K, Verhovsek M, Barcellini W, Chonat S, Judge MP, Zagadailov E, Xu R, Hawkins P, Beynon V, Gheuens S, van Beers EJ, Investigators A. Mitapivat versus Placebo for Pyruvate Kinase Deficiency. N Engl J Med 2022;386(15):1432-1442.
91. Glenthoj A, van Beers EJ, Al-Samkari H, Viprakasit V, Kuo KHM, Galacteros F, Chonat S, Porter J, Zagadailov E, Xu R, Oluyadi A, Hawkins P, Gheuens S, Beynon V, Barcellini W, investigators A-T. Mitapivat in adult patients with pyruvate kinase deficiency receiving regular transfusions (ACTIVATE-T): a multicentre, open-label, single-arm, phase 3 trial. Lancet Haematol 2022;9(10):e724-e732.
92. Aizawa S, Harada T, Kanbe E, Tsuboi I, Aisaki K, Fujii H, Kanno H. Ineffective erythropoiesis in mutant mice with deficient pyruvate kinase activity. Exp Hematol 2005;33(11):1292-1298.
93. Matte A, Kosinski PA, Federti E, Dang L, Recchiuti A, Russo R, Siciliano A, Riccardi V, Janin A, Mucci M, Leboeuf C, Iolascon A, Brugnara C, De Franceschi L. Mitapivat, a pyruvate kinase activator, improves transfusion burden and reduces iron overload in beta-thalassemic mice. Haematologica 2023;108(9):2535-2541.
94. Matte A, Federti E, Kung C, Kosinski PA, Narayanaswamy R, Russo R, Federico G, Carlomagno F, Desbats MA, Salviati L, Leboeuf C, Valenti MT, Turrini F, Janin A, Yu S, Beneduce E, Ronseaux S, Iatcenko I, Dang L, Ganz T, Jung CL, Iolascon A, Brugnara C, De Franceschi L. The pyruvate kinase activator mitapivat reduces hemolysis and improves anemia in a beta-thalassemia mouse model. J Clin Invest 2021;131(10):e144206.
95. Kuo KHM, Layton DM, Lal A, Al-Samkari H, Bhatia J, Kosinski PA, Tong B, Lynch M, Uhlig K, Vichinsky EP. Safety and efficacy of mitapivat, an oral pyruvate kinase activator, in adults with non-transfusion dependent alpha-thalassaemia or beta-thalassaemia: an open-label, multicentre, phase 2 study. Lancet 2022;400(10351):493-501.
96. Taher A, Al-Samkari H, Aydinok Y, Bartolucci P, Besser M, Dahlin JL, Estepp JH, Gheuens S, Gilroy KS, Glenthøj A, Goh AS, Iyer V, Kattamis A, Loggetto SR, Morris S, Musallam KM, Osman K, Ricchi P, Salido-Fiérrez E, Sheth S, Tai F, Tevich H, Uhlig K, Urbstonaitis R, Viprakasit V, Cappellini MD, Kuo KHM. ENERGIZE: A Global Phase 3 Study of Mitapivat Demonstrating Efficacy and Safety in Adults with Alpha- or Beta- Non–Transfusion-Dependent Thalassemia [abstract]. Hemasphere 2024;8(S1):11-12.
97. Kuo KHM, Al-Samkari H, Aydinok Y, Besser M, Boscoe AN, De Luna G, Estepp JH, Gheuens S, Gilroy KS, Glenthøj A, Goh AS, Kattamis A, Loggetto SR, Morris S, Musallam KM, Osman K, Ricchi P, Salido-Fiérrez E, Sheth S, Tai F, Uhlig K, Viprakasit V, Cappellini MD, Taher A. Improvements in Fatigue and 6-Minute Walk Test in Adults with Alpha- or Beta-Non–Transfusion-Dependent Thalassemia: The Phase 3 ENERGIZE Trial of Mitapivat [abstract]. Hemasphere 2024;8(S1):2830-2831.
98. Cappellini MD, Sheth S, Taher AT, Al-Samkari H, Antmen AB, Beneitez D, Cannas G, Coates TD, Czapla L, Dahlin JL, Estepp JH, Feenstra E, Georgiev P, Gheuens S, Gilroy KS, Glenthøj A, Musallam K, Osman K, Porter JB, Shao H, Uhlig K, Van Beers EJ, Viprakasit V, Kuo KHM, Kattamis A. ENERGIZE-T: A Global, Phase 3, Double-Blind, Randomized, Placebo-Controlled Study of Mitapivat in Adults with Transfusion-Dependent Alpha- or Beta-Thalassemia. Blood 2024;144(Suppl 1):409-411.
99. Forsyth S, Schroeder P, Geib J, Vrishabhendra L, Konstantinidis DG, LaSalvia K, Ribadeneira MD, Wu E, Kelly P, Kalfa TA. Safety, Pharmacokinetics, and Pharmacodynamics of Etavopivat (FT-4202), an Allosteric Activator of Pyruvate Kinase-R, in Healthy Adults: A Randomized, Placebo-Controlled, Double-Blind, First-in-Human Phase 1 Trial. Clin Pharmacol Drug Dev 2022;11(5):654-665.
100. Lal A, Clark Brown R, Coates T, Kalfa T, Kwiatkowski J, Brevard J, Trenor C, Wood K, Sheth S. Trial in progress: a phase 2, open-label study evaluating the safety and efficacy of the erythrocyte pyruvate kinase activator etavopivat in patients with thalassemia or sickle cell disease [abstract]. Hemasphere 2022;6(S3):2102-2103.

17 THE VALUE OF PATIENTS' ENGAGEMENT: THE EXAMPLE OF THE THALASSAEMIA INTERNATIONAL FEDERATION

Authors: **Androulla Eleftheriou**[1], **Angelo Loris Brunetta**[1,2,3], **George Constantinou**[1,4]

[1] Thalassaemia International Federation (TIF), Nicosia, Cyprus
[2] Fondazione Italiana "Leonardo Giambrone" per la Guarigione dalla Talassemia e Drepanocitosi (FITHAD), Caserta, Italy
[3] Associazione Ligure Thalassemici, Genoa, Italy
[4] United Kingdom Thalassaemia Society (UKTS), London, United Kingdom

Competing interest: None to disclose.

1. INTRODUCTION

Transforming healthcare in the 21st century is a difficult and an extremely challenging task due to the many intricate layers that form part of and influence the system. Political, environmental, economic, and public health factors combined with cultural differences are often constrained by value conflicts, resistance, and delays to proceed to changes. Amongst the key stakeholders that can contribute productively to changes and reforms in the healthcare environment, leading to improvements aiming to better patient outcomes and to a more inclusive society, are the patients themselves, and more particularly those with chronic illnesses, along with their families who live and feel on an everyday basis the benefits, challenges, and gaps of the health and social care systems in their country. They are those who fear most for their future, who have uncertainties for the availability of and accessibility to the next days' care. They are those who are the first to be adversely affected during periods of crises whether health, economic, environmental, climatic, or geopolitical in nature. Patients with transfusion-dependent β-thalassaemia (TDT), are among the ones to be severely influenced because of their lifelong, daily dependency on diverse medical, public health, social care, and other interventions.

It is a well-known fact that for decades, the decisions leading to health and social care systems' reform and changes were taken by those who served mainly political goals and agendas, and when seeking for guidance and advice, these were provided solely through scientific and medical recommendations. Healthcare professionals across disciplines thus possessed for decades the role of providing both scientific input and patients' perspectives to decision makers, without or with very confined patient contribution.

