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Review

Benefits and Risks in Polypathology and Polypharmacotherapy Challenges in the Era of the Transition of Thalassaemia from a Fatal to a Chronic or Curable Disease

Annita Kolnagou[1], Marios Kleanthous[1], George J. Kontoghiorghes[1,*]

[1]Postgraduate Research Institute of Science, Technology, Environment and Medicine Limassol, 3021 Limassol, Cyprus
*Correspondence: kontoghiorghes.g.j@pri.ac.cy (George J. Kontoghiorghes)
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Abstract

Beta thalassaemia major (TM), a potentially fatal haemoglobinopathy, has transformed from a fatal to a chronic disease in the last 30 years following the introduction of effective, personalised iron chelation protocols, in particular the use of oral deferiprone, which is most effective in the removal of excess iron from the heart. This transition in TM has been achieved by the accessibility to combination therapy with the other chelating drugs deferoxamine and deferasirox but also therapeutic advances in the treatment of related co-morbidities. The transition and design of effective personalised chelation protocols was facilitated by the development of new non-invasive diagnostic techniques for monitoring iron removal such as MRI T2*. Despite this progress, the transition in TM is mainly observed in developed countries, but not globally. Similarly, potential cures of TM with haemopoietic stem cell transplantation and gene therapy are available to selected TM patients but potentially carry high risk of toxicity. A global strategy is required for the transition efforts to become available for all TM patients worldwide. The same strategy could also benefit many other categories of transfusional iron loaded patients including other thalassaemias, sickle cell anaemia, myelodysplasia and leukaemia patients.

Keywords: thalassaemia major; iron overload; iron toxicity; organ damage; polypathology; polypharmacotherapy

1. Introduction

The thalassaemia diseases are haemoglobinopathies, the most common group of inherited diseases in humans. Adult haemoglobin in normal individuals is composed of two alpha and two beta polypeptide globin chains, each containing an iron molecule embedded in a protoporphyrin ring, which is responsible for the transport of oxygen to all cells of the body [1]. More than 200 mutations of the haemoglobin genes are known, causing a range of pathological abnormalities from asymptomatic to fatal states [1,2].

Beta thalassaemia major (TM) is an autosomal recessive inherited haemoglobinopathy with serious pathological complications and a high morbidity and mortality rate [1–3]. In TM patients insufficient or none of the beta globin chains of haemoglobin are produced and the abnormal haemoglobin composed of alpha globin chains cannot deliver oxygen efficiently to the tissues. Beta thalassaemia major is a fatal disease if it is not treated. The main form of treatment of TM patients is chronic red blood cell (RBC) transfusions and iron chelation therapy [4,5]. Bone marrow transplantation is also available for young TM patients in developed countries provided a compatible donor is found [6,7].

Excess iron accumulated from chronic RBC transfusions is toxic to many organs and becomes fatal unless it is removed by chelating drugs. Iron overload toxicity in TM and other transfusional iron loaded conditions has one of the highest metal related morbidity and mortality rates globally [8].

The geographic distribution and prevalence of TM is in developing countries found in the Mediterranean, Middle East and South East Asia, where over 90% of TM patients are born [1,2]. It is estimated that there are 100 million thalassaemia heterozygote asymptomatic carriers and more than 100,000 TM are born worldwide every year [1,2]. As an example, the annual birth rate of TM patients in India is estimated at 9000 [9].

Thalassaemia is considered as orphan disease in the European Union (EU) and the United States due to the low number of patients compared to the total population, which is mainly of Caucasian origin [10]. The treatment of TM patients in the EU countries (e.g., Cyprus, Greece and Italy) is supported by the state, whereas in most other countries support is not available [10].

The survival prospects of a newborn TM is directly related to the treatment options available in each country. In countries without the ability to provide regular RBC transfusions, TM patients die from ineffective erythropoiesis and other related complications usually by the age of 2–7 years [1,2]. In contrast, if regular RBC transfusions are available, survival in transfused TM patients is expected to increase to about 15–20 years [3]. In such cases, TM patients usually die from cardiac failure due to excess iron deposition in the myocytes and subsequent damage to myofibers [11–13].

The introduction of effective chelation therapy within a period of a few years of having the RBC transfusions, substantially increases the life expectancy of TM patients. There is an increasing number of TM patients who exceed 50 years of age, most of whom have previously adhered to effective chelation therapy protocols of subcutaneous (sc) deferoxamine (DF) and to oral deferiprone (L1), as well as effective combinations of these two drugs [4,13–15]. In general, compliance with sc DF for the majority of TM patients is poor and the average life expectancy much shorter, e.g., the mean life span of TM patients in the United Kingdom before the introduction of L1 was estimated to be 30–35 years [16]. Following the introduction of L1 in 1999, an improvement in compliance and efficacy in iron removal and a reduction in the number of cardiac deaths was observed in many countries [17–19]. Further increase in compliance in relation to chelation therapy was anticipated following the introduction of oral deferasirox (DFRA) in 2007, which may benefit TM patients intolerant of or with complications to DF and L1 therapy [19–24].

Overall, many aspects influence the morbidity and mortality rate of TM patients including the efficacy, toxicity and availability of chelating drugs [10,14–17]. Many other pathological effects in TM patients also need therapeutic intervention in addition to chelation therapy. Auxiliary therapeutics are being used in most TM patient cases in addition to iron chelating drugs for improving organ function and for treating other co-morbidities [15].

