β-Thalassemia intermedia: morbidity uncovered

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Optimal management of β thalassaemia intermedia

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Summary

Our understanding of the molecular and pathophysiological mechanisms underlying the disease process in patients with β thalassaemia intermedia (TI) has substantially increased over the past decade. The hallmark of disease process in patients with TI includes ineffective erythropoiesis, chronic haemolytic anaemia, and iron overload. There are a number of options currently available for managing patients with TI including splenectomy, transfusion therapy, iron chelation therapy, modulation of fetal haemoglobin production, and several other agents targeting specific clinical complications. Limited studies assessed the efficacy and safety of these modalities; hence, there are currently no clear guidelines for managing patients with TI. Until solid evidence-based guidelines are available, individualised treatment should be entertained.

Keywords: thalassaemia intermedia, splenectomy, transfusion, iron chelation, fetal haemoglobin induction.

β thalassaemia is an inherited disorder of haemoglobin (Hb) synthesis wherein mutations of the β globin gene lead to various degrees of defective β chain production, an imbalance in α/β globin chain synthesis, ineffective erythropoiesis, and a spectrum of anaemia (Weatherall & Clegg, 2001). Extremely diverse phenotypes exist within the β thalassaemia syndromes. At one end of the spectrum is β thalassaemia minor, a clinically silent, mildly hypochromic and microcytic anaemia. At the other end is β thalassaemia major (TM) which refers to those patients whose clinical course is characterised by profound anaemia, who present to medical attention in the first year of life, and who subsequently require regular blood transfusions and iron chelation therapy for survival (Weatherall & Clegg, 2001; Cao & Galanello, 2010). The term β thalassaemia intermedia (TI) was suggested to describe patients who had clinical manifestations that are too severe to be termed minor yet too mild to be termed major, although there remains substantial overlap between the three conditions (Sturgeon *et al*, 1955). Our understanding of the molecular and pathophysiological mechanisms underlying the disease process in patients with TI has substantially increased over the past decade. However, significant challenges towards the management of TI still exist, as the severity of anaemia and disease span an extremely broad spectrum. Thus, despite the availability of several treatment options, clear management guidelines are lacking (Taher *et al*, 2010c). With these limitations in mind, we herein aim to overview current evidence on the effectiveness and risks of common medical treatment approaches and recommend optimal scenarios for their incorporation into the care of patients with TI.

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Understanding the genotype/phenotype relationship in TI

Description of the various forms of β thalassaemia is based on the clinical severity of the condition rather than the underlying genetic abnormality. Although the term TI lacks specific molecular correlates, and the diagnosis remains largely clinical, a genotype/phenotype association has been observed (Galanello & Cao, 1998). The β thalassaemias, including TI, arise from defective gene function leading to the partial suppression of β globin protein production. Most TI patients are homozygotes or compound heterozygotes for β thalassaemia, meaning that both β globin loci are affected (Galanello & Cao, 1998). Hb E/β thalassaemia results from co-inheritance of a β thalassaemia allele from one parent, and the structural variant Hb E from the other. The latter is by far the commonest form of TI, accounting for about 50% of moderate or severe cases (Olivieri *et al*, 2008). Less commonly, only a single β globin locus is affected, the other being completely normal, that is, TI is dominantly inherited (Weatherall & Clegg, 2001).

In the case of TI, the multilayered complexity of the genetic basis for phenotypic diversity is best explained in terms of primary, secondary, and tertiary genetic modifiers (Weatherall, 2001). The primary modifiers represent the broad diversity of mutations that affect the β globin genes, ranging from

extremely mild promoter mutations that cause a very slight reduction in β globin–chain production to the many different mutations that result in the β thalassaemias; that is, a complete absence of β globin product. Compound heterozygosity for these different mutations can provide a very broad spectrum of clinical phenotypes. The secondary genetic modifiers are those that are involved directly in modifying the degree of globin-chain imbalance in β thalassaemia. The coinheritance of α thalassaemia has this effect, and, since there are numerous different molecular forms of α thalassaemia of different severity, this interaction provides further scope for a wide range of different β thalassaemia phenotypes. Similarly, the degree of globin-chain imbalance can be reduced by the more effective synthesis of the γ chains of fetal Hb (HbF) after birth. There are several genes involved in modifying the γ chain response, some that are encoded in the β globin–gene cluster, others that are on different chromosomes. Some of the variability in HbF levels and clinical severity in patients with TI is determined by the Xmn-$I^{+/+}$ genotype at position −158 of *HBG2* (Thein *et al*, 1987). The quantitative trait loci *HBS1L-MYB* intergenic region on chromosome 6q23 and *BCL11A* on chromosome 2p16, have also been recently identified as modulators of HbF production (Menzel *et al*, 2007; Sankaran *et al*, 2008, 2009; Wahlberg *et al*, 2009). Well-designed studies in cohorts of patients with β thalassaemia have demonstrated that these variants contribute significantly to the clinical course of the disease and may explain up to 75% of the variation in clinical severity (Uda *et al*, 2008; Galanello *et al*, 2009; Nuinoon *et al*, 2010). Another described secondary modifier would be the alpha-haemoglobin stabilising protein (AHSP) which is an abundant, erythroid-specific protein that forms a stable complex with free α Hb but not with β Hb or Hb A. AHSP specifically protects free α Hb from precipitation in solution and in live cells, probably acting to block the deleterious effects of free α Hb precipitation. AHSP gene dosage has been predicted to modulate pathological states of α Hb excess, such as in patients with β thalassaemia (Gell *et al*, 2002; Kihm *et al*, 2002). The tertiary modifiers, those that are not related to globin chain production but that may have an important effect on the complications of the disease, include genes involved with iron absorption, bilirubin metabolism, bone metabolism, cardiovascular disease, and susceptibility to infection (Weatherall, 2004). Environmental factors, such as malaria infection, may also be of considerable importance (Olivieri *et al*, 2008).