Patients' opinion, impressions, concerns, expectations, and challenges in assessing the range and quality of services they considered essential to address their multiple medical and social needs, characterizing in particular a chronic disease such as β-thalassaemia, were not sought for or acknowledged to any level for their value. No effort was made in the past to even identify the challenges and gaps of the system as seen and felt by the patients (and their families) to allow their reflection in recommendations provided by the healthcare professionals community to the regulators. Undoubtedly, we have all lived through such paternalistic approaches and quite sadly, there is ample evidence through patients and families testimonies, known to the Thalassaemia International Federation (TIF) for example, that such an approach still prevails to a smaller or larger extent across the world. Often, the absence of a structured patient engagement approach prior to

decision making led, and certainly continues to lead in many countries across the world (particularly of the developing economies), to health and social care reforms that are associated with great costs and that could jeopardise the sustainability and resilience of the health and social care systems without in fact effectively and sustainably addressing the needs of patients (especially those with chronic disease). Recognition of the need and value of listening to patients and taking on board their views, concerns, and real-life experiences came rather slow through the years, since resistance from the medical community was indeed very powerful. This was coupled with limited action to put forward procedures to productively engage and involve, in a meaningful and official way, patients at the decision-making level (national, regional, and international).

2. THE HISTORY OF PATIENT ENGAGEMENT IN THALASSAEMIA: HOW IT ALL BEGAN

In the field of thalassaemia, a number of decent and quite successful efforts were started by patients and their families in the 1950s and 1960s in countries in Southern (Mediterranean) Europe, where the disease was quite prevalent in the indigenous population. However, achieving patient and family involvement and participation in decision-making processes and discussions on policy reforms often meant confronting physicians, national health authorities, politicians, and other involved stakeholders. Patients and parents' confessions reveal that such movements had been extremely difficult in those early days and indeed such efforts caused them considerable pain, stress, and agony and certainly prevented or considerably delayed any real progress to be made in addressing their needs more effectively.

The unmet needs and the challenges of patients with thalassaemia were (and still are) evident in many countries worldwide, whether medical or social in nature including the burden of the 'stigma' carried by a genetic, hereditary disorder. Confronting the healthcare system and society in a country thus seemed like undertaking a 'masked' revolution. Patients in those year, were sub-optimally or not treated at all in most countries across the world, leading to very poor health-related quality of life (HR-QoL) and early, premature death. Knowledge about the pathophysiology and hence the effective management of the disease was very confined and research interest by the academia and industry was also very limited. Patients and their families felt the need to take their fate in their hands. Patient engagement and involvement in ways as known to us today were quite unknown concepts then. Patients and their families navigated through the system trying to find solutions to their problems, often with huge delays, enduring wrong or belated diagnosis and inappropriate interventions for years. They were also often faced with resistance and/or non-responsive behaviours from the authorities or poor awareness from healthcare professionals concerning the needs of their condition. There was no other way for patients and families to address their situation except to unite their efforts to make their voice heard. That is how and why patient organisations started to develop and that is how patient involvement began to evolve (in the case of thalassaemia).

The achievements (mostly disease-specific but also many in the larger context of public health) of some of the first national thalassaemia associations established in the 1960s and 1970s in Southern Europe (mainly Cyprus, Greece and Italy; but also in the United Kingdom), are considered milestones in the history of the development of disease-specific national programmes in these countries; particularly in the areas of spreading awareness about the disease to the community at large,

promoting voluntary blood donation practices, ensuring safety of blood, and sensitising the competent authorities about the value and contribution of effective, nationally-funded, and coordinated prevention programmes for improvement of the quality of services provided to the patients. Although the competent authorities and healthcare professionals community led these efforts by law, the voluntary contribution of patient organisations proved to be substantial and invaluable.

The work and official involvement of the patients/parents at the decision-making level were considered at the time (i.e., in the 1960s) as an innovative, extremely bold movement; referred to by many stakeholders as a revolution in health. The medical community and political decision makers initially met this movement with scepticism and great reservation, one that they had to fight, subside, and strongly resist. They were not able to realise back then that this is going to be one of the most significant steps ever taken to compliment and strengthen their own work, rather than oppose or harm it.

3. HOW DID THE MEDICAL COMMUNITY PERCEIVE THIS MOVEMENT

A veteran Greek Professor of Haematology, the late Professor Dimitris Loukopoulos, who made considerable academic and research contributions to the history of haemoglobin disorders in Greece and internationally stated during the Thalassaemia International Conference in 1999:

'The patient/parent movement that started in Greece, Cyprus and Italy in the 1960s and 1970s and followed by others through the years, the determination and persistence of the patients and their families to be heard by competent authorities and the involved healthcare professionals community through becoming involved in all stages of planning and decision taking with regards to their disease were well beyond our understanding and well above healthcare professionals comprehension. At the same time, we realised that this was also well beyond our power to either ignore or stop.

The strength of their action alarmed and impressed us but also scared us at the beginning, feeling we may lose our power and our key role as decision makers or key contributors to decision making in healthcare. But as time passed, along with many others involved colleagues across the world, we recognised and greeted the added value of this new development. The role of healthcare professionals was not threatened at all but in fact was greatly strengthened and we came to realise and appreciate that patient advocacy and involvement could in fact significantly support our academia and research communities' efforts. Utilising and building on the extremely valuable information and real-world perspectives obtained from the patient and parent community itself and reflecting these into our recommendations to the competent authorities contributed immensely to taking decisions more focused and tailored to the patient's needs and to make changes and reforms in the services and policies that were more appropriate for the effective management and control of these disorders.

At the same time, such approach proved to be more cost-effective for the health and social care systems themselves, protecting sustainability and resilience. Patients were indeed more 'independent' to move things along than us, the professionals, who had restrictions in confronting the system. Patients and their families could argue, disagree, and access concerned authorities without permission and bureaucratic procedures, acknowledging their right as humans and patients for quality, decent, and appropriate care and to be respected(as safeguarded by the United Nations (UN) and the World Health

Organization (WHO) resolutions and declarations. In fact, very shortly, we as health specialists not only greeted with relief but also began to request the collaboration and active involvement of the patient community gradually in our efforts, and in a more structured and official way. In conclusion, we welcomed patients' engagement in the decisions taken regarding their disease and also in other related public health and social care aspects.'

4. NATIONAL PATIENT/PARENT SUPPORT GROUPS LED TO THE BIRTH OF THE THALASSAEMIA INTERNATIONAL FEDERATION

Throughout the years, patients united their voice and efforts and started establishing non-governmental organisations (NGOs) across the world largely relying on the format, mission, and vision of the first thalassaemia associations in Southern Europe which had already demonstrated successful outcomes. After recognizing (i) the strength of the voice of the patients and the value of their participation and contribution at the national level in achieving disease-specific improvements, (ii) the worldwide occurrence of these disorders, (iii) the need to transfer knowledge and expertise gathered mainly from countries in Southern Europe which were first to successfully implement prevention and management approaches through the development of specific national programmes, and (iv) the urgent need to strengthen research for achieving further improvements, a small group of patients, parents, and healthcare professionals with the support and guidance of the WHO, established TIF in 1986. The support of the WHO working group was instrumental to provide the necessary environment for creation of TIF. At first, TIF had a very confined membership of patient organisations from around the world. However, membership expanded over the years with TIF enjoying to date (2024) membership of more than 200 patient organisations from 68 countries across the six different regions of the world as defined by the WHO.

