β-Thalassemia intermedia: morbidity uncovered

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β-Thalassemia Intermedia *Morbidity Uncovered* K.M. Musallam & A.T. Taher

β-Thalassemia Intermedia

Morbidity Uncovered

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&
Ali T. Taher

“The knowledge of anything, since all things have causes, is not acquired or complete unless it is known by its causes.”

Ibn Seena (Avicenna)

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CONTENTS

Chapter 1

Introduction

INTRODUCTION

Two major groups of inherited hemoglobin-related disorders exist. The first group is composed of structural hemoglobin variants, the most common of which are hemoglobins S, C and E. The second group includes the two forms of thalassemia (alpha [α]- and beta [β]-thalassemia), which result from the defective synthesis of the α- or β-hemoglobin chains that comprise adult hemoglobin. All these conditions are inherited in a Mendelian-recessive manner such that asymptomatic heterozygous parents, or carriers, both pass on one copy of a gene for a hemoglobin variant to their affected children. Far less commonly, only a single gene is affected, resulting in a dominantly inherited hemoglobinopathy [1].

In β-thalassemia, mutations in the β-globin gene cause deficient or absent synthesis of β-globin chains, an imbalance in α/β-globin chain synthesis, ineffective erythropoiesis, and a spectrum of anemia depending on the type and amount of globin synthesized and additional genetic modifiers [2]. More than 200 different mutations have been associated with β-thalassemia, and these mutations affect different levels of gene regulation and expression. The majority of molecular defects are point mutations involving one or a limited number of nucleotides in functionally important regions of the β-globin gene. Deletions of the β-globin gene are uncommon [3].

Extremely diverse phenotypes exist within the β-thalassemia syndromes. At one end of the spectrum is β-thalassemia minor, a clinically silent, mildly hypochromic and microcytic anemia. At the other end is β-thalassemia major which refers to those patients whose clinical course is characterized by profound anemia, who present to medical attention in the first year of life, and who subsequently require regular blood transfusions and iron chelation therapy for survival [3]. The term β-thalassemia intermedia was first suggested to describe patients who had clinical manifestations that are too severe to be termed minor yet too mild to be termed major [4]. Patients with β-thalassemia intermedia present to medical attention in later childhood, sustain more favorable hemoglobin levels, and remain largely non- or only occasionally-transfused [3].

Thus, classification in the various forms of β-thalassemia is based on the clinical severity of the condition rather than the underlying genetic abnormality. Although the term β-thalassemia intermedia lacks specific molecular correlates, and the diagnosis remains largely clinical, a genotype/phenotype association has been observed [5]. Most β-thalassemia intermedia patients are homozygotes or compound heterozygotes for β-thalassemia, meaning that both β-globin loci are affected, and the disease had a recessive genetic pattern [5]. Less commonly, only a single β-globin locus is affected, the other being completely normal, so in these instances, β-thalassemia intermedia is dominantly inherited [3]. The phenotype

of β-thalassemia intermedia may also result from the increased production of α-globin chains by a triplicated or quadruplicated α-genotype associated with β-heterozygosity [1].

In the case of β-thalassemia intermedia, the multilayered complexity of the genetic basis for phenotypic diversity is best explained in terms of primary, secondary, and tertiary genetic modifiers [6]. The primary modifiers represent the broad diversity of mutations that affect the β-globin genes, ranging from mild promoter mutations that cause a slight reduction in β-globin chain production to the many different mutations that result in the β°-thalassemias; that is, a complete absence of β-globin product. Compound heterozygosity for these different mutations can provide a 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-thalassemia. The coinheritance of α-thalassemia has this effect, and, since there are numerous different molecular forms of α-thalassemia of different severity, this interaction provides further scope for a wide range of different β-thalassemia phenotypes. Similarly, the degree of globin chain imbalance can be reduced by the effective synthesis of the γ-chains of fetal hemoglobin 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 [7]. The tertiary modifiers are those that are not related to globin chain

production but that may have an important effect on the complications of the disease [8]. Environmental factors, such as malaria infection, may also be of considerable importance [9].

Considerable advances have been made towards understanding the molecular basis of phenotypic diversity within the β-thalassemia syndromes in general (β-thalassemia intermedia versus β-thalassemia major) and within β-thalassemia intermedia. Phenotype, in this instance, is mainly represented by the severity of anemia and transfusion requirement, where a β-thalassemia intermedia patient is said to be at an advantage compared with a β-thalassemia major peer. Nonetheless, recent work started highlighting that transfusion-independence in β-thalassemia intermedia does not come without its own side effects. Although β-thalassemia intermedia patients sustain levels of anemia that are generally adequate for growth and development without transfusions, several other pathogenic mechanisms remain in play. Ineffective erythropoiesis and a secondary drive for increased intestinal iron absorption, extramedullary bone marrow expansion, intra- and extravascular hemolysis, and hypercogulability have all been described [10]. However, the study of the various clinical morbidities that could emanate from these underlying unique mechanisms has so far been limited. Moreover, lack of knowledge on the exact clinical profile of β-thalassemia intermedia patients left management completely arbitrary and solely based on clinical expertise, without solid

evidence on the beneficial or harmful roles of available treatment modalities.