The phenotype of TI may also result from the increased production of α globin chains by a triplicated or quadruplicated α genotype associated with β heterozygosity (Sampietro *et al*, 1983; Camaschella *et al*, 1997; Premawardhena *et al*, 2005).

Table I summarises the main molecular forms and interactions that result in the TI phenotype (Steinberg *et al*, 2009).

There is no adequate clinical definition of TI. The haematological findings in heterozygous β thalassaemia patients (β thalassaemia minor) are remarkably uniform, and are characterised by a mild degree of anaemia; splenomegaly is extremely unusual. Hence, any thalassaemic patient with a Hb level persistently below 90–100 g/l, particularly if there is associated splenomegaly, falls into the phenotype of TI. It is at the more severe end of the spectrum that the difficulty in definition arises. Some children survive early life with Hb levels in the 50–60 g/l range. Although they are often classified as having TI, particularly if they present relatively late, many do not thrive or develop normally, and many grow up with gross skeletal deformities. It is now believed that these children should be transfused to avoid these distressing complications. Whether they should be classified as having severe TI or as having TM is, therefore, a question that is of little importance. Some children with β thalassaemia have Hb values between 60

Table I. Thalassaemia intermedia.

Mild deficit in β globin chain production
Homozygous mild β^+-thalassaemia
Compound heterozygousity for severe β^0 or β^+ and mild β^+-thalassaemia
Interactions of β^0 with 'silent' β thalassaemia
Homozygosity for 'silent' β thalassaemia
Reduced globin chain imbalance due to coinheritance of α and β thalassaemia
Homozygous or compound heterozygous β^0 or β^+ thalassaemia with 2 or 3 α gene deletions
Homozygous or compound heterozygous severe β^0 or β^+-thalassaemia with nondeletion α2 gene mutation
Homozygous or compound heterozygous severe β^+-thalassaemia with 1 or 2 α gene deletions
Severe β thalassaemia with increased capacity for γ chain synthesis
Homozygous or compound heterozygous β^0 or β^+-thalassaemia with heterocellular HPFH
Homozygous or compound heterozygous β^0 or β^+-thalassaemia with particular β globin RFLP haplotype
Mechanism unknown
Deletion forms of δβ thalassaemia and HPFH
Homozygous $(\delta\beta)^0$ or $(^A\gamma\delta\beta)^0$ thalassaemia
Compound heterozygosity for β^0 or β^+ and $(\delta\beta)^0$ or $(^A\gamma\delta\beta)^0$ thalassaemia
Homozygosity for Hb Lepore (some cases)
Compound heterozygosity for Hb Lepore and β^0 or β^+-thalassaemia (some forms)
Compound heterozygosity for $(\delta\beta)^0$, $^G\gamma\beta^+$ or $^A\gamma\beta^+$ HPFH and β^0 or β^+-thalassaemia
Compound heterozygosity for $(\delta\beta)^0$ thalassaemia $(\delta\beta)^0$ HPFH
Compound heterozygosity for β or δβ thalassaemia and β chain structural variants
Hb S, C, E/β or δβ thalassaemia
Many other rare interactions
Other β thalassaemia alleles or interactions
Dominant β thalassaemia
β thalassaemia trait associated with ααα or αααα globin gene duplications
Highly unstable β globin chain variants

Hb, haemoglobin; HPFH, hereditary persistence of fetal haemoglobin; RFLP, restriction fragment length polymorphism.

and 90 g/l. They grow and develop reasonably well, and reach adult life, and it is also useful to retain the term TI for this type of patient. It should be remembered that they may require transfusions if complications develop, or if the disorder is complicated by other factors such as folate deficiency or intercurrent infection. Clearly, the term TI can cover a broad and shifting clinical spectrum, from almost complete health to a condition characterised by severe growth retardation and skeletal deformity that requires transfusion therapy; it is a diagnosis that can be made only after a considerable period of observation and that often requires revision (Steinberg *et al*, 2009).