TIF, since its establishment, in shaping its Constitution opted for a Board of Directors where patients were represented in equal number with parents (non-patients), and recognised from the beginning of its existence that three activities were of highest priority to be included in its mission and mandate, in order to have effective patient engagement and valuable contribution to achieve disease-oriented improvements at all levels, national, regional, and international:

1. Education of patients and families about the disease and its medical and social needs and the serious repercussions if it is left unaddressed or sub-optimally controlled. By the 1990s, considerably more scientific evidence became available on how to effectively prevent and appropriately manage this disorder. TIF established an extensive educational programme for patients, and in more recent years, its Global Patient Advisory/Advocacy Group comprised of patients from across various countries and regions of the world. These patients not only represent their country but also TIF and they provide the patients' perspective in discussing unmet needs and challenges and in advocating for improvements. The added value of such interaction and engagement has been recognised and immensely appreciated and acknowledged by the scientific community and regulatory and competent authorities at any level.
2. Empowerment of national patient organisations towards the establishment of a line of productive interaction, communication, and collaboration with the medical/scientific community in their countries. This would support patient-oriented improvements in every country with

patient involvement at the national decision-making level. At the international and regional levels, TIF worked in a parallel way: urging and empowering the international medical/scientific community to put together the knowledge and experience they gathered throughout the years into developing guidelines and recommendations for the prevention and the management of the disease. The collaboration and networking of patients (i.e., TIF) with the healthcare professionals community constituted a great step forward by enabling the patients through evidence-based guidelines authored by international experts, to pass on to the competent authorities what was/is considered as 'optimal care' to address the needs of the disease and the patients. This covered management aspects well beyond conventional medical care for a complex, genetic, hereditary disorder with multiorgan impact and significant challenges in social inclusiveness. In addition, TIF through collaboration with the medical/scientific community, had the opportunity to develop various educational programmes including conferences, workshops, preceptorships, webinars, courses, fellowships, and other programmes (virtual, physical, or hybrid) aiming to provide lifelong educational opportunities to healthcare professionals, patients, their families, and the community at large. The programmes and agendas of these educational programmes whether implemented at national, regional, or international level are developed jointly by patients/parents' representatives and healthcare professionals, and they are organised jointly to address the needs of both communities alike.

3. The third activity recognised by TIF towards achieving effective and meaningful patient involvement in decision making was the establishment of official collaborations with health-related official bodies at the international, regional, and national levels including but not limited to:
    - Official European Union (EU) competent bodies including the European Blood Alliance (EBA), the European Centre for Disease Prevention and Control (ECDC), the European Directorate for the Quality of Medicines & HealthCare (EDQM), European Medicines Agency (EMA), as well as the European Reference Network (ERN) dedicated to haematological disease (EuroBloodNET) and its European Patient Advocacy Group (ePAG)
    - The WHO (in official relations since 1996), the UN, the UN Economic and Social Council (ECOSOC, special consultative status in April 2017), the European Commission (official partner in the field of health since 2018), the Association of Southeast Asian Nations (ASEAN) (since 2013), and others while at the national level this is achieved through official agreements (Memorandums of Understanding) with Ministries of Health.
    - National, regional, and international scientific professional bodies including the European Hematology Association (EHA, with a TIF member involved in the EHA Patient Advocacy Committee), the International Society of Blood Transfusion (ISBT), and the European Association for the Study of the Liver (EASL).
    - Other organisations including the European Organisation for Rare Diseases (EURORDIS), Rare Diseases International, the International Alliance of Patients' Organisations (IAPO), and the European Patients' Forum (EPF).
    - Industry and academia involved in research.
    - Multistakeholder initiatives focused on strengthening patients' voice and position on critical matters aiming to achieve further improvements in the health and social care of patients.

5. KEY OBSERVATIONS CAPTURED THROUGH THE WORK AND ACTIVITIES OF THE THALASSAEMIA INTERNATIONAL FEDERATION

Patient involvement with the process of healthcare delivery can vary according to the patient's age, sex, and education level, and possibly ethnicity and culture. Younger patients tend to want more involvement than older patients, females prefer a more active role than males, and highly educated patients opt for greater engagement than their less academic peers. It has been demonstrated through TIF's work that some of these effects are due to differences in health literacy levels – in general younger and more educated patients tend to have a greater capacity for obtaining, processing, and comprehending basic health information needed to make appropriate health decisions.

In addition, TIF's experience at the grassroots, demonstrates or suggests that patients with less severe conditions may take on a more active role in their healthcare than patients who live with more severe symptoms.

The direction of advocacy and depth of engagement are certainly different in each country and even within a country, depending on the challenges and gaps existing or perceived by patients and their families, and on whether patients' involvement is acknowledged for its value and 'allowed' to happen in a national system with regards to thalassaemia care for example.

6. A STRONG EXAMPLE OF A VERY POWERFUL MOVEMENT: THE HUMAN IMMUNODEFICIENCY VIRUS STORY

In the early 1990s and during those same years that thalassaemia patient engagement initiatives began to evolve and strengthen, another patient movement, perhaps the strongest and most powerful ever, became the focus of attention and interest of global academic and research communities, the industry and international health bodies, and regulatory drug authorities. This was the 'revolution' of patients with human immunodeficiency virus (HIV) which constitutes another excellent example of how patients involvement achieved significant success in improving the management of a very complex and devastating disease, and in achieving progress in patients' HR-QoL and social inclusion. Patients infected with HIV had to demand and 'oblige' all relevant stakeholders to 'LISTEN' to their odyssey and understand their needs and journey, as they otherwise went through a labyrinth of poor services with unknown effectiveness. They demanded investment in research and drug development to effectively address the tragic consequences of the infection which constituted one of the most severe global public health threats ever. Although it took years of fight, success was indeed achieved, ultimately leading to the development of drugs that have the power today, and for some time now, to convert this infectious disease with immense medical, public, social, and economic repercussions into a chronic, well-managed one. No other disease has ever received more interest and funding for research, trials, and drug development than HIV.

This development, however, could not have happened without the continuous, active, united, persistent, strong, and on occasions aggressive yet well-documented patient advocacy and active involvement at national, regional, and international decision-making levels. Despite the burden of the huge stigma carried by this disease, patients worldwide opened up, talked about, and published their personal stories and experiences during their tragic disease journey. Research on HIV will also be remembered for another major contribution in addition to demonstrating the

power of patients' engagement. This concerns the development of molecular biology tools that can achieve early and accurate diagnosis and monitoring of the progression of HIV infection. These tools also later supported numerous other medical diseases and scientific applications. Similarly, the thalassaemia patient involvement and engagement contributed immensely to the development and strengthening of many other medical and public health areas such as voluntary, non-remunerated, blood donation practices and optimisation of blood transfusion services.

7. PATIENT ENGAGEMENT WITH REGULATORY AGENCIES

The HIV movement coincided or was followed by many other successful examples especially for rare diseases in Europe. The active and meaningful engagement of patients with rare diseases at all levels in decision making in the EU has led to the recognition by the EU competent bodies of the multiple unmet patients' needs and the development of specific recommendations and policies to be implemented in every EU country. The aim was, and still is, to improve early and accurate diagnosis, strengthen research and drug development, and provide effective management while acknowledging the poor HR-QoL and social exclusion of many if not most patients with rare diseases. The various challenges and special needs of these patients on account of their complexity and rarity got to be addressed in a more focused and patient-centric manner when patient engagement became more structured and recognised for its value by official bodies and competent authorities. In the EU, contrary to other regions, patient engagement and participation in essential committees has become mandatory throughout the years.

Patient engagement with the European Medicines Agency (EMA) for example has evolved and diversified over the years in parallel with the expansion of EMA's remit. There are several categories of patient representation at EMA; those who represent all patients in the European community as members of the EMA Management Board and scientific committees, those who represent their organisation via membership of EMA's Patients' and Consumers' Working Party (PCWP), or participation in workshops and responding to EMA public consultations. Patients also represent themselves as individual experts for medicine approval-related activities such as scientific advice, scientific advisory groups (SAGs), and the review of documents destined for the public such as medicines overviews, safety communications, and package leaflets. Various engagement methodologies have been tested and implemented over the years, resulting in established procedures to include the patient voice all along the medicines regulatory lifecycle at EMA.

Patient involvement in scientific advice began in 2005 when rare disease patients requested to be involved in protocol assistance procedures for medicines with an orphan designation. Success of this activity led to the inclusion of patients in scientific advice for medicines without an orphan designation from 2013 as well as parallel procedures of scientific advice and health technology assessment (HTA) bodies (Figures 1-3) [1].