One of the first studies to examine the wide scope of clinical morbidities in patients with β-thalassemia intermedia was conducted on patients attending two specialized thalassemia centers, the Chronic Care Center (affiliated with the American University of Beirut) in Lebanon and the Hereditary Anemia Center, University of Milan in Italy. This exploratory study clearly identified that patients with β-thalassemia intermedia could experience greater morbidity than previously recognized and that these morbidities are dissimilar from those observed in patients with β-thalassemia major (Table) [10]. The collaboration was propelled forward to produce much of the work presented in this thesis, and extended to include several centers from the Middle East (Iran, United Arab Emirates, Oman) and North Africa (Egypt).

The collaborations were driven by our sense of responsibility to advance knowledge in this field, also because our centers observe a large number of β-thalassemia intermedia patients compared to other centers in the west and even Asia (where the dominant forms are α-thalassemia and hemoglobin E/β-thalassemia). In the Middle East region and North Africa the estimated carrier prevalence of β-thalassemia is ~3% and with the high rate of consanguinity the annual β-thalassemia major birth prevalence averages 0.4 per 1,000

across nations from the Western Sahara to Iran, although the numbers are in decline due to implementation of preventive strategies [11]. A smaller, as yet ill-defined number of these individuals are born with β-thalassemia intermedia.

Table. Clinical complications in β-thalassemia intermedia versus β-thalassemia major patients [10].

Complication (% of patients affected)	**β-thalassemia intermedia**		**β-thalassemia major**	
	Lebanon	**Italy**	**Lebanon**	**Italy**
	(n = 37)	**(n = 63)**	**(n = 40)**	**(n = 60)**
Splenectomy	90	67	95	83
Cholecystectomy	85	68	15	7
Gallstones	55	63	10	23
EMH	20	24	0	0
Leg ulcers	20	33	0	0
Thrombotic events	28	22	0	0
Cardiopathy*	3	5	10	25
Pulmonary hypertension†	50	17	10	11
Abnormal liver enzymes	20	22	55	68
HCV infection	7	33	7	98
Hypogonadism	5	3	80	93
Diabetes mellitus	3	2	12.5	10
Hypothyroidism	3	2	15	11

*Fractional shortening <35%. †Defined as pulmonary artery systolic pressure >30 mm Hg; a well-enveloped tricuspid regurgitant jet velocity could be detected in only 20 patients, so frequency was assessed in these patients only.
EMH = extramedullary hematopoiesis; HCV = hepatitis C virus.

OUTLINE OF THIS THESIS

The aim of the research presented in this thesis is to explore the various clinical morbidities experienced by β-thalassemia intermedia patients and determine their association with patient and disease characteristics, laboratory and radiological indices, as well as treatment modalities. The work is divided into five chapters (**Chapters 2 to 6**) that each contain several papers on a specific subject. Dr. Khaled Musallam takes responsibility of **Chapter 2** and **Chapter 5** while Dr. Ali Taher takes responsibility of **Chapter 6**. For **Chapter 3**, Dr. Khaled Musallam takes responsibility of the second and last papers while Dr. Ali Taher takes responsibility of the remaining papers. For **Chapter 4**, Dr. Ali Taher takes responsibility of the first two papers while Dr. Khaled Musallam takes responsibility of the remaining papers.

Chapter 2 identifies the association between two laboratory markers related to erythropoiesis and the occurrence of morbidity. **Chapter 3** focuses on non-transfusional iron overload. It starts by identifying the presence of elevated levels of toxic iron species in transfusion-independent patients with β-thalassemia intermedia. It then describes the association between hepatic iron concentration, an established marker of total body iron status, and the occurrence of vascular, endocrine, and bone morbidity. Cardiac iron overload is then evaluated in two separate studies to confirm an unexpected

negative finding. The contribution of iron overload to renal disease is then explored. Vascular disease is the highlight of **Chapter 4**. The first study dissects the association between splenectomy and thrombosis in β-thalassemia intermedia, to identify patients at highest risk. The second study diverts to evaluate risk factors for pulmonary artery hypertension, a common finding in β-thalassemia intermedia. Both studies are sub-analyses of the larger OPTIMAL care study described in **Chapter 6**. The subsequent three studies in **Chapter 4** present the results of three imaging modalities applied to the brains of β-thalassemia intermedia patients to evaluate the occurrence of cerebrovascular pathology, and discuss the mechanisms and risk factors for the observed findings. In **Chapter 5** the association between multiple morbidity and quality of life in patients with β-thalassemia intermedia is investigated. **Chapter 6** first highlights the natural history of morbidity in β-thalassemia intermedia patients who never received any form of therapy. It then presents the OPTIMAL CARE study, a key evaluation of a large group of patients that determines the associations between different treatment modalities and β-thalassemia intermedia-related morbidities. The chapter concludes with a review that aims to provide a guideline for therapy in light of the recent evidence.