Pathophysiology and clinical complications

If left untreated, three main factors are responsible for the clinical sequelae of TI: ineffective erythropoiesis, chronic haemolytic anaemia, and iron overload (Taher *et al*, 2006a). The degree of ineffective erythropoiesis is the primary determinant of the severity of anaemia, while peripheral (intra- and extravascular) haemolysis of mature red blood cells (RBCs) remains secondary (Olivieri, 1999). Ineffective erythropoiesis is also associated with skeletal deformities and osteopenia attributed to erythroid marrow expansion as well as compensatory extramedullary haematopoiesis (EMH) leading to tumour formation anywhere throughout the body (Haidar *et al*, 2010). Haemolysis has mainly been associated with splenomegaly; however, recent evidence suggests that haemolysis, along with other factors, is also the hallmark of a hypercoagulable state in TI (Table II) (Ataga *et al*, 2007; Taher *et al*, 2008b). Hypercoagulability justifies the high rate of thromboembolic phenomena in patients with TI (Taher *et al*, 2006b, 2008b) and may explain other complications such as pulmonary hypertension (PHT) with secondary right heart failure (HF) (Aessopos *et al*, 2001; Taher *et al*, 2002). Ineffective erythopoiesis and chronic anaemia also lead to an increase in gastrointestinal iron absorption (Taher *et al*, 2009a), resulting in non-transfusional iron overload (similar to patients with hereditary haemochromatosis), in the liver and less so in the heart (Roghi *et al*, 2010; Taher *et al*, 2010e). Figure 1 highlights the proposed mechanism of iron overload in non-transfused patients with TI. Involvement of the liver can eventually lead to cirrhosis and hepatocellular carcinoma (Restivo Pantalone *et al*, 2010).

A recent report on 120 treatment-naive patients with TI revealed a significant role for advancing age (even among paediatric and adult patients) in acquiring clinical complications. The study demonstrated a decreasing trend in Hb level and a progressive increase in iron accumulation with advancing age. Thus, despite being considered as having a milder form of the disease at initial presentation and diagnosis, TI patients are still at risk of acquiring several serious complications with the passage of time which warrants optimal and early intervention extremely essential (Taher *et al*, 2010a).

Table II. Factors contributing to hypercoagulability in thalassaemia.

Factor	Mechanisms
Red blood cells	Formation of reactive oxygen species
	Expression of negatively charged phospholipids
	Enhanced cohesiveness and aggregability
Platelets	Increased platelet aggregation
	Increased expression of activation markers
	Presence of platelet morphologic abnormalities
Peripheral blood elements	Expression of endothelial adhesion molecules and tissue factor on endothelial cells
	Formation of microparticles
Splenectomy	High platelet counts and hyperactivity
	High levels of negatively charged red blood cells
Nitric oxide	Decreased levels leading to vasoconstriction
Thrombophilia	Decreased levels of antithrombin III, protein C and protein S
	Anti-phospholipid antibodies
	No role for prothrombotic mutations
Other factors	Cardiac dysfunction
	Hepatic dysfunction
	Endocrine dysfunction

Management

It is very important before embarking on any form of treatment to establish the particular variety of β thalassaemia and to obtain full blood group genotype of the patient. It is also essential to assess the patient carefully over the first few months after the diagnosis is established and not to embark on any treatment modality, especially transfusion therapy, too hastily. Many patients with TI, who may not need regular transfusion, embark on a life of unnecessary treatment of this kind, particularly if they present with an unusually low Hb level during a period of intercurrent infection. Even if a few transfusions have been administered in the acute situation, immediate commitment to a transfusion program should not be undertaken. It is worthwhile to attempt to evaluate the patient in the non-emergency situation from the untransfused baseline: that is, to withdraw transfusions and carefully observe. Moreover, the importance of indications for transfusion or other forms of therapy do not only rely on the steady-state Hb level. In fact, some children with TI, specifically with Hb E/β thalassaemia, have a remarkable facility to adaptation for low Hb levels (O'Donnell *et al*, 2007). Instead, the patient's well being, particularly with respect to activity, growth, development, and the early appearance of skeletal changes or other disease-complications are the factors to be taken into consideration.

Hitherto the management of TI has relied on careful observations together with intermittent transfusion for complications or splenectomy. Recent work has suggested that

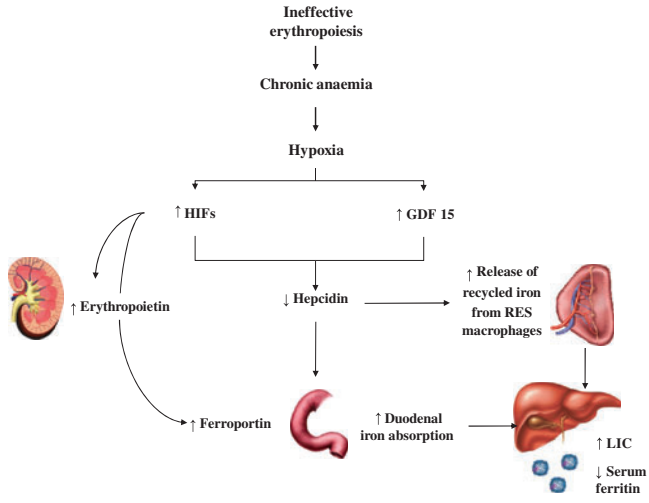

Fig 1. Iron metabolism in transfusion-independent patients with thalassaemia intermedia. The combination of ineffective erythropoiesis [leading to increased growth and differentiation factor 15 (GDF 15)] and chronic anaemia/hypoxia [altering the expression of hypoxia-inducible transcription factors (HIFs)] results in hepcidin suppression, increased iron absorption from the gut and increased release of recycled iron from the reticuloendothelial system (RES). This results in depletion of macrophage iron, relatively low levels of serum ferritin, and preferential portal and hepatocyte iron loading leading to an increase of liver iron concentration (LIC) (Taher *et al*, 2009a).

complications, particularly later in life, appear to be less common in regularly transfused patients and more common in those that have undergone splenectomy; and hence, in the future regular transfusion may become a more common option for management and prevention of late complications. However, until more is known about these later complications, the management of this condition will continue to be personalised. A detailed approach has been summarised recently in the case of the commonest form of thalassaemia intermedia, Hb E/β thalassaemia (Olivieri *et al*, 2008).