2. The Polypathology of Thalassaemia

There are many clinical complications arising from the main treatment of TM patients using chronic RBC transfusions and the removal of excess iron by chelation therapy, as well as other complications related to the underline disease. Similarly, many other clinical effects are observed as patients get older, which may be related to familial disorders, ageing or a combination of their effects and also side effects of different drugs and other therapeutic interventions. The treatments of each of the above mentioned complications are widely available to TM patients in developed countries. However, different conditions apply to TM patients in developing countries, where in most cases the patients receive irregular or no RBC transfusions or chelation therapy [1,2,9].

2.1 The Pathological Effects of Transfusional Iron Overload Toxicity in Thalassaemia

Regular RBC transfusions and iron chelation therapy is the mainstay therapy for the vast majority of TM patients worldwide. This form of therapy is also used for many other types of transfusion dependent thalassaemias and also for millions of patients with refractory anaemias such as sickle cell disease, myelodysplasia, aplastic anaemia and hematopoietic stem cell transplantation (HSCT) [6–8,25].

Thalassaemia major patients are usually transfused every 1–4 weeks, with 1–3 packed units (1 unit = 200 mg of iron) of RBC in order to maintain haemoglobin levels above 9–10 mg/dL. This rate of transfusion maintains the normal physiological bodily functions and activities of TM patients, but at the same time causes the rapid increase in body iron accumulation and progresses to iron overload. These effects in TM patients are also found in other categories of transfused patients with refractory anaemias [6–8,25].

Body iron levels in normal individuals are mainly regulated through iron absorption and the erythropoietic activity of the bone marrow. In TM patients the excess iron accumulated from transfusions cannot be excreted and it is stored in the cells of different organs in the form of ferritin and especially as haemosiderin [26–28]. The latter protein increases in concentration in many organs, particularly in the liver, as well as the heart and spleen of the transfused patients (Fig. 1) [28–30].

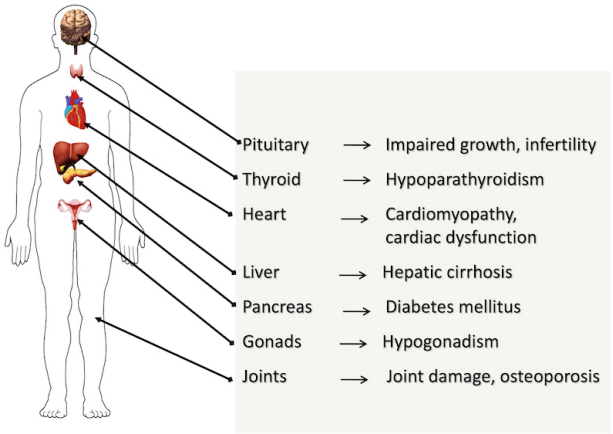

Fig. 1. The major organs susceptible to iron overload toxicity and associated pathological effects in Thalassaemia major patients. The accumulation of iron in the organs is caused from chronic red blood cell transfusions. Similar toxic side effects of iron overload in affected organs are also observed in other categories of chronically transfused patients.

Iron overload toxicity in TM on the cellular level has been previously studied using electron microscopy [11–13,27]. It has been shown that in iron overloaded conditions both cardiomyocytes and hepatocytes in TM patients contain iron loaded ferritin arrays, which are formed intracellularly mainly in primary lysosomes and also haemosiderin iron aggregates in secondary lysosomes [11–13,27,28]. Furthermore, ultrastructural observations of samples from heavily iron loaded TM patients suggest the presence of iron-laden lysosomes, which rupture into the cell sap causing intracellular damage. Similarly, several other forms of sub-cellular damage have been identified for example in cardiomyocytes of TM patients who suffered congestive cardiac failure [12,13]. In this case the sub-cellular dam-

age includes the presence of large cytoplasmic vacuoles, swollen mitochondria with loss of their cristae but with no iron deposits within them, substantial loss of myofilaments, an increase in the electron density of nuclei and also increased amounts of heterochromatin [12].

The rupture of iron-laden lysosomes into the cell sap following deposition of excess iron is considered to be the cause of the release of hydrolytic enzymes and also of potentially toxic forms of labile, redox active iron, which can catalyse the production of free radical cascades and cause further damage [31–33]. These damaging effects can progressively lead to ferroptosis and a vicious cycle of cellular, tissue and organ damage [34–39].

Organ damage in iron overload is generally detectable when approximately 50–100 units of red blood cells have been transfused [3,8,28,29]. The organ damage at the early stages can be reversible provided effective iron chelation therapy protocols are applied. In the absence of effective chelation therapy and following repeated transfusions, the organ damage caused by increasing iron load can progressively become irreversible, e.g., in liver fibrosis and cardiac failure.

The major cause of mortality in iron loaded TM patients is iron overload toxicity related cardiomyopathy [3,8,11,16,17]. Magnetic resonance imaging (MRI) diagnostic studies of iron load deposition in the heart of TM patients have suggested the existence of a correlation between cardiac damage and the level of iron overload [29,30,40–43]. In particular, there is increased risk of congestive cardiac failure in TM patients with excess cardiac iron deposition levels [41–43].

In addition to the heart complications in TM patients, increased iron deposition and iron toxicity has also been observed in many other organs, affecting their function. These include liver, spleen and endocrine damage (Fig. 1) [29,30]. Excess iron deposition and associated toxicity in organs can be prevented using effective chelation therapy and by achieving as well as maintaining normal iron levels from childhood [44].

2.2 Organs Susceptible to Iron Overload Toxicity Caused by Chronic Transfusions

Several organs have been identified as being susceptible to iron overload toxicity from chronic RBC transfusions and the extent of damage in each of these organs contributes to the overall morbidity and mortality rate observed in TM patients (Fig. 1). The diagnosis and extent of damage in these organs has been identified and monitored using mainly histopathological and magnetic resonance imaging (MRI) techniques, which can monitor structural feature changes in organs and also other tests related to the function of these organs, such as liver enzyme levels [13,29,30,40–43,45,46].