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Chapter 2

Ineffective Erythropoiesis

Levels Of Growth Differentiation Factor-15 Are High And Correlate With Clinical Severity In Transfusion-independent Patients With β Thalassemia Intermedia

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Levels of growth differentiation factor-15 are high and correlate with clinical severity in transfusion-independent patients with β thalassemia intermedia

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ABSTRACT

Transfusion-independent patients with β thalassemia intermedia (TI) experience a variety of clinical complications attributed to the underlying ineffective erythropoiesis and subsequent anemia, hemolysis, and iron overload. Growth differentiation factor-15 (GDF-15) was recently investigated as a marker of ineffective erythropoiesis in several anemias. In this work, we evaluated GDF-15 levels in 55 patients with TI. The mean GDF-15 level was 25,197.8 ± 16,208.9 pg/ml which is lower than values reported for patients with thalassemia major, yet considerably higher than those reported in patients with other congenital and acquired anemias. GDF-15 levels were significantly higher in splenectomized compared to non-splenectomized patients and correlated with anemia, markers of iron overload, and a pre-defined clinical severity score. Further studies are needed to determine the practical utility of GDF-15 measurement and its potential to reflect the severity of the clinical course in TI patients.

Introduction

A diversity of phenotypes exists within the β thalassemia syndromes. Patients with β thalassemia intermedia (TI) are those who usually present to medical attention beyond the age of two years, and maintain hemoglobin values between 70 and 90 g/l without the need for a regular transfusional regimen [1]. However, it is now established that the diagnosis of TI carries higher morbidity than previously recognized, with clinical complications involving most organ systems and a disease severity that worsens with advancing age [2–4]. Ineffective erythropoiesis is the hallmark of disease process in TI, leading to anemia, hemolysis, and iron overload due to intestinal hyperabsorption [5,6]. Measurement of growth differentiation factor-15 (GDF-15), a member of the transforming growth factor-β superfamily, is thought to predict ineffective or apoptotic erythropoiesis [7]. GDF-15 levels were found to be significantly elevated in patients with β thalassemia major (TM). Furthermore, sera from those patients suppressed expression of the iron regulatory protein hepcidin, with a subsequent reversal of suppression when GDF-15 was depleted [8]. Data on GDF-15 levels in patient with transfusion-independent TI are lacking. Moreover, correlation between GDF-15 level, as a marker of ineffective erythropoiesis, and clinical severity in thalassemia patients was never explored.

Materials and methods

This was a cross-sectional study of all TI patients treated at two centers in Milan, Italy and Beirut, Lebanon. All patients were diagnosed with TI based on previously described criteria [2]. After excluding all patients with any history of transfusion, iron chelation, or fetal hemoglobin induction therapy, 55 patients were available for analysis. The study received institutional review board approval and written informed consent was provided by all patients. Patient charts were reviewed to retrieve data on demographics (age and gender), splenectomy status, and clinical complications known to be common in patients with TI [2] defined according to criteria described in the OPTIMAL CARE study [4]. None of the patients had hemoglobin S, C, E/β or δβ thalassemia. Moreover, none of the patients had co-inheritance of α thalassemia [α^+ ($-\alpha^{3.7}$ and $-\alpha^{4.2}$) or α^0 ($-^{Med}$ and $-^{SEA}$)] or determinants associated with increased γ chain production [*Xmn*-I +/+ genotype at position −158 of *HβG2*].

Blood samples were obtained for assessment of hemoglobin and serum ferritin levels. For LIC, direct determination of iron burden was performed using R2 MRI in Beirut and R2* MRI in Milan using established methodologies, calibrated to mg/g of iron by dry weight (dw) in fresh liver biopsy specimens [9,10]. LIC measurements using R2* MRI are unbiased with respect to those using R2 MRI [11].

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Serum GDF-15 was evaluated using DuoSet Sandwich ELISA Kit (R&D Systems, Minneapolis, MN). Briefly, serum samples were centrifuged to remove residual cells; 96-well plates were coated with 2 μg/ml monoclonal mouse anti-human GDF-15 capture antibody and blocked with 1% bovine serum albumin in phosphate-buffered saline. After incubation with serum, the wells were washed and bound GDF-15 was detected using a biotinylated goat anti-human GDF-15 antibody. Recombinant human GDF-15 protein was used to generate a standard curve.

Patients were also assigned a clinical severity score as described in Table 1, inspired by recently published literature recently reviewed [2] as well as from clinical experience. Patients were further classified as having mild (severity score ≤5), moderate (severity score 6–10), or severe TI (severity score >10) (Table 1).

Statistical analysis

Descriptive statistics are expressed as means ± (standard deviation, SD), medians, or percentages. Bivariate correlations between GDF-15 levels and study parameters were evaluated using the independent samples *t*-test for categorical variables and the Pearson's correlation coefficient (r) for continuous variables. To determine the best GDF-15 cut-offs for discriminating patients with mild, moderate, or severe TI; the maximum sum of sensitivity and specificity was calculated from receiver-operating characteristic (ROC) curve analysis. All *P*-values are two sided with the level of significance set at <0.05.

Results and discussion

The mean age of patients was 30.7 ± 15 years (range, 8–67 years) with 25 patients (45.5%) being males. Thirty-five patients (63.3%) were splenectomized. The mean hemoglobin level was 87 ± 17 g/l (range, 51–131 g/l), while the mean serum ferritin level was 763.6 ± 501.5 μg/l (range, 18–1772 μg/l) and the mean LIC was 7.2 ± 6.6 mg Fe/g dw (range, 0.6–32 mg Fe/g dw). The mean GDF-15 level was 25,197.8 ± 16,208.9 pg/ml (range, 1126–58,262 pg/ml). GDF-15 levels increased with age (r = 0.23, $P = 0.043$), but were comparable in males and females. Mean GDF-15 levels were significantly higher in splenectomized compared to non-splenectomized patients (29,266.5 ± 15,914.8 vs. 18,077.6 ± 14,477 pg/ml, $P = 0.012$). GDF-15 levels also correlated positively with serum ferritin levels (r = 0.36, $P = 0.007$) and LIC (r = 0.63, $P = 0.014$), yet correlated negatively with hemoglobin level (r = −0.312, $P = 0.048$).