Figure 1. *Number of patients involved with the European Medicines Agency from 2008 to 2020, by procedure type (protocol assistance, scientific advice and parallel procedures with health technology assessment bodies). Data collection on patient involvement in scientific advice procedures began in 2008. Reproduced with permission from [2].*

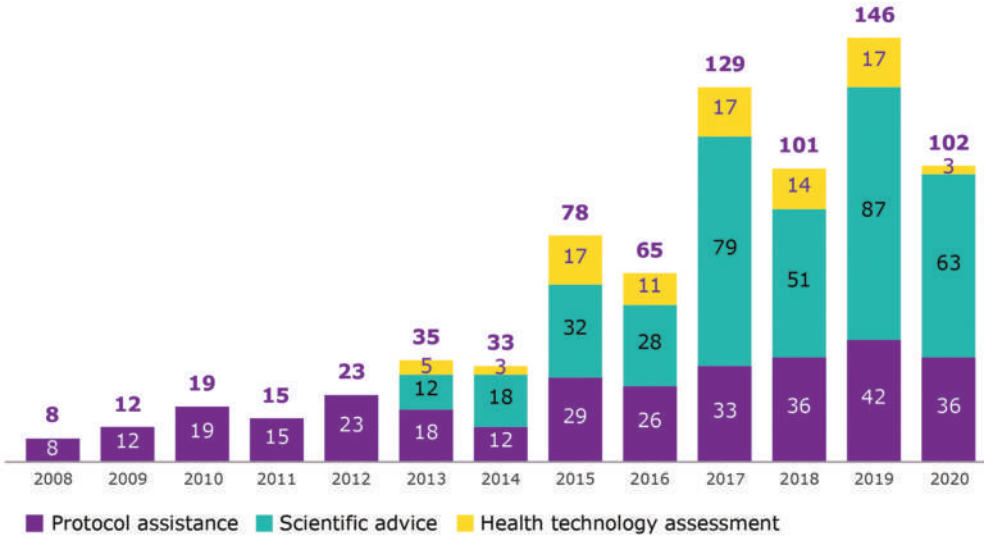


Figure 2. *Areas of development plan where patients provided input as part of the European Medicines Agency scientific advice. More than one category could be selected for each survey question. Reproduced with permission from [2].*

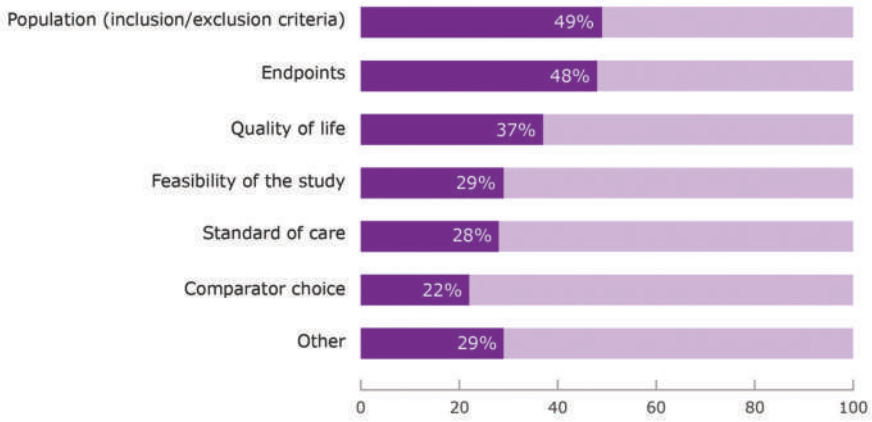
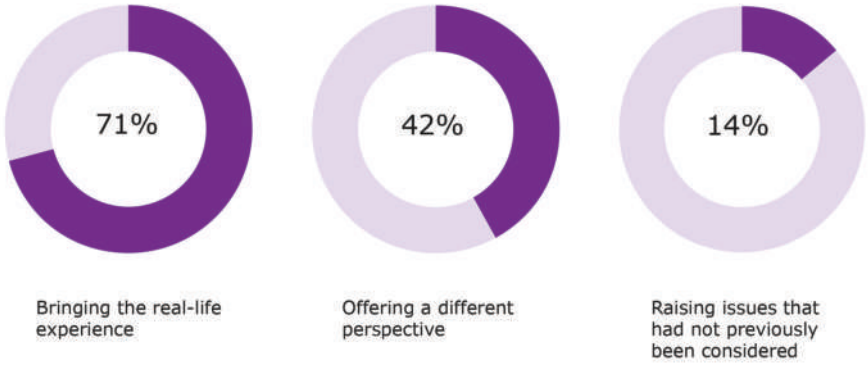


Figure 3. *Additional input by patients on aspects such as real-life experience as part of the European Medicines Agency scientific advice, different perspectives and other considerations were also measured (2017-2020). Reproduced with permission from [2].*



Several criteria were used to select patients, usually one and sometimes two patients are invited to participate in a scientific advice procedure. English is the working language at EMA, and all patients must have a level of understanding that would enable them to read the relevant documents and comment in writing or in person. Depending on the questions raised, the level of experience can vary from a newly diagnosed individual, a carer, or a long-term patient advocate representing the condition. Having followed a training course on medicines' development is beneficial but not a pre-requisite for involvement. As with all other experts participating in EMA activities, patients were required to complete a confidentiality agreement and declare any competing interests, which were assessed prior to formal invitation. EMA experts were generally residents of an EU Member State.

Similarly, earlier in 2012 and as part of the reauthorisation of the Prescription Drug Use Fee Act (PDUFA V), the US Food and Drug Administration (FDA) established a programme to help ensure patients' experiences, perspectives, needs, and priorities are captured and meaningfully incorporated into the development and review process. This was formalised as Patient-Focused Drug Development (PFDD) [2]. A number of activities and meetings are involved in this novel approach so as to gather input from patients who are willing to share their personal experiences of living with a disease or condition.

Moreover, in the attempt to foster collaboration among the regulatory agencies across the world and to harmonise patient involvement, the EMA and FDA are pursuing several common activities where patients' representatives are involved, like the joint meeting of Clinical Trial Transformation Initiative (CTTI) as part of the Patient Engagement Collaborative (PEC) of the FDA and the EMA's PCWP. These two groups started their activity in 2022 to share the different perspectives and lessons learned from the COVID-19 pandemic and are now working on common definitions for patient reported outcomes (PRO) and HRQoL data and how they influence regulatory decisions.

8. PATIENT ENGAGEMENT IN RESEARCH

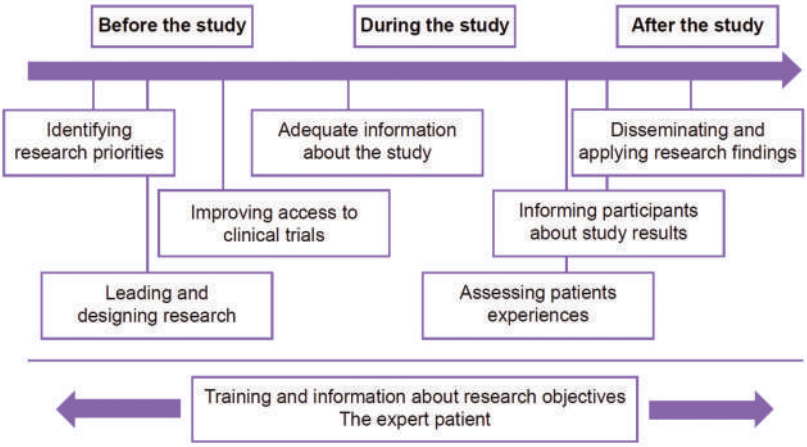
Involvement of patients in the planning and conduct of clinical research, beyond being study participants, ensures patient centricity in design and supports better recruitment and retention. Figure 4 demonstrates possible patient involvement in clinical research while Table 1 describes specific initiatives to engage patients in the research process [3].