The major organs associated with an increased mortality due to iron overload damage in TM patients are the heart and to a lesser extent the liver (Fig. 1, Ref. [13]). In general, the level of cardiomyopathy and cardiac dysfunction appears to be related to the level of iron deposition in the heart and in particular severe cases of cardiac iron deposition, e.g., of MRI T2* with signal intensity less than 9 ms, the TM patients are in danger of congestive cardiac failure (Fig. 2) [13,43]. Similarly, excess iron deposition in the liver, e.g., in severe siderosis can progressively cause hepatic cirrhosis, fibrosis and hepatocellular carcinoma (HCC), which can also lead to death [28,47–49].

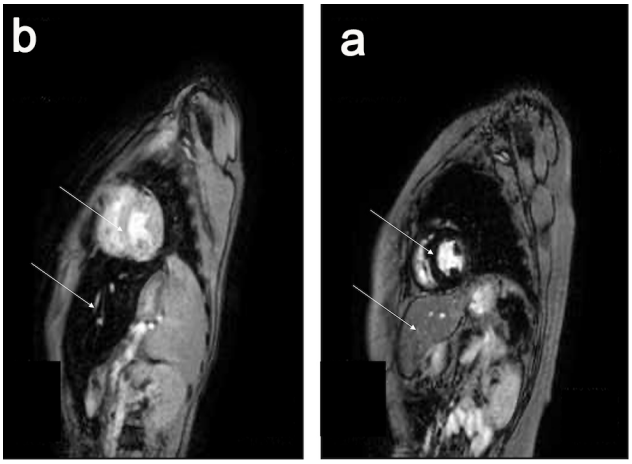

Fig. 2. Magnetic resonance imaging (MRI) images of two iron loaded thalassaemia patients, showing differential iron loading of the heart and liver. (a) Right picture. Heavy haemosiderosis of the heart [T2* = 6.32 ms (normal T2* $\geq$19 ms)] and normal T2* of the liver (T2* = 19.2 ms). The top arrow shows the abnormal iron deposition in the interventricular septum of the heart of the patient, which is shown with low signal intensity (dark). The bottom arrow shows the liver of the patient with no iron deposition (normal). (b) Left picture. Heavy haemosiderosis of the liver [T2* = 1.2 ms (normal T2* $\geq$6.3 ms)] and normal T2* of the heart (T2* = 20.6 ms). The top arrow shows the interventricular septum of the heart of the patient with no iron deposition (normal) where the bottom arrow shows the heavy iron loading within the liver parenchyma, demonstrated as low signal intensity (dark). Adapted from [13].

Several other organs are affected from excess iron, including the pancreas, which is associated with diabetes mellitus and is widely occurring in TM patients [50]. Impaired or stunted growth and infertility associated with excess iron deposition in the pituitary gland is also observed, as well as hypoparathyroidism and hypogonadism caused by the malfunction of the thyroid and the gonads respectively, all of which are important side effects affecting many TM patients [51–54].

Excess iron deposition appears to be at lower levels in the kidneys, skin, lungs and the brain of TM patients and accordingly their function is not affected to the same extent by iron toxicity in comparison to the heart, liver and other iron loaded organs. For example, a bronze colour appearance of the skin is observed in heavily iron loaded TM patients, which does not appear to be associated with any serious skin damage [55–58].

In some TM patients the presence of excess focal deposited iron, irrespective of the total body iron load can also cause tissue damage. Excess iron has for example been observed in the joints of some TM patients using MRI T2* with associated knee and ankle complications [59]. It should be noted that focal iron deposition and associated complications have also been observed in the brain of patients with neurodegenerative diseases, such as Friedreich ataxia, Alzheimer's and Parkinson's diseases [60–62].

In addition to organ toxicity observed in TM patients as a result of excess iron deposition, pathological effects are also suspected from non-transferrin bound iron, which is able to catalyse the formation of free radicals leading to oxidative stress toxicity and progressively to biochemical, subcellular, cellular and tissue damage [63–68]. All three chelating drugs and in particular L1 appear to inhibit effectively the iron catalysed free radical damage [69–73].

2.3 Pathological Effects in Thalassaemia Caused by Infections and Other Complications

Major clinical complications in TM are not directly related to iron overload toxicity but to several other factors including those caused by the underlying disease, transfusions from different blood donors, drugs and also many other common diseases affecting the general population. In most of these cases the treatments provided have different parameters and characteristics in comparison to the general population because of differences in the pathophysiology of TM patients.

Microbial infections are among the serious clinical complications affecting TM patients with estimated rate of 8.5 per 100 patient years and are becoming the major cause of death especially since the number of congestive cardiac failure incidences has dramatically declined in many countries following the introduction of effective chelation therapy protocols and primarily of L1 [16–18,74,75].

Although the level of iron overload and transferrin saturation are important parameters contributing to the growth and proliferation of microbes, many other pathological complications and factors are involved, some of which are similar to other categories of patients facing infection [75–79].

Thalassaemia major patients are subjected to various bacterial, fungal and viral infections. Many complications in TM patients include pneumonia, liver abscess, biliary tract and soft tissue infection, which are mostly caused by different bacteria species such as *Klebsiella pneumonia*, *Streptococcus pneumonia*, *Escherichia coli*, *Salmonella typhi* and *Yersinia enterocolitica* [74,76]. Viral infections involving for example Human parvovirus B19 (HBV), Cytomegalovirus (CMV) and Human immunodeficiency virus (HIV), as well as fungal infections such as Mucormycosis or Zygomycoses and *Pythium insidiosum* are also common in TM patients. The main predisposing factors for infection in addition to iron overload in TM are transmission from transfusions, splenectomy, diabetes, liver derangement, reduced immunity and chronic diseases [74,76,80].