Transfusion therapy

Current indications for transfusion therapy in TI are summarised in Table III. In general, the prevailing approach has been avoidance of early blood transfusions and the concomitant requirement for chelation therapy, reserving the introduction of transfusion until later in the disease course when complications manifest. Consequently, unlike TM, evaluation of the role of transfusion therapy in the management of TI has been limited. In the OPTIMAL CARE study, patients who were placed on transfusion regimens (intermittent or regular) suffered fewer complications relevant to chronic anaemia, ineffective erythropoiesis, and haemolysis (mainly EMH, PHT, and thrombosis); while suffering from a higher rate of iron overload related endocrinopathy (Taher *et al*, 2010c). Observational studies have also confirmed that transfused TI patients suffer fewer thromboemolic events,

Table III. Indications for transfusion therapy in thalassaemia intermedia.

Indication
Haemglobin level < 50 g/l
Declining haemoglobin level in parallel with profound enlargement of the spleen (at a rate exceeding 3 cm/year)*
Growth failure (height is more indicative of growth pattern than weight) or poor performance at school
Diminished exercise tolerance
Failure of secondary sexual development in parallel with bone age
Severe bony changes
Pregnancy
Infection
Other specific complications (e.g. Heart failure, pulmonary hypertension, thromboembolic disease, leg ulcers, priapism)

*At least in periods of maximal growth and development.

PHT, and silent brain infarcts as compared to transfusion naïve patients (Taher *et al*, 2006b, 2010b,d; Aessopos *et al*, 2007). This has been attributed to correction of the underlying ineffective erythropoiesis and the resulting damaged RBCs with thrombogenic potential (Taher *et al*, 2008b). As such, earlier introduction of transfusion therapy aimed at preventing the consequences of chronic haemolytic anaemia may benefit TI patients by prevention, rather than palliation of late and irreversible anaemia-related complications. Rather than enforcing the regular transfusion regimens implemented in

TM, blood transfusion, if initiated in patients with TI will require closer monitoring and should be individually tailored to meet patient needs. Alloimmunization is a relatively common observation in TI, and the risk is decreased if transfusion therapy is initiated before the age of 12 months (Spanos *et al*, 1990). Thus, early introduction of transfusion therapy will also help alleviate the increased risk of alloimmunization. Kell and Rhesus phenotyping prior to transfusion therapy is also recommended, depending on differences between the donor and recipient populations (Hmida *et al*, 1994). Although earlier introduction of blood transfusions will increase the rate of iron accumulation, effective methods of iron chelation are now available, and the benefits of transfusion therapy may greatly outweigh the cost and inconvenience of iron chelation therapy. In the OPTIMAL CARE study, patients who received both transfusion and iron chelation therapy had a lower incidence of complications (including endocrinopathy) than patients who received no treatment or either treatment alone (Taher *et al*, 2010c).

Splenectomy

The most widely practiced indications for splenectomy in TI are summarised in Table IV. Many patients who undergo splenectomy appear to restore Hb levels in the short term by about 10–20 g/l. Some of these patients demonstrate a marked improvement in growth and development. However, clinical observations have suggested that splenectomy in TI can contribute to an increased susceptibility to thrombosis (Cappellini *et al*, 2000; Taher *et al*, 2006b). The development of these complications has been ascribed to the presence of high platelet counts and platelet dysfunction following splenectomy (Atichartakarn *et al*, 2003a) and/or to increased number of RBCs with negatively charged membranes that carry thrombogenic potential (Atichartakarn *et al*, 2002). In splenectomised TI patients, thrombin generation is significantly higher than in control subjects and patients who had not undergone splenectomy (Cappellini *et al*, 2000). A high incidence of silent brain abnormalities (60%) has also been documented in splenectomised adults with TI, but their effects on neurocognitive functioning have not yet been evaluated (Taher *et al*, 2010d). Splenectomised TI patients also have a high frequency of PHT, mostly attributed to chronic thromboembolic disease (Atichartakarn *et al*, 2003b). It has been suggested that the intact spleen may be a reservoir of excess iron and may have a possible scavenging effect on iron free fractions including non-transferrin-bound iron (NTBI), which explains the higher serum level of NTBI in splenectomised TI patients (Tavazzi *et al*, 2001; Taher *et al*, 2009b). Most recently, a large retrospective overview on 584 TI patients managed in the Middle East and Italy, the OPTIMAL CARE study, assessed the rate of disease-associated complications in relation to currently practiced treatment options. The study confirmed an independent role for splenectomy in a higher occurrence of thromboembolism, PHT, HF, EMH, leg ulcers, and iron-related endocrinopathy (Taher *et al*, 2010c). This collective data calls for a review of splenectomy as a procedure of choice, especially with its potential role in increasing TI-related complications and the inherent risk of infection associated with the procedure even for individuals without haematological disorders (Cadili & de Gara, 2008). Infection among post-splenectomy patients carries a high mortality rate especially among children with haematological disorders (Bisharat *et al*, 2001). As such, a guarded approach to the need for splenectomy is recommended with delay in initiating the procedure unless considered extremely necessary. If undertaken, at least 6 weeks before splenectomy, patients should be vaccinated with pneumococcal, *Haemophilus influenzae* type B and meningococcal vaccines; and after surgery, daily prophylactic penicillin should be administered, at least during childhood and probably indefinitely. Antimalarials should be given to those travelling to or living in countries in which malaria is endemic.