Table 1. *Specific initiatives to engage patients in the research process. Modified with permission from [3].*

Aspect	Approach
Identifying research priorities	• James Lind Alliance (http://www.jla.nihr.ac.uk/) • PCORI (www.pcori.org)
Leading and designing research	• PatientsLikeMe (www.patientslikeme.com) • 23andME (www.23andMe.com) • OMERACT (www.omeract.org)
Improving access to clinical trials	• European Union (www.clinicaltrialsregister.eu) • Trials 4 Me (http://trials4me.lillycoi.com/) • NIH (https://clinicaltrials.gov/)
Adequate information about the study	• Readability of informed consent documents and patient information sheets
Assessing patients' experience	• Systematic collection of opinions and experiences of the participants
Informing participants about the study results	• Process to communicate the results at the end of the study
Disseminating and applying research findings	• Promoting transparency • Databases of randomised clinical trials • Information for patients in medical journals
Information and education	• FDA (www.fda.gov/ForPatients) • NIH (www.nih.gov/health/clinicaltrials/index.htm) • ASCO (www.cancer.net/) • Oxford University (www.healthtalkonline.org/) • EUPATI (www.patientsacademy.eu)

Abbreviations: EUPATI, European Patients' Academy on Therapeutic Innovation; FDA, Food and Drug Administration; NIH, National Institute of Health; PCORI, Patient-Centered Outcomes Research Institute; OMERACT, outcome measures in rheumatoid arthritis clinical trials; ASCO, American Society of Clinical Oncology.

Figure 4. *How to engage patients in clinical research? Reproduced with permission from [3].*



9. THE GLOBAL PERSPECTIVE OF PATIENT ENGAGEMENT: THE EXAMPLE OF THE WORLD HEALTH ORGANIZATION

At the global level, the WHO since the 1970s was perhaps the first official health body that acknowledged the value of patients' involvement and the inclusion of patients' representation in its work and different programmes at country, regional, and international levels. The WHO assigns NGOs including patient organisations which fulfil the criteria as non-state actors, in 'official relations with the WHO'. TIF enjoys the privilege of being one of such organisations and maintains this status since 1996.

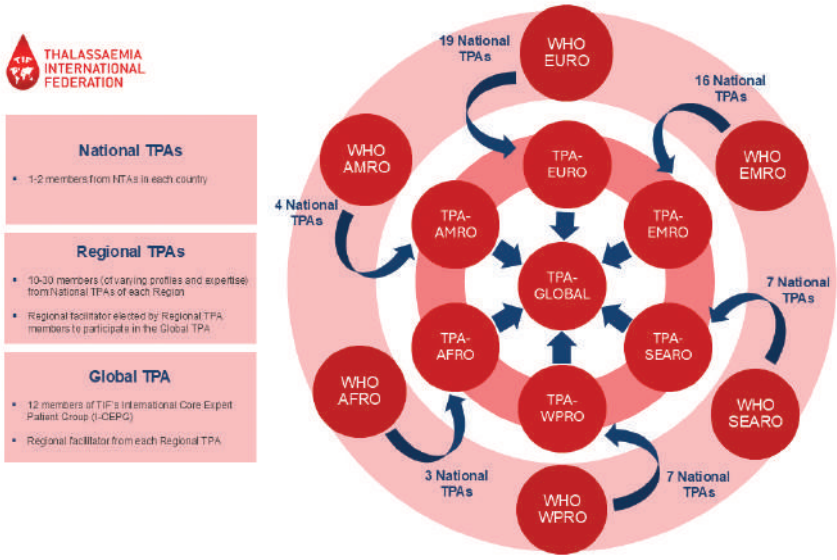
In this context, patients can become involved in the works of the regional and general assemblies and can contribute to public consultations and the development of publications as well as engage in other activities related to the various programmes of WHO. Patients in official relations with the WHO often advocate for the implementation of the WHO's and UN's significant work and resolutions by governments and competent authorities across the world, while they also undertake joint projects with the WHO's headquarters, regional, national, or collaborating centres. The development of TIF's Global Thalassaemia Review [4], capturing patients' perspective on the extent and quality of the services provided in their countries (members of TIF), is an example of such joint work with the WHO. It proved to be an asset for better understanding of the unmet needs and challenges faced by patients, and of the gaps and weaknesses of the health and social care systems across the world as perceived by patients and their families.

Globally, there is an ongoing effort by patients and patient organisations to follow the example of successful patient movements, especially those from the West. The journey to successful patient involvement and engagement of most patient organisations in the developing world where more than 80% of the thalassaemia patient community live, is indeed very challenging. Patients' engagement at the decision-making level in these countries is still not recognised for its value,

despite TIF's immense efforts to support this transformation. In these countries, with very few exceptions, decision makers are still only looking for healthcare professionals to provide recommendations and support their decisions for improvements, just like the early days in the Europe.

The establishment of TIF's national, regional, and global patient advocacy groups in 2016, involving patients with knowledge and experience mainly obtained through TIF's educational programmes, has indeed started to contribute albeit slowly towards this direction (Figure 5). Considerable work is still needed to 'copy' the successful patient engagement and productive advocacy model of patients in the EU.

Figure 5. *Thalassaemia International Federation's patient advocacy groups' structure and membership. Abbreviations: TPA, thalassaemia patient advocates; NTA, national thalassaemia association; TIF, Thalassaemia International Federation; WHO, World Health Organization.*



10. PATIENT ENGAGEMENT IN THALASSAEMIA INTERNATIONAL FEDERATION ACTIVITIES

The work of TIF mandates patients' engagement in every activity undertaken and at all levels. Some key examples of such activities at the international level, with patients/parents participation, are provided below:

- WHO, as an NGO in official relations (since 1996)
  - Three-year joint plan of activities supporting and promoting WHO's work, recommendations, and resolutions
  - Participation and contribution to the work of General and Regional Assemblies and activities
  - Contribution to disease-specific and other relevant International Days

- Distribution of recommendations and WHO material to patients across the world during public health and other health-related crises
- Providing feedback and support to public consultations for relevant programmes
- Contribution to WHO projects, programmes, and development of publications
- Collaboration with countries and regional WHO offices and WHO collaborating centres with relevant disease expertise, across the world

Examples of such activities at the regional level (e.g., in Europe) are provided below:

- TIF collaborates with the European Commission and European Parliament through
  - Coordination, contribution, and participation in EU-funded projects
  - Contribution to public consultations (EU) concerning the revision of existing or development of new recommendations, directives, regulations, and position papers
  - Participation in relevant committees during the development or reviewing of policy papers
  - Providing the patients' perspective in events organised by the European Commission and European Parliament on relevant topics
  - Providing the patients' perspective through participation in committees and to the work of European Commission bodies or agencies: ECDC, EDQM, EMA, EuroBloodNet ePAG
- TIF provides the patients perspective and position through active participation of patients in national, regional, and international medical/healthcare professionals bodies including (but not limited to)
  - EHA: a patient (member of TIF), holds the Vice Chair of EHA's Patient Advocacy Committee
  - ISBT
  - EASL
  - Providing the patients' perspective through participation in the programmes of conferences organised by many other professional associations across medical disciplines
- TIF provides the patients' perspective through the official collaborations established in the context of a Memorandum of Understanding with
  - Governments, health, and social competent authorities
  - Universities (medical schools)
- TIF undertakes projects jointly with the academic and research community and the industry by providing the patients' perspective living with the disease, supporting and empowering the development of drugs, recruitment of patients to clinical trials, and contributing to the collection of real-world data essential to establish evidence for long-term safety and efficacy of innovative therapies
- TIF collaborates with medical/scientific communities and contributes, at national, regional, and international levels to
  - The development and continuous update of guidelines for the prevention and management of thalassaemia (α and β, transfusion- and non-transfusion-dependent)

  - The development of educational materials, courses, programmes, and events including national, regional, and international conferences, workshops, fellowships, and preceptorships covering thalassaemia and other haemoglobin disorders
- TIF undertakes to collect and analyse the patients' perspective, through surveys, questionnaires, semi-interviews, and other tools concerning their challenges, concerns, HRQoL, safety, and effectiveness of the medical and social care services they receive aiming to alert and advocate to national competent authorities, health care professionals' community for reforms and changes for improvements
- TIF develops, in collaboration with expert scientists in the field, tools to facilitate improvements at the national level including:
  - A generic model of a national registry and cost of illness
  - Recognition of treating centres across the world that can become TIF's Collaborating Centres and in the establishment of regional networks to connect and reach out to patients and healthcare professionals more effectively in every region (e.g., ASEAN)