Infections and other clinical complications arising from chronic RBC transfusions in TM patients are also found in many other categories of regularly transfused patients with refractory anaemias. These additional complications can range from short term effects such as anaphylactic reactions to long term effects such as RBC antibodies arising from blood transfusions obtained from multiple blood donors. The latter effect makes blood selection for transfusions difficult, especially in older TM patients [81,82]. Acute haemolytic reactions and delayed reactions are also frequent in patients with chronic transfusions [81–84]. Some other side effects of transfusions may be very serious or even fatal, such as the accidental transfusion of the wrong blood type group, or rare cases like transfusion-related acute lung injury (TRALI) [85,86]. Chronic RBC transfusions are also implicated in many other serious pathological effects such as cardiac hypertrophy and pulmonary hypertension, which are mainly observed in older TM patients [87,88].

Many TM patients experience anaemia symptoms to a variable degree, which in general depends on the rate of RBC transfusions. In developing countries the absence or insufficient number of RBC transfusions can cause severe anaemia in TM patients, with many associated pathological changes and reduced survival. In such cases chronic haemolysis, bone marrow expansion and extramedullary erythropoiesis are observed, which can result in bone pain, deformities and fractures, facial abnormalities, as well as splenomegaly and hepatomegaly [2,16].

Several other pathological effects are also observed in the older TM patient population, which are related to familial diseases such as diabetes and heart disease and also in ageing populations such as osteoporosis, musculoskeletal/joint problems and malignancy. In general these effects appear at a much younger age in TM patients in comparison to normal individuals of similar ages in the general population.

2.4 Clinical Complications Arising from the Toxic Side Effects of Chelating and Other Drugs

The need for daily chelation therapy for the treatment of iron overload, as well as many other drugs for the treatment of other pathological conditions in TM patients requires continuous vigilance for the possibility of toxic side effects. In this context, regular monitoring and prophylactic

measures for chelating and other drug toxicity are generally recommended. Clinical examination and biochemical tests are regularly carried out to identify drug toxicities including tests for organ function such as liver enzyme and urine creatinine levels, blood cell counts, echocardiography, MRI, serum ferritin, serum iron and zinc levels [3,16,40,45,46].

The general toxic side effects of chelating and other drugs are listed in each drug's label information following their regulatory approval. The monitoring of possible toxic side effects related to the daily use of DF, DFRA and L1 is important for the safety of TM patients (Fig. 3) [89–91]. In particular, the prospect of chelating drug toxicity increases in TM patients with low or normal iron stores [91]. Within this context, the use of DFRA is not recommended for iron loaded patients with serum ferritin lower than 500 μg/L [92–96]. Some toxic side effects have also been reported in iron loaded TM patients treated with DFRA, which however are less frequent than in non-iron loaded categories. These include renal, liver and bone marrow failure and agranulocytosis, skin rashes and gastric intolerance [92,97–99]. Kidney function is regularly monitored in TM patients treated with DFRA and withdrawal of the drug is recommended for patients with persistent rise in serum creatinine levels [92].

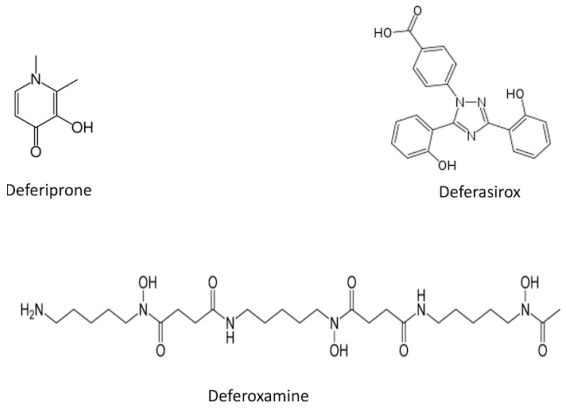

Fig. 3. The chemical structure of the iron chelating drugs used for the treatment of iron overload in Thalassaemia major patients. Deferiprone (L1), deferasirox (DFRA) and deferoxamine (DF) and their combinations are used for the treatment of thalassaemia and other transfusional iron loading conditions.

Similar limitations and restrictions apply in the use of DF in TM patients with low iron stores as those of DFRA, despite that the incidence of serious toxicity is much lower in the case of DF. The use of DF in non-heavily iron loaded TM patients or other categories of patients with normal iron stores is not recommended due to toxicity implications. Several toxicities were reported in different categories of patients using DF including cases of mucormycosis, acute respiratory distress syndrome and *Yersinia enterocolitica*. Furthermore, auditory and ocular toxicity has also been reported in non-heavily iron loaded TM patients using DF [89,90,100–103].

Low toxicity has been observed in TM and other categories of patients with low or normal iron stores using L1 in thousands of patients in the last 25 years and also in studies in patient categories with normal iron stores, with an excess of 100 patients years [89,90,104]. The safety record of L1 in TM patients increased the prospect of its wider clinical use as a universal chelator/antioxidant in non-iron overloading diseases related to free radical pathology [33]. The chelator/antioxidant effects of L1 have been investigated in clinical trials involving many categories of patients including neurodegenerative, cardiovascular, renal, infectious diseases, cancer, AIDS and ageing [33,60–62,105–108].