The median severity score was 7 (range, 1–15). There was a strong positive correlation between GDF-15 levels and the severity score (r = 0.830, $P < 0.001$, Fig. 1A). On ROC curve analysis, a GDF-15 cut-off of 16,000 pg/ml differentiated patients with mild from moderate TI with an area under the curve (AUC) of 0.864 ± 0.067 ($P < 0.001$), a specificity of 88.9%, and a sensitivity of 90%. Moreover, a GDF-15 cut-off of 32,000 pg/ml differentiated patients with moderate from severe TI with an AUC of 0.924 ± 0.042 ($P < 0.001$), a specificity of 85%, and a sensitivity of 88.2%.

This is the first report on GDF-15 levels in transfusion-independent patients with TI. Despite being lower than patients with TM, levels of

Table 1
Clinical severity score for patients with transfusion-independent β thalassemia intermedia.

Factor	Score
Age (years)	
≤10	0
11–20	1
21–32	2
>32	3
Splenectomy	
No	0
Yes	1
Hemoglobin level (g/dl)	
≥9	0
<9	1
LIC (mg Fe/g dry weight)	
<3	0
3 to <7	1
7 to <15	2
≥15	3
Clinical complications	
Extramedullary hematopoiesis	1
Leg ulcers	1
Thrombosis	1
Pulmonary hypertension	1
Heart failure	1
Abnormal liver function	1
Diabetes mellitus	1
Hypothyroidism	1
Osteoporosis	1
Hypogonadism	1
Minimum score	0
Maximum score	18
Mild TI	≤5
Moderate TI	6 to 9
Severe TI	≥10

LIC, liver iron concentration; TI = β thalassemia intermedia.

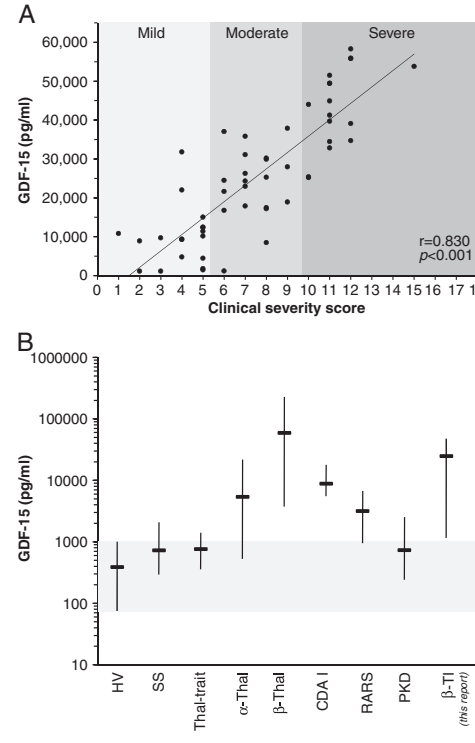

Fig. 1. A) Correlation between growth differentiation factor-15 (GDF-15) levels and clinical severity score in β thalassemia intermedia. B) GDF-15 levels from healthy volunteers (HV; n = 37), sickle cell anemia (SS; n = 13), thalassemia trait (Thal-trait; n = 12), α thalassemia (α-Thal; n = 20), β thalassemia (β-Thal; n = 40), congenital dyserythropoietic anemia type I (CDA I; n = 17), refractory anemia with ring sideroblasts (RARS, n = 20), pyruvate kinase deficiency (PKD, n = 22) [7] and transfusion-independent β thalassemia intermedia (β-TI; n = 55) (this report). Bars show the range and mean (dash) of GDF-15 concentrations.

GDF-15 in patients with TI are considerably higher than those reported in patients with other congenital and acquired anemias (Fig. 1B) [7]. Since GDF-15 has been regarded as a marker of ineffective erythropoiesis, our findings also confirm the substantial role of ineffective erythropoiesis in the pathophysiology and clinical severity of TI. Ineffective erythropoiesis, and the subsequent chronic anemia and hypoxia, lead to hepcidin suppression, increased iron absorption from the gut, and increased release of recycled iron from the reticuloendothelial system. This results in depletion of macrophage iron, relatively low levels of serum ferritin, and preferential portal and hepatocyte iron storage. This in turn leads to considerable hepatic iron overload (suggested by a positive correlation between GDF-15 levels and LIC in this report) and release into the circulation of toxic iron species like non-transferrin-bound iron, which can lead to target-organ damage [5,12–14]. Ineffective erythropoiesis is also directly related to the occurrence of osteoporosis in thalassemia [15]. Moreover, ineffective erythropoiesis results in the secondary release into the circulation of damaged red blood cells with thrombogenic potential that reflect in a high rate of thromboembolic events in TI [4,16,17], as well as an increased release of placenta growth factor, endothelin-1, which may contribute to pulmonary hypertension [4,18].