11. REACHING OUT

TIF keeps patients and healthcare professionals across the globe well-connected and actively engaged throughout the year through the existence and ongoing updates of its webpage, the regular provision of reports on activities and clinical trials, a bimonthly newsletter, use of social media, and development of a mobile learning application (THALIA App) (Table 2). It is expected that the use of artificial intelligence in the very near future will significantly strengthen and facilitate TIF's efforts further in reaching out more effectively to its global patient community and succeed to achieve even stronger and more active patient engagement at the national decision-making level. This is while acknowledging the fact that, based on TIF's observations, this is still very limited in the majority of its member countries across the world. Based on TIF's long-term collaboration and networking with patient organisations (n = 240) in its member countries across the world (68 countries), a gross division is observed amongst them with regards to their level of engagement at the decision-making level in their countries, and consequently, on the extent of their achievements in co-deciding mainly (and not only) on disease-specific reforms and improvements (Table 3).

Table 2. *Examples of Thalassaemia International Federation's educational programmes and resources.*

For patients	For healthcare professionals

• Online e-courses
  - Thalassaemia e-course for patients and parents
  - SCD course for patients, families and carers
• TIFLIX
• Conferences
• Capacity building workshops
• THALIA Mobile App
• Publications
• Podcasts
	• Online e-courses
	  - eThalED course (thalassaemia prevention)
	  - eThalED course (thalassaemia management)
	  - SCD course for healthcare professionals
	  - HPLC screening in the service of prevention and diagnosis
	• TIFLIX
	• Publications
	• Fellowships
	• Conferences
	• Capacity building workshops
	• Bimonthly update on advances and clinical trials
	• Scientific literature overviews

Abbreviations: SCD, sickle cell disease; HPLC, high-performance liquid chromatography.

Table 3. *Level of patient engagement across Thalassaemia International Federation's member countries.*

Level of engagement
Group A: United Kingdom, Cyprus, Greece, Italy, USA, Malaysia, Brazil, Iran, Canada, India. Countries that have achieved a very good level of engagement with successful patient contribution to disease-specific and public health improvements.
Group B: Germany, Sweden, Austria, Norway, Belgium, Netherlands. Countries that have quite newly established patient organisations. The national health systems of these countries are very well organised and responsive to unmet needs of patients with haemoglobin disorders, making their active engagement and continuous involvement at the decision-making level not so essential as their needs and challenges are timely and appropriately addressed. These are countries with haemoglobin disorders included in their national lists of rare diseases or countries with very small numbers of patients; although, haemoglobin disorders having been introduced to their indigenous population through migration and population movements either some time ago or more recently, except for Germany. Germany has a large population with haemoglobin disorders introduced into the indigenous population decades ago, but strong patient engagement, advocacy, and involvement have not yet been achieved to a satisfactory level. Considerably more efforts are needed to establish true, active, and official patient engagement at the level of decision making (as has been achieved in the United Kingdom and France which historically also hosted migrant populations).

Group C: Bulgaria, Pakistan, Egypt, Romania, Türkiye, Indonesia, Palestine, Yemen.
Countries that have struggled for years now for active patient engagement and which, despite the robust infrastructure of their patient organisations and some significant achievements in establishing improvements, their official patient involvement and participation at the decision-making level has only been achieved to a confined level. Palestine and Yemen sadly and despite their work and huge focus on achieving improvements, political and war conflicts have prevented any further progress. Palestine indeed had met the criteria of Group A prior to the conflicts.

Group D: Bangladesh, Cambodia, Vietnam, Thailand, Morocco, Algeria, Argentina, Albania.
Countries where patient organisations had very little or no success in achieving to any length, patient engagement and acknowledgement of its value at any level. Thailand, a country with high disease (thalassaemia) prevalence and with huge improvements made in research, control, and management of thalassaemia (both α and β) throughout the years constitutes a unique example of a case where patients and families' involvement and official engagement are absent.

12. FINAL THOUGHTS

Considering the current global landscape with its numerous challenges, geopolitical, economical, public health, environmental, climatic, and others as well as the multiple unmet needs of patients with β-thalassaemia and other haemoglobin disorders, as demonstrated through the 2023 Global Thalassaemia Review [4], in the greatest majority of the countries where these disorders occur, including those with high disease prevalence, the official, mandatory, and meaningful engagement of patients becomes essential. This involvement is crucial to drive and support progress in the control, management, and research of these conditions.

Despite the advanced knowledge, scientific achievements, and successful experience and outcomes through the years, disease and other medical and public health-specific policies still need to be developed and/or strengthened where they exist, in particular in low- and middle-income countries where more than 80% of the patient population is born and lives. Patients need to be heard and their perspectives to be highly acknowledged so as to have patient-centred policies. On the other hand, effective control of chronic conditions for obvious reasons, benefit or contribute positively and significantly to the sustainability and resilience of health and social care systems. Moreover, the very recent scientific advances, despite bringing hope and great optimism to patients including those with β-thalassaemia, need to yet be made available, accessible, and affordable to the patient as this remains a huge challenge that the world is facing currently. Engagement and contribution of patients in the research, clinical trials, and collection of real-world data to demonstrate the long-term safety, durability, and efficacy of these medicines is critical and invaluable, especially considering that many obtained a conditional authorisation with long-term commitment of the developers to collect reliable and comprehensive data.

We as TIF hope that healthcare professionals who take an interest to read this Chapter join our efforts towards ensuring that the patients' voice and involvement is taken seriously onboard by regulators and decision makers in general.

REFERENCES

1. Murphy A, Bere N, Vamvakas S, Mavris M. The Added Value of Patient Engagement in Early Dialogue at EMA: Scientific Advice as a Case Study. Front Med (Lausanne) 2021;8:811855.
2. Perfetto EM, Burke L, Oehrlein EM, Epstein RS. Patient-Focused Drug Development: A New Direction for Collaboration. Med Care 2015;53(1):9-17.
3. Sacristan JA, Aguaron A, Avendano-Sola C, Garrido P, Carrion J, Gutierrez A, Kroes R, Flores A. Patient involvement in clinical research: why, when, and how. Patient Prefer Adherence 2016;10:631-640.
4. Eleftheriou A, Angastiniotis M. Global Thalassaemia Review 2023. Nicosia, Cyprus: Thalassaemia International Federation, 2023.

SUMMARY OF MONITORING RECOMMENDATIONS

The below table summarises routine monitoring recommendations for morbidities and manifestations of transfusion-dependent β-thalassaemia featured in these Guidelines. These represent screening (first level) assessments. Additional assessments may be required to confirm the diagnosis of specific complications. The frequency of assessment may also need to be adjusted (lower/higher) based on previous findings. Further details on these recommendations and their grading can be found in respective Chapters. Recommendations for special scenarios and populations such as fertility and pregnancy are detailed in respective Chapters.