Several toxic side effects have been reported for L1, with the most serious those of agranulocytosis (less than 1%) and neutropenia (less than 5%) [89,90,104,109]. Both toxicities are reversible and weekly or fortnightly mandatory blood count monitoring is recommended for prophylaxis for all patients using L1. Several other, less serious toxic side effects include gastric intolerance, joint pains and Zn deficiency [104,109–111].

It appears that the rate of morbidity and mortality for each chelating drug is different and also the target organ of toxicity varies in each case [90]. Furthermore the iron complex of chelating drugs is less toxic than the non-bound chelator in all three drug cases.

The toxicity of the less frequently used drugs for the treatment of other co-morbidities in TM patients, in addition to iron chelation is rather rare and similar to that observed in other categories of patients. However, toxicity vigilance including drug interactions and prophylactic measures are important parameters for ensuring the safety of TM patients treated with chelating and also all other drugs.

3. The Polypharmacotherapy of Thalassaemia

The polypathology of TM requires the continuous biochemical and clinical monitoring of patients in specialised clinics, which includes the regular assessment of haemoglobin levels and arrangements for RBC transfusions every 1–4 weeks, as well as the adjustment of chelation therapy following serum ferritin estimations. Many more clinical and pharmacological interventions are also needed for the different co-morbidities and pathological effects related to the underlying condition, all of which contribute to the survival prospects and overall health status of TM patients.

Many different drugs are intermittently used by TM patients in addition to daily treatment by chelating drugs. The different pharmacological treatments help in improving the quality of life and in decreasing the overall morbidity and mortality of TM patients. In particular, the therapeutic approach used in the last two decades has significantly im-

proved the life expectancy of TM patients, some of whom are grandparents and most are professionals contributing to many sectors of society [13,15].

3.1 The Importance of Effective Iron Chelation Therapy Protocols in Thalassaemia

The main aim of chelation therapy in chronically transfused TM patients is the prevention or minimisation of iron overload toxicity and the decrease of the associated high mortality and morbidity rate [44]. This goal can only be achieved if effective chelation treatments are available, which can maintain the general body iron load and iron in the affected organs to normal or near normal levels [14,90].

Despite the common goals for the substantial reduction or complete elimination of iron overload in TM patients, there is no consensus in the use of iron chelation protocols, which differ between countries and even clinics in each country [14,90,112]. In general, chelation treatment protocols in TM involve the administration of DF, L1 and DFRA, as well as different combinations of these chelating drugs (Fig. 3) [90,113–117]. All drugs including the iron chelating drugs have different pharmacological activity including absorption, distribution, metabolism, elimination and toxicity (ADMET) characteristics, mode of action, efficacy, and cost [118]. Similarly, variable pharmacological activity of DF, L1, DFRA and their combinations are observed in TM patients, the availability and use of which affects the overall survival of TM patients in each country [90,113–117].

The recommended doses for use of the chelating drugs in TM patients are 40–60 mg/kg/day for DF, 75–100 mg/kg/day for L1 and 20–40 mg/kg/day for DFRA [90]. Various dose protocols and combinations are used in the context of personalised therapies, which are based on variations in general body iron overload and also individual organ targeting effects [90,113–117,119]. A range of therapeutic protocols are selected for different categories of patients including intensive chelation, e.g., combinations of DF (40–60 mg/kg/day) and L1 (75–100 mg/kg/day) in heavily iron-loaded TM patients to intermittent withdrawal of chelation in less heavily iron-loaded TM patients [120–123].

The most tolerable, safe and effective chelation protocol, which has been identified in achieving negative iron balance and normalisation of the iron stores in TM patients is that of the International Committee on Chelation (ICOC) combination of oral L1 (80–100 mg/kg/day) and sc DF (40–60 mg/kg/day, at least 3 days per week) [4,13–15,113,114]. The time period required for achieving the normalisation of the iron stores in TM patients as assessed by MRI T2* and serum ferritin levels varies and depends mainly on the iron load of patients and the overall dose of the chelating drugs. For example, the complete elimination of iron overload in TM patients with initial serum ferritin of 700–4000 μg/L using the ICOC protocol was estimated to be about 6–30 months [120,122,123].

The importance of the selection of ICOC and similar protocols on TM patient survival has also been shown in epidemiological studies, which suggested that primarily the use of L1 and also its combination with DF can cause a substantial reduction in morbidity and mortality [15,17,124]. The ICOC and similar protocols appear to be effective therapeutic options in significantly reducing or eliminating gross iron overload and also for decreasing the associated high mortality and morbidity observed in TM. Overall, the role of L1 in reducing primarily excess cardiac iron and also excess body iron is considered as one of the major factors in the transition of TM from a fatal disease to a chronic disease [15,125–128].

The safety of L1 in TM patients with normal iron stores has also been confirmed following its introduction for the treatment of non-iron loaded patients by targeting focal toxic iron deposits, e.g., in Parkinson's disease and Friedreich ataxia and also in diabetic and non-diabetic glomerular disease patients affected by toxic labile iron [60–62,105–108]. In particular, the long term safety of L1 has been shown in the glomerular disease patients using doses of 50–75 mg/kg/day for 6–9 months with no serious toxic side effects [105]. Similar findings from clinical trials on the safety of L1 have been observed in many other categories of non-iron loaded diseases including the anaemia of chronic disease, renal dialysis, infections and several other neurodegenerative diseases [89,90].

3.2 Pharmacotherapies in Thalassaemia not Related to the Elimination of Excess Iron

Many other drugs are regularly used for the treatment of different pathologies in TM, in addition to the use of chelating drugs for the removal of excess iron. Most of these drugs are used for the prevention or treatment of abnormalities observed in the various organs and their function, some other drugs for microbial infections and immune reactions and many more for other abnormalities also affecting the general population.