Further studies are needed to confirm the association between GDF-15 levels and erythropoietic drive in TI, since its measurement could potentially reflect the severity of ineffective erythropoiesis and subsequent clinical picture. This could allow better understanding of the phenotype heterogeneity in TI and the relative contribution of the various mechanisms implicated in the disease process.

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Fetal Hemoglobin Levels And Morbidity In Untransfused Patients With β-thalassemia Intermedia

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Fetal hemoglobin levels and morbidity in untransfused patients with β-thalassemia intermedia

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To evaluate the association between fetal hemoglobin (HbF) levels and morbidity in β-thalassemia intermedia (TI), we analyzed data from 63 untransfused patients who had also never received HbF induction therapy. Patient records were reviewed for any history of 10 predefined morbidities. Laboratory measurements for markers of ineffective erythropoiesis were also obtained. The mean age of patients was 32.1 years, 47.6% were males, and the median HbF level was 37.2%. HbF levels correlated positively with total hemoglobin, yet negatively with growth differentiation factor-15 and non–transferrin-bound iron levels. Median HbF levels were significantly lower in patients with the majority of evaluated morbidities than in those without. There was a strong negative adjusted linear correlation between the HbF level and the total number of morbidities ($R^2 = 0.825$, $P < .001$). The HbF threshold of 63.7% had 95.5% sensitivity and 100% specificity for ensuring absence of morbidity. There exists a strong association between HbF levels and morbidity in the subset of untransfused patients with TI. (*Blood*. 2012; 119(2):364-367)

Introduction

Patients with β-thalassemia intermedia (TI) usually present to medical attention after 2 years of age and maintain hemoglobin values between 70 and 90 g/L without the need for a regular transfusion regimen.[1] Nonetheless, the diagnosis of TI can be associated with a number of serious complications involving several organ systems.[1,2] Although the mechanisms underlying the disease process have been studied extensively, our understanding of the risk factors that govern the clinical heterogeneity of the disease remains limited, and only a few genetic and environmental modifiers of disease severity have been elucidated.[1] Some of the variability in the observed morbidities in this patient population has been attributed to the choice of treatment approach, which in most instances does not follow clear guidelines, in contrast to approaches used in patients with β-thalassemia major.[2] Fetal hemoglobin (HbF) levels have been shown to be an important modifier of morbidity and mortality in adults with sickle cell disease.[3-5] Furthermore, it is known that patients with the same β-thalassemia mutations are more likely to have thalassemia major if they have lower production of HbF.[6] HbF is clearly an important contributor to the clinical variability in β-thalassemia; however, the extent to which interindividual variation in HbF levels contributes to the clinical heterogeneity observed in TI patients has never been evaluated. Here, we examine the association between HbF levels and morbidity in untransfused patients with TI. By selecting patients who have never received transfusions or HbF induction therapy, we could ensure that the effects seen were more likely to be caused by the variation in HbF and not by other confounding variables.

Methods

This was a cross-sectional study of all TI patients treated at 2 centers in Milan, Italy, and Beirut, Lebanon ($n = 260$). At both centers, an age of diagnosis > 2 years and hemoglobin values between 70 and 90 g/L without the need for a regular transfusion regimen, with or without splenomegaly, were the main criteria to define the TI phenotype on presentation.[1] It should be noted, however, that TI patients may have changes in the total hemoglobin level as they grow, and some patients may eventually require transfusion therapy.[1] After the exclusion of all patients with any history of blood transfusion, iron chelation, or HbF induction therapy, 63 patients were available for analysis. The current study utilized a completely de-identified dataset. Data was collected as part of now completed clinical studies, and which were approved by the Institutional Review Board at both institutions. All patients had signed an informed consent form for participating in the original studies in accordance with the Declaration of Helsinki. By review of genetic records, none of the patients had coinheritance of α-thalassemia (α^+ [$-\alpha^{3.7}$ and $-\alpha^{4.2}$] or α^0 [$--^{Med}$ and $--^{SEA}$]) or determinants associated with increased γ-globin chain production (*Xmn*-I +/+ genotype at position −158 of *HβG2*). β-Globin and α-globin genotypes of the study sample are described in supplemental Table 1 (available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article). None of the patients had hemoglobin S, C, E/β-thalassemia, or δβ-thalassemia. Medical charts were reviewed to retrieve data on demographics (age and sex), splenectomy status, and presence of morbidities relevant in patients with TI[1] (extramedullary hematopoiesis, pulmonary hypertension, venous thromboembolism, heart failure, leg ulcers, abnormal liver function, diabetes mellitus, hypothyroidism, hypogonadism, and osteoporosis), defined according to criteria described in the OPTIMAL CARE study.[2] Patients were also assigned a morbidity score, which was the total number of morbidities experienced up to the time of study. For each patient, we also retrieved the HbF level

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determined at the time of diagnosis (measured at a median age of 9 years, range 5-21 years; the date of measurement preceded the dates of splenectomy, other laboratory measurements, and morbidity occurrence). Without intervention, HbF levels are thought to remain stable throughout the course of the disease. To adjust for the confounding effect of ineffective erythropoiesis on any observed association, we also retrieved data on the following relevant laboratory indices from all available laboratory records: mean total hemoglobin, nucleated red blood cells, growth differentiation factor (GDF)–15, serum ferritin, and non–transferrin-bound iron (NTBI) levels. In both centers, GDF-15 and NTBI levels were determined as described elsewhere.[7,8]