WHAT	WHEN/WHO*
Diagnosis	
Clinical examination and family history, complete blood count with red cell indices and morphology, iron studies, and capillary electrophoresis or HPLC	At suspicion of thalassaemia in patients with hypochromic microcytic anaemia
Molecular studies to confirm β-genotype and α-thalassaemia status, HLA typing	At diagnosis
Blood transfusion	
Extended red cell antigen typing at least for Rh C, c, D, E, e, and Kell (K, k), and if available, a full red cell pheno/genotype	Before first transfusion
New antibodies and indirect antiglobulin test crossmatch or electronic crossmatch	Before each transfusion
Haemovigilance and adverse events monitoring and reporting	Ongoing with transfusion
Haemoglobin	Before/after each transfusion
Blood units, volume, and haematocrit	With each transfusion
Iron overload and chelation	
Calculated transfusional iron intake	Annual
Serum ferritin	At least every 3 months, with the start of transfusion therapy
LIC (MRI)†	Annually, starting 8-10 years
Myocardial T2* MRI†	Annually, starting 8-10 years
Iron chelation adverse event monitoring as provided with the drug when receiving authorisation from internationally or nationally recognised competent regulatory authorities (e.g., US FDA, EMA)	Ongoing with iron chelation therapy
Iron chelation adherence monitoring	Ongoing with iron chelation therapy

WHAT	WHEN/WHO*
Cardiac disease	
Echocardiography (including TRV)	Annually, starting 10 years
Electrocardiogram	Regular as clinically indicated
VTE risk assessment	In medical and surgical settings based on local guidelines, especially in older patients who are splenectomised, with low pretransfusion haemoglobin, or pregnant
Liver disease	
AST, ALT, alkaline phosphatase, γGT, and bilirubin	Every 3 months
Prothrombin time and albumin	Every 3 months, in patients with severe hepatic impairment
Hepatic ultrasound	Annually, starting 18 years Every 6 months, in patients with advanced liver damage, cirrhotic patients, and in patients with severe hepatic iron overload, age >45 years, or chronic viral hepatitis
Alpha fetoprotein	Every 6 months, in cirrhotic patients and in patients with severe hepatic iron overload, age >45 years, or chronic viral hepatitis
Transient elastography and fibrosis-4 score (in non-splenectomised patients) to assess for fibrosis/cirrhosis	In patients with advanced liver damage
Height and growth disorders	
Clinical and auxological evaluation including weight, height, BMI, height when sitting, growth rate/velocity (charted), and Tanner stage	Every 6 months, starting from patient first access at the treatment centre, until adult height achievement and completion of pubertal development
Disorders of pubertal development	
Testicular volume, growth rate, and Tanner stage	Every 6 months, starting from the normal age of puberty, until the completion of pubertal growth
Female hypogonadism	
Menses rhythm for oligo/amenorrhoea	Every 6 months
FSH, LH, oestradiol, and pelvic ultrasound	In patients with oligo/amenorrhoea
Male hypogonadism	
Testosterone, FSH, and LH	Annually, starting puberty
Hypothyroidism	
FT4 and TSH	Annually, starting 9 years Every 6 months, in patients with suboptimal iron overload management Every 3 months, in patients receiving drugs interfering with thyroid function (e.g., amiodarone)

WHAT	WHEN/WHO*
Glucose metabolism disorders	
Fasting blood glucose levels and/or blood glucose during OGTT, and HOMA-IR index	At least every 2 years, from 10-18 years Annually, starting 18 years
Serum fructosamine	Periodic (e.g., every 6-12 months)
Hypocorticosurrenalism	
Sodium, potassium, and ACTH/cortisol at 8 a.m.	Annually, starting adolescence
Hypoparathyroidism	
Serum calcium (corrected for albumin) and phosphorus	Annually, starting 10 years
PTH, magnesium, serum creatinine, 25OH vitamin D, and 24-hour urine calcium	In patients with hypocalcaemia
Growth hormone deficiency (adults)	
IGF-1 level	Annually, starting 25 years or earlier in case of severe iron overload and other pituitary deficits
Bone disease	
BMD by DXA	Every 1-2 years, starting 10 years
Cholelithiasis	
Gall blader ultrasound	As part of routine hepatic ultrasound
Malignancy (other than liver)	
Screening for solid and haematologic malignancies	Per local screening standards and guidelines‡
Skin manifestations and leg ulcers	
Skin inspection	Every visit
Renal disease	
Serum creatinine and urine analysis with spot ratio of protein/creatinine	Every 6 months
Serum calcium, phosphorus, magnesium, uric acid, and 24-hour urine collection for protein/creatinine and calcium/creatinine ratio	Annually, starting 10 years
Renal ultrasound	Every 2 years, starting adolescence and in case of laboratory abnormalities
Splenomegaly	
Spleen size assessment on physical exam	Every visit
Infectious disease	
HBsAg Anti-HBs Anti-HCV Anti-HIV	Annually, in unvaccinated patients Annually, in vaccinated patients Annually, followed by HCV RNA if positive Annually

WHAT	WHEN/WHO*
Oral and dental care	
Dental assessment	Regular
Nutrition	
25OH vitamin D	Every 6 months, to maintain circulating levels above 30 ng/mL (75 mmol/L)
Copper, magnesium, selenium, zinc, folate, vitamin C, vitamin E, cholesterol, and triglycerides	Annually, starting 10 years
Patient reported outcomes	
QOL and psychological well-being	Regular

**Recommendations apply to all patients unless otherwise indicated.*
†Using validated method with appropriate calibration, acquisition, and processing.
‡If MRI is done regularly for LIC, images can also be checked for abnormalities and tumours, but this does not replace standard cancer screening guidelines.
Abbreviations: LIC, liver iron concentration; MRI, magnetic resonance imaging; FDA, Food and Drug Administration; EMA, European Medicines Agency; HPLC, high-performance liquid chromatography; TRV, tricuspid valve regurgitant jet velocity; VTE, venous thromboembolism; AST, aspartate transaminase; ALT, alanine transaminase; γGT, gamma glutamyl transferase; FIB-4, fibrosis-4 score; BMI, body mass index; FSH, follicle stimulating hormone; LH, luteinising hormone; FT4 free thyroxine; TSH, thyroid-stimulating hormone; OGTT, oral glucose tolerance test; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; PTH, parathyroid hormone; ACTH, adrenocorticotropic hormone; 25OH, 25-hydroxy; PTH, parathyroid hormone; IGF-1, insulin-like growth factor 1; BMD, bone mineral density, DXA, dual-energy X-ray absorptiometry; HBsAg, hepatitis B surface antigen; Anti-HBs, hepatitis B surface antibody; HCV, hepatitis C virus; HIV, human immunodeficiency virus; QOL, quality of life.

TIF'S COMPREHENSIVE EDUCATIONAL PROGRAMME

EDUCATIONAL EVENTS

TIF organises physical and virtual educational events (conferences, seminars and workshops) held at local, national, regional and international levels.

- TIF's International Conferences on Thalassaemia and Other Haemoglobinopathies constitute the biggest educational events in the field of haemoglobin disorders, attracting over 2,000 participants from over 60 countries around the world.
- TIF's Pan-European, Pan-Asian and Pan-Middle East Regional Conferences are held in the years between the international conferences to shed light onto regional challenges and actively engage local actors in disease-specific education.

RENZO GALANELLO FELLOWSHIP PROGRAMME

Offered each year through the Joint Red Cell Unit, Haematology Department of the University College London NHS Foundation Trust in London, UK, under the leadership of Dr Perla Eleftheriou, Consultant Haematologist, the Renzo Galanello Fellowship programme covers all aspects of the clinical management of haemoglobinopathies and is addressed to physicians, specialists in the field of haematology, paediatrics or internal medicine.

e-ACADEMY

- **eThalED Course for Medical Specialists**

  The eThalED course offers specialised knowledge on the prevention and clinical management of thalassaemia to medical specialists who have an interest and/or are involved in these areas. Based on the "Guidelines for the Management of Transfusion-Dependent Thalassaemia (4th edition, 2021)", the course offers valuable insights on a number of topics, incl. genetic counselling, patient adherence and the changing doctor-patient relationship.

- **SCD e-Course for Healthcare Professionals**

  The Sickle Cell Disease Course is an online educational course for healthcare professionals around the world. This course covers all aspects of SCD clinical management, with content developed by eminent international medical experts, with extensive experience in treating patients with SCD. The course has been reviewed and endorsed by the European Haematology Association (EHA).

- **HPLC Screening in the Service of Prevention and Diagnosis**

  The course, HPLC Screening in the Service of Prevention and Diagnosis, offers specialised knowledge on interpreting HPLC chromatograms to identify haemoglobinopathy cases. This course is comprised of a series of educational videos covering in-depth key issues related to understanding and analysing HPLC chromatograms.