Table IV. Indications for splenectomy in thalassaemia intermedia.

Indication	Comments
Poor growth and development	As an alternate to transfusion therapy, although the latter is preferred particularly where iron chelation therapy is available
Increased transfusion demand	Annual blood requirements exceed 1·5 times those of splenectomised patients, provided that they are on the same transfusion scheme and have no other reasons for increased consumption (e.g. new alloantibodies, infection, or changes in the haematocrit of the transfused units) The rate of iron overload should also be taken into consideration. For patients who maintain effective chelation therapy despite increased blood requirements, splenectomy may be unnecessary
Hypersplensim	Leucopenia or thrombocytopenia causing clinical problems such as recurrent bacterial infection or bleeding
Splenomegaly	Accompanied by symptoms such as left upper quadrant pain or early satiety Massive splenomegaly causes concern about possible splenic rupture

Iron chelation therapy

Iron loading in TI patients is derived from two sources: increased intestinal absorption and transfusion therapy (Taher *et al*, 2009a). Iron overload in non-transfused patients with TI develops more slowly than transfusional iron overload (Pippard *et al*, 1979). In either case, iron overload can be monitored and readily controlled with chelation therapy. The initiation of chelation therapy in TI patients depends primarily on the extent of iron overload and rate of iron accumulation but, as with other aspects of the management of TI, clear

guidelines are not available. The observation that serum ferritin levels do not accurately reflect the level of iron overload in patients with TI (Taher *et al*, 2008a) has important implications for patient management. Reliance on serum ferritin alone may result in a delay in initiating chelation therapy and may therefore prolong patient exposure to high iron levels and the associated risks of morbidity and mortality. Unlike TM, where transfusion history can be a useful indicator of the need for iron chelation therapy, patients with TI will require direct assessment of body iron levels in order to guide therapy. As assessment of serum ferritin is evidently inappropriate in these patients, disease-specific recommendations for the management of patients with TI should include direct assessment of liver iron concentration (LIC) by biopsy, or preferably by non invasive imaging methods like MRI every 1–2 years. The reciprocals of MRI T2 and T2*, known as R2 and R2*, are directly proportional to iron and demonstrate the most promising results (St Pierre *et al*, 2005; Wood *et al*, 2005). Both techniques have been validated across various haemoglobinopathies including TI (Voskaridou *et al*, 2004; Taher *et al*, 2008a; Kirk *et al*, 2010). The available studies on MRI T2* assessment of cardiac iron in patients with TI failed to document cardiac siderosis despite significantly elevated LIC (Roghi *et al*, 2010; Taher *et al*, 2010e). Thus, in patients with TI, further research is still needed to better understand if (and when) detectable cardiac iron deposition can occur, and its correlation with cardiac morbidity and mortality.

Chelation therapy should be initiated when LIC exceeds 7 mg Fe/g dry weight (Taher *et al*, 2009a). Where LIC measurement is not possible, threshold serum ferritin values of 400–500 μg/l (which are lower than those generally accepted in patients with TM) could be considered as an indicator for initiation of iron chelation therapy (Taher *et al*, 2009a). In such cases, serial serum ferritin level determination is advised and values should be confirmed in at least two separate samples. Iron chelation therapy in patients with TI may not necessarily be life-long. It may be relatively easy to reduce iron burden in these patients; hence, intermittent periods of iron chelation with careful assessment of iron indices throughout the course of the disease could be sufficient in many cases.

Data on the use of deferoxamine (DFO) in patients with TI are limited and our knowledge and understanding of the efficacy and application of DFO relies mainly on the extensive experience gained from studies of the TM population. The practical limitations and inconvenience of frequent, prolonged subcutaneous therapy with DFO is a key consideration, impacting on quality of life and compliance (Treadwell & Weissman, 2001; Cappellini, 2005). Small studies of DFO in patients with TI have been performed, providing some useful insights. In one 6-month study of 10 transfusion-independent patients, a significant decline in serum ferritin levels was seen, accompanied by substantial iron excretion despite modest serum ferritin levels (Cossu *et al*, 1981). The authors consequently noted that serum ferritin levels were of no value in predicting iron excretion. Observations during the study indicated that patients may have positive iron balance from the age of 5 years, even in the absence of transfusions, and the authors recommended that iron chelation therapy be initiated in patients over this age to prevent ongoing accumulation. They also concluded that treatment should be tailored to individual patients, guided by serum ferritin and DFO-induced excretion, a concept that is increasingly recognised as a key element of effective iron chelation therapy today. A second small study investigated DFO in patients with TI, but despite demonstrating efficacy, the authors' conclusions focused on the need for oral iron chelation therapy, which at the time of the study was not widely available (Pippard & Weatherall, 1988).