Statistical analysis

Data are described as mean ± SD, median (interquartile range), or percentage. Bivariate correlations to determine the association between HbF levels and study parameters were conducted with the Mann-Whitney *U* test for categorical variables and the Spearman correlation coefficient for continuous variables. Linear regression analysis, with adjustment for clinically relevant variables, was used to evaluate the independent correlation between HbF levels and the occurrence of morbidity. Receiver operating characteristic curve analysis was used to determine the HbF level threshold with the highest sum of sensitivity and specificity to discriminate patients who experienced morbidity from those who did not. All *P* values were 2 sided, with the level of significance set at $< .05$.

Results and discussion

The mean age of patients in the present study was 32.1 ± 16.2 years (range 8-78 years). A total of 30 patients (47.6%) were males, and 37 (58.7%) were splenectomized. The mean total hemoglobin level was 89.0 ± 16.1 g/L (range 57-131 g/L), and the median HbF level was 37.2% (interquartile range 57.8%, range 1.1%-100%). Bivariate correlations between HbF level and study parameters are summarized in Table 1. Clinical and laboratory quantification of the severity of ineffective erythropoiesis remains a challenge. Ineffective erythropoiesis is the hallmark of the disease process in TI, leading to anemia, hemolysis, and iron overload because of excessive intestinal absorption.[1,9] Measurement of GDF-15, a member of the transforming growth factor-β superfamily, is thought to indicate the extent of ineffective erythropoiesis.[10] NTBI levels are thought to reflect the erythropoietic rate and are increased by ineffective erythropoiesis.[11,12] The negative correlation between HbF levels and both of these markers in the present report, as well as the positive correlation with total hemoglobin levels, suggests an ameliorating role of HbF level on the severity of ineffective erythropoiesis, probably through an effect on globin chain imbalance. Previous work on small cohorts of TI patients showed that high levels of circulating HbF ($> 40\%$) could be associated with higher erythropoietin activity and expansion of erythropoiesis.[13,14] The present study suggests that this increase in erythropoietic drive is also less "ineffective." The exact mechanisms that could explain such an association, however, merit further investigation.

A total of 41 patients (65.1%) experienced at least 1 morbidity, and 30 patients (47.6%) experienced multiple morbidities. The median HbF level was significantly lower in patients with the majority of evaluated morbidities than in those without (Table 1). On linear regression analysis, and after adjustment for age, splenectomy, total hemoglobin, nucleated red blood cells, GDF-15, serum ferritin, and NTBI levels, there was a strong negative linear correlation between HbF level and the morbidity score (β −0.050, 95% confidence interval [CI] −0.059 to −0.042; $R^2 = 0.825$, $P < .001$; Figure 1A). These results remained unchanged when the analysis was stratified for splenectomized and nonsplenectomized patients (splenectomized: beta −0.053, 95% CI −0.063 to −0.042, $R^2 = 0.785$, $P < .001$; nonsplenectomized: beta −0.045, 95% CI −0.060 to −0.030, $R^2 = 0.852$, $P < .001$). A regression analysis was also performed with the total HbF level in grams per liter rather than the % HbF, and the results remained essentially unchanged (beta −0.040, 95% CI −0.048 to −0.033, $R^2 = 0.882$, $P < .001$). An even stronger correlation was found using the log of the % HbF (beta −3.401, 95% CI −3.915 to −2.886, $R^2 = 0.933$, $P < .001$; Figure 1B). The HbF threshold 63.7% had 95.5% sensitivity and 100% specificity for ensuring absence of morbidity on receiver

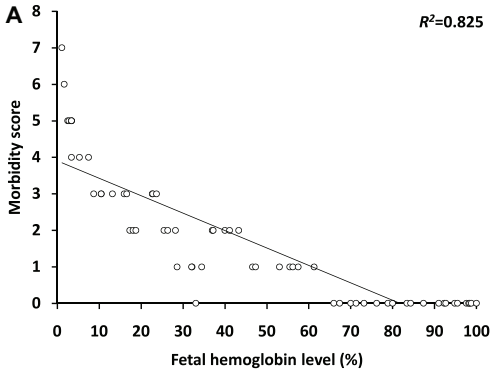
Table 1. Bivariate correlations between fetal hemoglobin level and study parameters