Chronic RBC transfusions in TM are associated with clinical complications and side effects [129–131]. Most of the complications of RBC transfusions are related to reactions in response to blood from donors, which is contaminated with white blood cells and other antigenic factors [129,132]. In such cases the treatment depends on the side effects and other co-morbidities of the patient. For example, mild allergic reactions such as itching and erythema are treated by corticosteroids and antihistaminic drugs. However, more intensive and complex therapies are required in severe allergic reactions which can cause anaphylaxis, hypotension and bronchospasm, or in delayed transfusion reactions, which may occur 1–2 weeks after transfusion and can cause anaemia, jaundice and fatigue. Acute haemolytic reaction is an additional side effect resulting from errors in blood typing and compatibility testing, which can cause

fever, chills, shock, dyspnea, and haemoglobinuria. In such cases transfusion is stopped and patients are treated with intravenous fluids, diuretics and heparin [81–84]. Alloimmunisation from transfusions and the occurrence of related alloantibodies causing reduction in haemoglobin levels is estimated to affect 10–20% of mainly splenectomised TM patients [81,132,133]. In addition, autoimmune haemolytic anaemia may occur in some patients with alloantibodies who can be treated with steroids, intravenous immunoglobulin and immunosuppressive drugs [84,133]. Transfusion-related acute lung injury (TRALI) and transfusion-induced graft versus host disease are very rare cases in TM and other transfused patients but with very serious multi-pathological effects including fatalities, which require intensive and multidisciplinary treatment [85,86,133,134].

Myocardial damage and dysfunction is the most serious toxic side effect of iron overloaded TM patients, which requires regular monitoring and follow up, as well as many and different drugs for improving cardiac function [43,135–139]. Cardiac complications include arrhythmias, cardiac failure, pulmonary hypertension and peripheral vascular disease. Therapeutic approaches include anti-arrhythmic drugs such as beta-blockers, angiotensin converting enzyme (ACE) inhibitors such as ramipril, captopril and enalapril, aldosterone antagonists such as spironolactone and eplerenone, anticoagulants, cardiotonic agents, vasodilator agents and also diuretics [88,135–139].

The liver complications in TM are mostly related to the level of iron overload, which may lead to cirrhosis, fibrosis and hepatomegaly. Liver damage and function are also affected by viral infections including chronic hepatitis B and C, and also fatty liver disease and hepatocellular carcinoma [48,49,140–142]. Hepatitis B is treated with interferon, the nucleoside analogs lamivudine and entecavir and the nucleotide analogs adefovir and tenofovir. Hepatitis C is treated with ribavirin and pegylated interferon and also bocepreviror and telaprevir [143–147]. There are also rare cases of hepatocellular carcinoma in TM, which are treated with chemotherapy and surgery [48,49,148,149]. In a few cases of TM with severe liver damage, treatment with liver transplantation has also been considered and carried out.

Multiple clinical complications including infections and those related to splenomegaly and splenectomy, which occur in addition to iron overload toxicity, are also a major target of therapeutic interventions in TM [29,47,74,80,150–155]. In general, TM patients prior to splenectomy are treated for immunoprophylaxis with vaccination against the pneumococcal, *Haemophilus influenza* and *Neisseria meningitides* viruses [156]. Splenectomised patients receive a number of prophylactic and other treatments including penicillin and other antibiotics for infections and sepsis, aspirin for thrombocytosis and anti-coagulant prophylaxis for hypercoagulability and thromboembolic complications [157,158]. Further complications and therapies arise following splenectomy, such as increasing iron loading of other organs and particularly the liver and the heart [29,47,80,159,160]. In such cases, personalized chelation and other therapeutic protocols can be designed for overcoming individual associated health risk problems.

Endocrine damage is a major complication in young TM patients, which can lead to growth retardation and arrested puberty [50–55]. In males, the management of the delayed puberty and hypogonadotrophic hypogonadism involves the intramuscular administration of depot-testosterone and in pubertal arrest the intramuscular administration of testosterone esters or topical testosterone gel. In females the treatment involves the administration of oral estradiol, low oestrogen and progesterone [161–164]. In relation to hypothyroidism the treatment involves L-thyroxine and in cases of subclinical hypothyroidism and cardiomyopathy the drug amiodarone. In hypoparathyroidism management and also prophylaxis for prevention of acute and chronic complications of hypocalcemia, the affected patients receive oral vitamin D, calcium and synthetic human parathyroid hormone [164–166]. The management of osteoporosis and osteopenia involves calcium and vitamin D supplementation, hormonal replacement, prevention of hypogonadism, continuous hormonal replacement, and also the use of calcitonin and bisphosphonates [166,167]. Lastly, the management of adrenal insufficiency involves treatment with glucocorticoids [164]. In many cases endocrine damage can be prevented, reversed or minimized using effective chelation protocols [168–171].

In the majority of cases clinical complications with several co-morbidities can occur simultaneously as shown in Table 1 (Ref. [8,14,29,30,43,47–58,74,80,88,90,113,114,120–128,135–170]), especially in older iron loaded TM patients, where multiple therapeutic drug interventions and other treatments are required in addition to chelation therapy [172]. Despite the complexity of such co-morbidities in TM patients, the clinical outcome in many cases has been improved with the introduction of effective iron chelation protocols and other new targeted therapeutics.