Data reporting the use of the first oral iron chelator, deferiprone, in patients with TI are also limited. Published literature includes a case report (Olivieri *et al*, 1992) and a small clinical trial that demonstrated effective management of iron levels (Pootrakul *et al*, 2003). In the latter, deferiprone was studied in nine intermittently transfused TI patients, demonstrating significant reductions in mean serum ferritin, hepatic iron, red cell membrane iron and serum non-transferrin-bound iron levels (Pootrakul *et al*, 2003). A significant rise in serum erythropoietin was also observed and in three patients there was an increase in Hb values. Transfusion requirements were reduced in four patients. Adverse events were mild and included gastrointestinal symptoms in six patients and arthralgia in one, none requiring withdrawal of treatment. However, the study sample was relatively small and similar data were never reproduced.

Deferasirox is the most recent addition to the iron chelator options. With a pharmacokinetic profile suitable for once-daily oral dosing, it can provide 24-h chelation coverage, and an extensive clinical development program has demonstrated efficacy in a wide range of patient categories (Cappellini *et al*, 2006; Vichinsky *et al*, 2007; Porter *et al*, 2008). In a pilot study, deferasirox doses up to 30 mg/kg per day provided effective control of iron levels in eleven minimally transfused TI patients (Voskaridou *et al*, 2010). Mean aspartate aminotransferase and alanine aminotransferase levels progressively decreased during the study. There were no significant changes in mean serum creatinine, cystatin-C, or 24-h proteinuria. In general, adverse events were mild and consistent with that documented throughout the registration studies of deferasirox. Nausea was reported in eight patients (73%) and diarrhoea was reported in two patients (18%) within the first month of deferasirox therapy. These adverse events were treated conventionally and did not re-occur within the 12 months of this study (Voskaridou *et al*, 2010). Recently, a boxed warning was added to the US deferasirox prescribing information, although this amendment has not been adopted by the European Health Authority or applied globally. The warning indicates that it may cause renal and hepatic impairment, including failure, and gastrointestinal haemorrhage. In some reported cases, these reactions were fatal. However, these reactions were more frequently observed in patients with advanced age, high risk myelodysplastic syndromes, underlying renal or hepatic impairment, or

low platelet counts. A 1-year study of more than 150 patients with TI is currently ongoing, which will represent the first large-scale study of an iron-chelating agent in this specific population. The primary objective of this placebo-controlled study is to determine the efficacy of deferasirox in patients with non-transfusion-dependent thalassaemia as determined by changes in LIC. Adult and paediatric patients (≥10 years of age) will be included (Taher *et al*, 2009c).

Modulation of fetal haemoglobin production

The clinical picture of TI could be greatly improved by an even partial reduction in the degree of the non-α to α globin chain imbalance. Several drugs have been tried in an attempt to reactivate γ chain synthesis and HbF production (Borgna-Pignatti, 2007).

Hydroxycarbamide, also known as hydroxyurea, an S-phase-specific and non-DNA-hypomethylating chemotherapeutic agent is capable of inducing HbF synthesis. Hydroxycarbamide may also have a more general role in increasing globin synthesis (Atweh & Loukopoulos, 2001). Large series of TI patients have been reported from Iran (Karimi *et al*, 2005) and India (Dixit *et al*, 2005; Panigrahi *et al*, 2005). The results were impressive, especially in the first study, where most patients were reported to have become transfusion independent. In patients who were not transfused, the Hb concentration increased. The same Iranian group more recently showed that the combination of hydroxyurea with L-carnitine or magnesium could be more effective in improving haematologic parameters and cardiac status in patients with TI than hydroxyurea alone (Karimi *et al*, 2010). However, whether hydroxycarbamide therapy can prevent rather than treat cardiac complication including PHT is not yet evaluated. The OPTIMAL CARE study also documented a beneficial role for hydroxycarbamide in TI patients especially when combined with transfusion and iron chelation therapy (Taher *et al*, 2010c). Previous studies from Europe had documented a constant increase of the erythrocyte volume and in HbF, but only a modest effect on total Hb concentration. Co-inheritance of α thalassaemia, the Xmn-1 *HBG2* polymorphism (Panigrahi *et al*, 2005) and the underlying β globin genotype may be predictive of a good response to hydroxycarbamide; Hb E/β thalassaemia patients generally have a good response (Singer *et al*, 2005). However, one study from Italy reported a decrease in the efficacy of hydroxycarbamide in TI patients after a long-term follow-up (Mancuso *et al*, 2006).