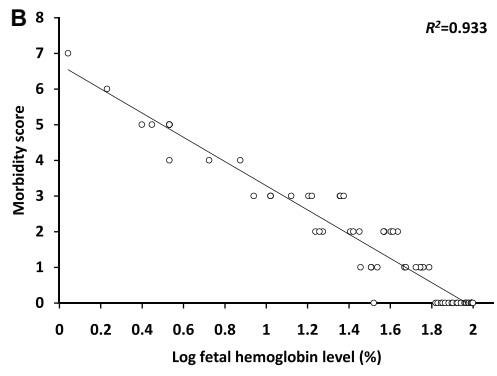
Parameter	Value	*P*
Age (y)	r_s −0.164	.199
Sex		
Male (n = 30)	37 (54)	
Female (n = 33)	42.3 (62.4)	.625
Splenectomy		
No (n = 26)	18.5 (51)	
Yes (n = 37)	47.3 (51.4)	.007
Total hemoglobin level (g/L)	r_s 0.595	< .001
NRBC count (× 10^6/L)	r_s −0.073	.567
GDF-15 level (pg/mL)	r_s −0.522	< .001
Serum ferritin level (μg/L)	r_s −0.092	.471
NTBI level (μmol/L)	r_s −0.444	< .001
Morbidities		
Extramedullary hematopoiesis		
No (n = 50)	50.2 (59.8)	
Yes (n = 13)	18.8 (31.1)	.003
Pulmonary hypertension		
No (n = 44)	58.8 (56.6)	
Yes (n = 19)	8.7 (25.9)	< .001
Venous thromboembolism		
No (n = 51)	47.3 (57.1)	
Yes (n = 12)	18.1 (17.8)	.003
Heart failure		
No (n = 57)	43.3 (56.2)	
Yes (n = 6)	4.4 (8.5)	.002
Leg ulcers		
No (n = 52)	45.0 (57.4)	
Yes (n = 11)	10.5 (24.9)	.002
Abnormal liver function		
No (n = 49)	55.5 (55.2)	
Yes (n = 14)	13.9 (20.1)	< .001
Diabetes mellitus		
No (n = 60)	40.6 (60.4)	
Yes (n = 3)	22.8 (0.5)	.309
Hypothyroidism		
No (n = 53)	47.3 (56.3)	
Yes (n = 10)	5.5 (15.0)	< .001
Hypogonadism		
No (n = 53)*	49.3 (49.7)	
Yes (n = 8)	8.1 (8.6)	< .001
Osteoporosis		
No (n = 49)	55.5 (57.0)	
Yes (n = 14)	9.6 (18.5)	< .001

Data are presented as median fetal hemoglobin in % (interquartile range) unless otherwise indicated.

NRBC indicates nucleated red blood cell; GDF, growth differentiation factor; NTBI, non–transferrin-bound iron; and r_s, Spearman correlation coefficient.

*Two patients were younger than the age-appropriate criteria to define hypogonadism.





Variable	beta (95% CI)	p-value
Fetal hemoglobin level (%)	-0.050 (-0.059 to –0.042)	<0.001
Age (years)	0.000 (-0.010 to 0.009)	0.937
Splenectomy	-0.026 (-0.345 to 0.292)	0.868
Total hemoglobin level (g/l)	-0.288 (-0.522 to -0.055)	0.017
NRBC count (×10^6/l)	0.002 (0.002 to 0.003)	<0.001
GDF-15 level (pg/ml)	0.000 (0.000 to 0.000)	0.695
Serum ferritin level (µg/l)	0.000 (0.000 to 0.000)	0.136
NTBI level (µmol/l)	0.005 (-0.073 to 0.083)	0.121

Variable	beta (95% CI)	p-value
Log fetal hemoglobin level (%)	-3.401 (-3.915 to -2.886)	<0.001
Age (years)	-0.003 (-0.001 to 0.006)	0.560
Splenectomy	0.039 (-0.266 to 0.343)	0.800
Total hemoglobin level (g/l)	-0.053 (-0.255 to 0.148)	0.596
NRBC count (×10^6/l)	0.000 (0.000 to 0.000)	0.850
GDF-15 level (pg/ml)	0.000 (0.000 to 0.000)	0.261
Serum ferritin level (µg/l)	0.000 (0.000 to 0.000)	0.516
NTBI level (µmol/l)	0.012 (-0.061 to 0.085)	0.747

Figure 1. Association between fetal hemoglobin level and the morbidity score. Figures show linear regression of (A) fetal hemoglobin level and (B) log fetal hemoglobin level against the morbidity score. Tables represent results of linear regression analysis after adjustment for indicated variables. NRBC indicates nucleated red blood cell; GDF, growth differentiation factor; NTBI, non–transferrin-bound iron; and CI, confidence interval.

operating characteristic curve analysis (area under the curve 0.986, 95% CI 0.957 to 1.000).

We were able to demonstrate here the quantitative ameliorating effect of HbF levels on morbidity in TI. Although we included a select group of TI patients not receiving transfusion or HbF induction therapy in the present study, we believe that our results will be more broadly applicable in TI. Despite the observation of a statistically independent effect of HbF levels on morbidity, these increased levels could still be acting to reduce the extent of ineffective erythropoiesis and increase total hemoglobin levels in the patients with TI. The mechanisms underlying these observations should be the target of future studies.

In the absence of intervention, the heterogeneity of HbF levels in these patients may be rooted at the molecular level. Recent genome-wide association studies have identified 3 loci (at *BCL11A, HBS1L-MYB*, and the β-globin locus) that carry DNA polymorphisms that modulate HbF levels.[15-19] These polymorphisms have been associated with higher levels of HbF in patients with sickle cell disease and were shown to correlate with a milder disease course.[16,18,19] They were found to occur at higher frequencies in patients with TI than in patients with β-thalassemia major[6,16,20] and were associated with milder disease severity in patients with hemoglobin E/β-thalassemia.[21] Whether these polymorphisms explain the spectrum of HbF levels and associated morbidity in TI patients will need to be explored in future studies.