4. Future Prospects including Transplantation and Gene Therapies

Major efforts have been undertaken by governments and international organisations for the worldwide prevention of TM, a potentially fatal inherited disease prevalent in developing countries [1,2,9,10,15]. In the meantime, further research is needed on all aspects of the pathological effects and therapeutic interventions for the new born and existing TM patients.

Despite that HSCT from a matched family or unrelated donor, may offer a successful therapy for selected young TM patients who have no secondary organ damage due to iron overload, the risks of death and serious toxic effects are still high for the majority of the remaining patients [7,173–177]. Further improvements on safety in HSCT transplantation and the introduction of safe gene therapy may offer a

Table 1. Organ damage in Thalassaemia major and therapeutic interventions.

Organ and associated clinical complications	Treatment	Ref
Heart		
Iron overload damage:	L1 and combinations with DF and DFRA	[8,14,30]
Cardiac failure. Arrhythmias. Arterial changes Complications unrelated to iron overload: Pulmonary hypertension. Arrhythmias and atrial Fibrillation. Thrombotic episodes. Cardiac function (restrictive or/and diastolic dysfunction/fibrosis). Arterial changes	Anti-arrhythmic drugs. Aldosterone antagonists. ACE inhibitors. Anticoagulants, Diuretics. Cardiotonic agents. Vasodilator agents	[43,88,90,120–128,135–139]
Liver		
Iron overload damage: Liver damage and malfunction, hepatomegaly cirrhosis and fibrosis	DF, L1 and DFRA. Combinations of all three	[29,30,48,49,113,114]
Complications unrelated to iron overload: Hepatitis B (HBV) and C (HCV). Hepatocellular carcinoma (HCC). Fatty liver disease	HBV: Interferon, nucleoside and nucleotide analog antiviral drugs. HCV: Pegylated interferon and antiviral drugs. HCC: Chemotherapy and surgery	[120–128,140–149]
Spleen		
Iron overload damage: Spleen damage and malfunction. Splenomegaly	DF, L1 and DFRA. Various combinations of all three drugs	[29,30,47]
Spenectomy. Prior to splenectomy:	Immunoprophylaxis with vaccines against viruses	[74,80]
Following splenectomy: Prophylaxis against infections and sepsis. Thrombocytosis. Hypercoagulability and thromboembolic complications	Antibiotics. Aspirin. Anti-coagulant prophylaxis	[150–160]
Endocrine glands		
Iron overload damage:	DF, L1 and DFRA. Various combinations of all three drugs from young age	[29,30]
Growth retardation and arrested puberty in young TM patients		[50–58]
In males: Delayed puberty and hypogonadotrophic hypogonadism	Intramuscular depot-testosterone Intramuscular testosterone esters or topical testosterone gel	[120–128]
Pubertal arrest		[161–170]
In females:	Oral estradiol, low oestrogen and progesterone	
Hypothyroidism	L-thyroxine	
Subclinical hypothyroidism and cardiomyopathy	Amiodarone	
Hypoparathyroidism and hypocalcemia	Vitamin D, calcium and parathyroid hormone	
Osteoporosis and osteopenia	Vitamin D, calcium, calcitonin, bisphosphonates, hormonal replacement	
Diabetes mellitus	Oral antidiabetic drugs, insulin (sc)	
Adrenal insufficiency	Glucocorticoids	

ACE inhibitors, angiotensin converting enzyme inhibitors; DFRA, deferasirox; L1, deferiprone; DF, deferoxamine; HBV, Hepatitis B; HCV, Hepatitis C; HCC, hepatocellular carcinoma; sc, subcutaneous.

complete treatment for TM patients [7,173–179].

There is increasing interest as well as many clinical trials in progress over the last 2 to 3 years concerning gene therapy and its comparison with allogeneic HSCT, both of which can potentially offer complete therapy for TM patients [6–8,173–180]. The risk/benefit assessment of these two HSCT methods and their comparison with the standard therapy of regular RBC transfusion and chelation therapy are likely to be the subject of future investigations.

Allogeneic HSCT in TM from human leukocyte antigen (HLA) matched siblings or unrelated bone marrow donors was initiated 40 years ago and is estimated to have been used so far in about 4000 TM patients worldwide [6–8,173–177,181]. This therapeutic method is subject to suitable donor availability and offers the complete treatment of TM especially for young TM children. The ther-

apy has been developed for TM following many years of monitoring and investigations mainly on improving the transplant procedure and also treating the short and long term toxic side effects of transplantation including graft rejection, chimerism, graft versus host disease (GvHD), infections, myeloablative conditioning regimens, the use of matched or mismatched donors and in patients of different ages, iron loading and with different underlying co-morbidities [7,8,182–185]. In this context, many different factors appear to influence the overall survival (OS) and thalassaemia –free survival (TFS) of HSCT TM patients in different countries and transplantation centers. For example, in a follow up of a maximum 30 years monitoring study, OS was estimated to be about 83% and TFS 78%. Furthermore, the probability of graft rejection was estimated to be about 7% and transplant-related mortality about 14%. Graft versus host disease was the major complication with grade II–IV acute and chronic incidence to range to about 24% and 13% respectively [186]. Similar results were obtained in other short and long term monitoring studies of HSCT TM patients [7,182–184,187]. Overall, it appears that in general young and non-iron loaded patients with non-underlying co-morbidities have the highest prospects of OS and TFS from the HSCT TM patients.