Treatment with hydroxycarbamide has also shown promising results in decreasing plasma markers of thrombin generation. Hydroxycarbamide may decrease coagulation activation by reducing phospholipid expression on the surface of both RBC and platelets and decrease RBC adhesion to thrombospondin. In addition to being a nitric oxide donor, hydroxycarbamide may also decrease haemostatic activation by its effect in decreasing the white blood cell count and particularly monocytes that express tissue factor (Ataga *et al*, 2007).

In splenectomised adults with TI, trials of recombinant human erythropoietin (EPO) showed a significant, dose-dependent increase in erythropoiesis, without an increase in HbF, mean corpuscular volume and mean Hb content, and without a change in the α to non-α ratio (Bohl *et al*, 2000). The most commonly used dose of EPO (500 U/kg × 3/week) is 5–10 times higher than the dose used for the anaemia of chronic renal failure. It is not clear if the simultaneous administration of iron, essential in patients with renal failure, is necessary.

The combination of hydroxycarbamide with EPO was effective in some patients, while the addition of sodium phenylbutyrate had no effect (Hoppe *et al*, 1999). In general, better responders were splenectomised, had a higher baseline HbF level and higher soluble transferring factor receptor and erythropoietin levels. In one study (Loukopoulos *et al*, 1998), the combination of a very high dose of EPO (50 000 U three times a week) with standard dose hydroxycarbamide for 12 weeks produced an increase in HbF and total Hb levels, but these results were not maintained when the dose of EPO was reduced. Trials to find other potent HbF inducers are ongoing.

Butyrate and butyrate derivatives are short chain fatty acids that inhibit the histone deacetylases and are believed to increase *HBG1/2* expression by increasing histone acetylation at the promoter level or by increasing the efficiency of translation of *HBG1/2* mRNA (Weinberg *et al*, 2005). Butyrate derivatives, such as arginine butyrate, sodium isobutyramide and sodium phenylbutyrate, have been studied in patients with TI. The first compound to enter a clinical trial, arginine butyrate, was reported to be effective in some patients when administered intravenously (Perrine *et al*, 1993). Unfortunately, the majority of treated patients continued to suffer from anaemia. It was not possible to predict which patients would respond to therapy, on the basis of baseline HbF, type of mutation or other parameters. The oral derivatives, sodium phenylbutyrate and sodium isobutyramide, are difficult to administer because of the large number of pills that need to be given and the poor taste of the compound. Some studies reported an increase of ≥10 g/l Hb in half of the patients (Dover, 1998). In another study (Domenica Cappellini *et al*, 2000), sodium isobutyramide was given to 12 patients with TI for 28 days. Little or no increase in the non-α to α ratio or the percentage of HbF was observed.

Thalidomide, a drug known for its immunomodulating and anti-angiogenic properties, has recently been demonstrated to induce γ globin gene expression and to increase the proliferation of erythroid cells (Moutouh-de Parseval *et al*, 2008). Two reports documented the successful treatment of TM patients with thalidomide (Aguilar-Lopez *et al*, 2008; Masera *et al*, 2010). Both patients achieved an increase in HbF and total Hb production. These findings encourage further efforts in this direction, especially in TI patients where mild increases in Hb level may be sufficient to ameliorate the chronic anaemia.

In summary, most trials on agents that modulate HbF production in TI patients are small, poorly controlled, or have

shown only modest benefit. Large, randomised, controlled trials are needed before these agents or their derivatives are widely used in TI management.

Other considerations

Antioxidants and vitamin supplements. Oxidative damage by reactive oxygen species (generated by free globin chains and labile plasma iron) is believed to be one of the main contributors to cell injury, tissue damage, and hypercoagulability in patients with thalassaemia (Amer & Fibach, 2004). Treatment with antioxidants, in mono- or combination therapy, may thus neutralise the deleterious effects of reactive oxygen species (Borgna-Pignatti, 2007). Few studies reported promising roles of vitamin E, N-acetylcysteine, and several other substances of plant origin in patients with TI (Tesoriere *et al*, 2001, 2006; Pace *et al*, 2003; Amer & Fibach, 2004; de Franceschi *et al*, 2004; Pfeifer *et al*, 2008). However, larger *in vivo* studies are needed before any recommendations can be made.

Supplementation with vitamin C is only recommended in regularly transfused patients receiving DFO, with demonstrated deficiency. Similar to patients with TM and thalassaemia minor, daily supplementation with 1 mg of folic acid may also be advised for patients with TI. Serum zinc levels have been found to be low in patients with TI; however, the benefit of supplementation has not been evaluated but may be necessary in heavily chelated patients (Borgna-Pignatti, 2007).

Anticoagulation. As a high rate of thromboembolic events has been observed in patients with TI (Taher *et al*, 2009d), anticoagulant and antiplatelet therapy merit consideration. The available data on the use of anticoagulants, antiplatelet, or other agents in TI are either lacking or involve small and poorly controlled studies (Taher *et al*, 2008b). However, in one study, TI patients who experienced a thromboembolic event and received aspirin afterwards had a lower recurrence rate compared with those who were not, although these differences were not statistically significant (Taher *et al*, 2006b). Moreover, in a subanalysis on the OPTIMAL CARE study, a platelet count of $\geq 500 \times 10^9$/l was an independent and significant predictor of thromboembolism in splenectomised TI patients (Taher *et al*, 2010b). As such, consideration of antiplatelet aggregants (e.g. aspirin) for the prevention of thromboembolic events in these patients remains logical.