All courses are offered free-of-charge, attested by a certificate issued by TIF, and can be accessed through a simple registration on TIF's e-Academy.

EDUCATIONAL RESOURCES

- **AI Knowledge Bot**

  The AI Knowledge Bot is a revolutionary tool designed to transform the way individuals access and engage with information about thalassaemia. Created with patients, caregivers, healthcare professionals, and the broader community in mind, this state-of-the-art solution leverages the power of artificial intelligence alongside TIF's extensive and trusted resources on thalassaemia.

  Trained exclusively on TIF's reputable resources and publications, the bot ensures that all provided information is both accurate and reliable. Available 24/7, it supports multiple languages to promote inclusivity and accessibility. Moreover, it dynamically tailors its responses based on the user's

profile-whether they are a healthcare professional, a patient, or a member of the community-delivering personalized and relevant support for everyone.

TIF Knowledge Bot can be accessed on TIF's Website: Go to https://thalassaemia.org.cy/

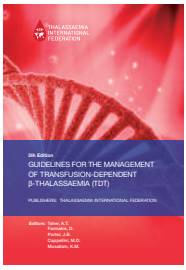
► TIF's LIbrary eXtended (TIFLIX)

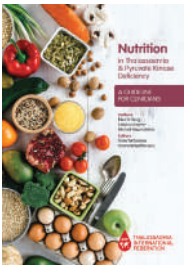
TIF's Library has been extended to provide its users with premium, on-demand educational video content on a variety of topics relevant to thalassaemia and other haemoglobin disorders. TIFLIX contains an extensive library of case studies and lectures addressed to healthcare professionals with an interest in the clinical management of haemoglobin disorders.

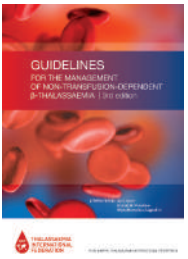
► Publications

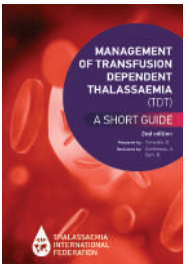
Since 1996, TIF has issued a vast number of diverse publications on thalassaemia and sickle cell disease, many of which have been and are still used as reference texts for academics, healthcare professionals, patient-support organisations and individual patients. New editions are regularly produced to keep up with scientific progress and novel concepts.

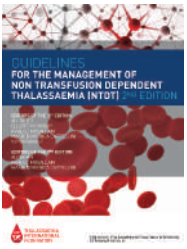
TIF PUBLICATIONS | HEALTH CARE PROFESSIONALS

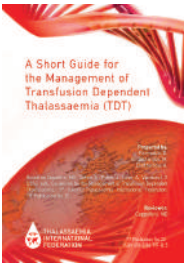


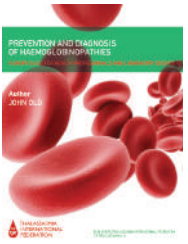


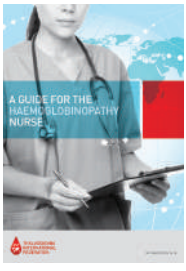


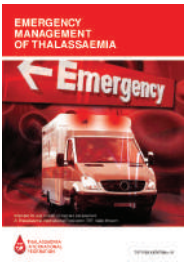






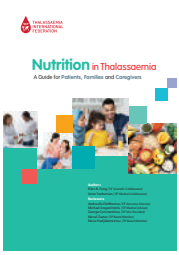


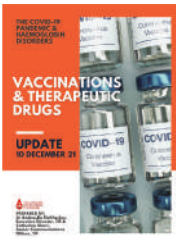


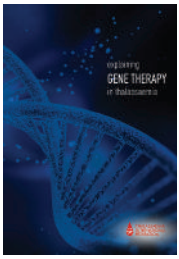


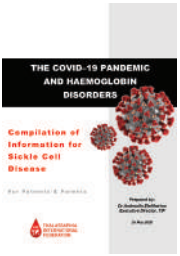
Please note that some of our publications are translated to a number of languages.

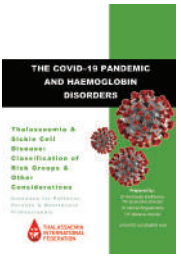
TIF PUBLICATIONS | **PATIENTS**

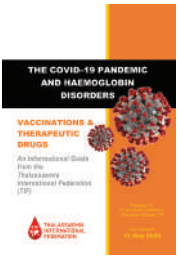


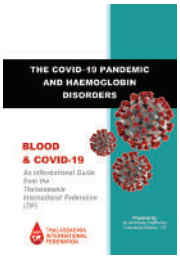


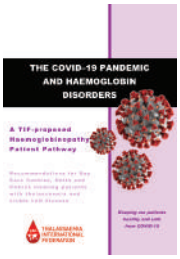


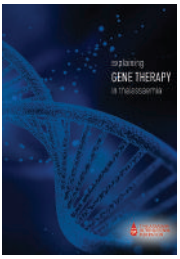


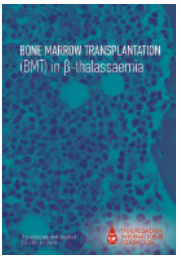


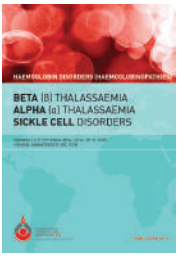


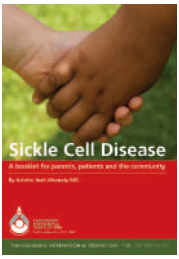


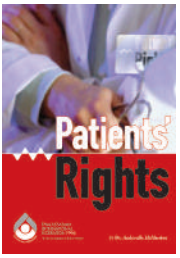












Visit our digital library: https://thalassaemia.org.cy/tif-publications/

ABOUT THE THALASSAEMIA INTERNATIONAL FEDERATION (TIF)

The Thalassaemia International Federation, a non-governmental, patient-driven umbrella organisation, established in 1986, supports to-date, the rights of patients for access to quality health, social and other care through its work with over 200 national thalassaemia associations in 64 countries across the world. It was founded by a small group of doctors and patients/parents who represented National Patient Associations, mainly from Cyprus, Greece, Italy, UK and USA, i.e. countries where thalassaemia had been recognized early as a genetic, hereditary disorder with huge medical, public health, social and economic repercussions if left unaddressed in terms of both effective prevention and management. Thus, these were the countries where strong research activity was initiated and the first control programmes were implemented in the early 1980s, with measurable success. The rationale of these founding members lay on the establishment of an international umbrella organisation to build on the accumulated experience and the knowledge gained, aiming to support the efforts of other countries since by the mid-1980s the worldwide prevalence of the diseases had been well verified.

Our Mission: The prioritisation of thalassaemia on national health agendas and the development programmes within national healthcare systems based on universal coverage

Our Vision: To support the provision of equal access of every patient with thalassaemia to high quality health, social and other care in a truly patient-centred healthcare setting

Our Values: Transparency, reliability, ethos, accountability, independence and patient-centredness

Our Work:

- Education
- Advocacy
- Collaborations / Networking
- Research
- Raising Awareness

Our Partners:

- World Health Organisation: in official relations since 1996
- United Nations: in special consultative status with the United Nations Economic and Social Council (ECOSOC) since 2017
- Council of Europe: participatory status in the Conference of International NGOs since 2019
- European Union: official partners of the European Commission in the field of Health since 2018
- European Hematology Association (EHA): Members since 2010

Our Motto: Unity & Knowledge constitute our Strength!

Thalassaemia International Federation
P.O.Box 28807, Nicosia 2083, Cyprus
Tel: +357 22319129
Fax: +357 22314552
Email: thalassaemia.org.cy

THALIA

Co-funded by the European Union

ISBN: 978-9963-717-30-9