Genetically modified autologous HSCT via gene addition has recently received a conditional approval by the European Medicines Agency (EMA) for the treatment of TM patients [178–180]. This gene therapy option offers the potential for the treatment of all TM patients without the need of a bone marrow donor. The method is based on a gene addition using lentiviral vectors which can introduce a beta-globin gene into autologous hematopoietic stem cells. The product approved by EMA, betibeglogene autotemcel (beticel), has reached phase 3 trials with some promising results. Several other products and gene editing techniques are under investigation and development. Many clinical trials for these agents are ongoing and full assessment of the results is expected in the next few years [178–180,188–195].

Despite the initial encouraging results there are at least three major drawbacks, which are likely to limit the gene therapy option for TM patients and will require further research and development in the coming years. One of the major drawbacks is that most of the TM patients do not achieve transfusion independence, e.g., in a recent study six of nine patients failed to achieve this goal [180,193,196]. Another drawback is the risk of hematological malignancies due to different factors including insertional mutagenesis. Lastly, public expenditure concerns appear to limit the overall number of TM patients that can have access to gene therapy [180,196].

Inducers of increased haemoglobin F production or other drugs than can cause reduction in the rate of RBC transfusion and subsequently to iron loading are important research areas for further improving the therapeutic prospects of TM [190,197–199]. The anticancer and iron binding drug hydroxycarbamide (hydroxyurea) is widely used in thalassaemia indermedia for increasing HbF production but is not effective in TM [200–202]. Similarly, the recent introduction of erythropoietic biologics such as luspatercept (Reblozyl) has been considered for reducing RBC transfusions in TM and other haematological diseases [203–205]. Several studies have suggested that about 20% reduction in RBC could be achieved in some TM patients [203–205]. However, the use of luspatercept in TM is questionable, since even if haematopoiesis can be increased as suggested in other haematological patient categories, in the case of TM patients luspatercept can only increase the production of abnormal, non-functional haemoglobin [206]. Further studies are required to establish the mode of action and efficacy of luspatercept in TM. Similarly, serious concerns remain in other aspects of therapy, such as the safety and the availability of luspatercept and also of other biological therapies in TM and other diseases [206,207].

Regular RBC transfusions and chelation therapy remains the mainstay, realistic therapy for the vast majority of TM patients. In general, iron overload toxicity is considered as an independent adverse prognostic factor in all diseases mainly because of the ability of iron to catalyse the excess production of free radicals and other reactive oxygen species, as well as to promote microbial infections. Many other categories of regularly transfused patients are affected from iron overload toxicity in addition to TM, including patients of allogeneic HSCT such as sickle cell anaemia, myelodysplasia, and leukaemia [8,15,208–211]. Research efforts for the complete removal of excess iron and the achievement of normal iron stores is the ultimate aim for the treatment of iron toxicity in all these iron overload categories involving millions of patients [8,14,33,44,114].

Further improved therapeutic protocols, which can also decrease the overall morbidity and mortality in TM are expected from the introduction of new, personalised adjusted chelating drug combinations involving one to three drug combinations of L1, DF and DFRA as previously suggested [90,212].

One of the major contributing factors that resulted in the increased survival and the quality of life of TM patients is the organisation of multidisciplinary specialised team protocols for monitoring and intervening in all the pathological effects involving each of the affected organs and their function [15,172,213].

New efforts are required for the supply of chelating drugs for the treatment of iron overloaded patients worldwide. These efforts could include clinical development of natural and synthetic investigational new chelating drugs, combinations with L1, DF and DFRA and targeted chelation protocols for optimal therapies [214–217]. Similarly, advancements in the diagnosis and treatment of other co-morbidities, as well as improvements in the efficacy of polypharmaceutical treatment using new drugs could contribute further to the efforts in the transition of TM from

a fatal to a chronic disease [15,172,193,218–222]. Future research efforts and worldwide strategies targeting all comorbidities and the high mortality rate of TM in developing countries could signal the end of TM as a fatal disease worldwide.

5. Conclusions

The mainstay treatment of TM and other refractory anaemias is chronic RBC transfusions and chelation therapy. The introduction of effective personalised iron chelation therapy protocols involving mainly L1 and also DF, DFRA and their combinations, as well as new non-invasive diagnostic techniques for monitoring iron removal from major organs, resulted in the complete treatment of iron overload and the long term survival of TM patients in many developed countries. Similarly, new therapeutic approaches in relation to the polypathological and polypharmaceutical clinical challenges and the involvement of multidisciplinary specialist teams contributed to a great extent to the transition of TM from a fatal to a chronic disease in some developed countries. Future research efforts and worldwide strategies on all aspects of the polypathological clinical challenges, including the development of worldwide strategies for the supply of iron chelating and other drugs, as well as drug combinations could further improve the therapeutic outcomes of TM patients globally. Such efforts could also benefit many other categories of regularly transfused patients which are affected from iron overload toxicity including patients of allogeneic HSCT, sickle cell anaemia, myelodysplasia, and leukaemia. The complete removal of excess iron and the achievement of normal iron stores is the ultimate aim for the treatment of iron overload toxicity in TM and all other categories of chronically transfused patients.

Abbreviations

HSCT, allogeneic hematopoietic stem cell transplantation; ACE inhibitors, angiotensin converting enzyme inhibitors; DFRA, deferasirox; L1, deferiprone; DF, deferoxamine; HBV, Hepatitis B; HCV, Hepatitis C; HCC, hepatocellular carcinoma; HLA, human leukocyte antigen; MRI, magnetic resonance imaging; OS, overall survival; RBC, red blood cell; sc, subcutaneous; TFS, thalassaemia free survival; TM, beta thalassaemia major.

Author Contributions

GJK designed, wrote and edited the manuscript. AK reviewed the clinical aspects of Thalassaemia and polypharmacotherapy. MK reviewed the technical aspects and helped in the preparation of the manuscript.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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