Sildenafil for pulmonary hypertension. Pulmonary arterial hypertension is defined as a mean pulmonary artery pressure (PAP) ≥25 mmHg at rest, a pulmonary capillary wedge pressure, left atrial pressure, or left ventricular end-diastolic pressure ≤15 mmHg, and a pulmonary vascular resistance > 3 Wood units. Progressive PHT can eventually lead to right heart failure and death (McLaughlin *et al*, 2009).

Unlike patients with sickle cell anaemia, the pathophysiology of PHT in patients with TI has not been extensively studied. Nitric oxide pathway dysregulation has been suggested as a factor leading to hypercoagulability and PHT in patients with TI (Ataga *et al*, 2007). Sildenafil citrate, a potent inhibitor of cyclic guanosine monophosphate-specific phosphodiesterase-5 and a selective smooth muscle relaxant, has been evaluated for the management of PHT in patients with sickle cell anaemia with suboptimal results. In TI patients, the drug was first successfully used in a 34-year-old transfusion-dependent man (Littera *et al*, 2002) and in four patients who experienced reduction of pulmonary pressure, improvement of cardiovascular function, and a better exercise tolerance (Derchi *et al*, 2005). The drug is currently being evaluated in a large multicentre trial on patients with thalassaemia including TI.

The endothelin receptor antagonist bosentan was also used in a patient with TI complicated by chronic pulmonary thromboembolism and liver iron overload. The patients showed improvement in respiratory status without worsening of his liver disease (Pierre *et al*, 2006). This finding warrants further investigation, however, hepatotoxicity may still be a concern.

Extramedullary haematopoiesis. Among the various body regions reported for EMH tumour formation, paraspinal involvement is common (11–15% of cases) and receives special attention due to the debilitating clinical consequences secondary to spinal compression (Haidar *et al*, 2010). Aside from transfusion and HbF induction therapy which help decrease the demand for EMH, management options include radiotherapy and laminectomy. Many patients with paraspinal spinal EMH have been successfully treated with hydroxycarbamide alone (Saxon *et al*, 1998; Cario *et al*, 2002) especially in thalassaemic patients who are unable to receive blood transfusions due to alloimmunization. However, no data currently exists on the efficacy of hydroxycarbamide in preventing rather than treating EMH. Combinations of these modalities have also been used. Hydroxycarbamide is commonly used in conjunction with transfusion or radiotherapy (Haidar *et al*, 2010). There is no evidence as to the best treatment approach, and treatment remains individualised depending on severity of symptoms, size of the mass, patient's clinical condition, and previous treatment (Haidar *et al*, 2010).

Endocrine complications and pregnancy. Osteoporosis secondary to bone marrow expansion and 25-hydroxy vitamin D deficiency are highly prevalent in TI patients (Napoli *et al*, 2006; Taher *et al*, 2010c). Fractures and bone pain can be devastating consequences. Different regimens of vitamin D and calcium are frequently prescribed to patients with TI. In general, high doses are recommended (700–800 mg of vitamin D and 1200–1500 mg calcium), but with careful monitoring of renal function (Borgna-Pignatti, 2007). Although the efficacy and safety of bisphosphonates has been proven in patients with TM (Voskaridou *et al*, 2008b), data on patients with TI is limited (Voskaridou *et al*, 2008a).

Other endocrine complications are less frequent in patients with TI (Taher *et al*, 2010c) as compared to patients with TM who mainly developed severe anaemia or heavy iron-overload related dysfunction (Taher *et al*, 2009e). If present, management can follow the same approach as in patients with TM.

Delayed puberty is common, but fertility is usually normal. In pregnant women with TI, experience reveals an increased risk of abortion, pre-term delivery, intrauterine growth restriction, Caesarean section delivery, and thromboembolic events (Nassar *et al*, 2008). Although the use of blood transfusions may be required to address these complications, the risk of alloimmunization in transfusion-naive women should always be taken into consideration. Other approaches such as the use of EPO have been described (Bennett *et al*, 2005). Splenomegaly can interfere with the enlargement of the uterus and can be complicated by hypersplenism. Splenectomy can therefore become necessary during gestation or after delivery. Anticoagulation should be considered especially in women with additional prothrombotic risk factors (Nassar *et al*, 2006).

Conclusion

Until ongoing efforts optimise haematopoietic stem cell transplantation or gene therapy as a cure for patients with TI, medical therapy will be the corner stone for management. However, despite a number of available treatment options, there are currently no clear guidelines for managing patients with TI. Current practice follows recommendations extracted from expert opinion, small series, or studies that were not necessarily designed to investigate the role of various interventions. With these limitations in mind, we herein reviewed the available data, interpreted and presented them in a context that could be utilised in clinical practice. Until solid evidence-based guidelines are available, we recommend an individualised approach that takes into consideration all measures of the patient's disease status and accordingly determines the optimal treatment strategy. Future studies are expected to re-evaluate the role of splenectomy and assess the optimal timing; dose and duration of transfusion, iron chelation, or HbF induction therapy; and the added advantage of multimodal therapy.

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