In conclusion, the present study demonstrates that high HbF levels are associated with a milder disease course in patients with TI. The mechanisms underlying this observation should be evaluated in view of the recent advances in understanding HbF expression and could lead to potential therapeutic interventions.[22] Furthermore, our demonstration that the amelioration in the extent of morbidity is quantitatively associated with HbF levels suggests that therapeutic efforts aimed at even modest induction of these levels may have a profound effect on disease course in patients with TI.

Authorship

Contribution: K.M.M., V.G.S., M.D.C., D.G.N., and A.T.T. designed the study; K.M.M., V.G.S., and L.D. prepared and analyzed the data; K.M.M., V.G.S., M.D.C., L.D., D.G.N., and A.T.T. reviewed the analysis and prepared the manuscript; and K.M.M., V.G.S., M.D.C., L.D., D.G.N., and A.T.T. approved the final submission.

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Supplemental Table 1. Frequencies of the different β- and α-globin genotypes in the study sample (n=63).

β-globin genotype	**α-globin genotype**	**n (%)**
IVS-I-6/IVS-I-6	αα/αα	17 (27.0)
cd29/cd29	αα/αα	9 (14.3)
cd30/cd30	αα/αα	5 (7.9)
IVS-II-1/IVS-II-1	αα/αα	4 (6.3)
cd39/-87	αα/αα	3 (4.8)
cd39/β^A	ααα/αα	3 (4.8)
IVS-I-110/β^A	ααα/αα	3 (4.8)
IVS-II-1/cd29	αα/αα	2 (3.2)
IVS-II-745/IVS-II-844	αα/αα	2 (3.2)
-88/-88	αα/αα	2 (3.2)
IVS-I-1/IVS-I-101	αα/αα	1 (1.6)
IVS-I-5/IVS-I-5	αα/αα	1 (1.6)
IVS-I-6/IVS-I-110	αα/αα	1 (1.6)
IVS-I-110/IVS-I-110	αα/αα	1 (1.6)
IVS-I-101/cd39	αα/αα	1 (1.6)
IVS-I-110/cd39	αα/αα	1 (1.6)
IVS-I-1/β^A	ααα/αα	1 (1.6)
IVS-I-6/-88	αα/αα	1 (1.6)
IVS-I-110/-87	αα/αα	1 (1.6)
IVS-II-745/-87	αα/αα	1 (1.6)
IVS-I-1/-25bpdel	αα/αα	1 (1.6)
cd8/cd8	αα/αα	1 (1.6)
cd6/-87	αα/αα	1 (1.6)

Chapter 3

Iron Overload

Levels Of Non-transferrin-bound Iron As An Index Of Iron Overload In Patients With Thalassaemia Intermedia

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Levels of non-transferrin-bound iron as an index of iron overload in patients with thalassaemia intermedia

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Summary

Non-transferrin-bound iron (NTBI) was evaluated as an index of ironoverload in a cross-sectional study in 74 non-transfused patients with thalassaemia intermedia (TI). Mean NTBI (2·92 ± 3·43 μmol/l), serumferritin (1023 ± 780 ng/ml) and liver iron concentration (LIC; 9·0 ± 7·4 mg Fe/g dry weight) were increased above reference-range levels. Significant positive correlations occurred between mean NTBI and LIC (Pearson correlation 0·36; $P = 0·002$) and serum ferritin (Pearson correlation 0·421; $P < 0·0001$); with higher levels observed in splenectomised patients. NTBI assessment has potential as a simple reliable approach to determining iron status in TI.

Keywords: iron overload, non-transferrin-bound iron, thalassaemia intermedia.

Anaemia in patients with thalassaemia intermedia (TI) is typically mild and does not necessitate regular blood transfusion therapy until later in life. Nonetheless, patients remain prone to iron loading, primarily due to intestinal iron absorption and ineffective erythropoiesis (Origa *et al*, 2007; Taher *et al*, 2008), with the accumulation of approximately 2–5 g of iron per year, depending on the degree of bone marrow expansion and peripheral hemolysis (Origa *et al*, 2007). Patients with TI eventually develop complications of iron loading similar to those observed in thalassemia major (TM), including liver, heart and endocrine dysfunction (Origa *et al*, 2007; Taher *et al*, 2008).

The most commonly used methods for evaluating iron overload include measurement of serum ferritin and liver iron concentration (LIC). Assessment of serum ferritin levels is convenient, non-invasive and widely used (Pakbaz *et al*, 2007), but is likely to underestimate the severity of iron load in patients with TI (Origa *et al*, 2007; Pakbaz *et al*, 2007; Chirnomas *et al*, 2008; Taher *et al*, 2008). LIC and heart iron concentration assessed by R2 magnetic resonance imaging (MRI) are reliable approaches but their use is limited due to cost and the need for specialised equipment.

Non-transferrin-bound iron (NTBI) is a low-molecular-weight form of iron that is detected in conditions of iron overload when transferrin becomes fully saturated and is unable to bind excess iron. NTBI is thought to catalyse the formation of reactive radicals (Cighetti *et al*, 2002), and is known to cause direct oxidative damage (Cappellini *et al*, 2000). High levels of free intracellular iron are related to massive membrane damage and metabolic impairment in thalassaemia (Tavazzi *et al*, 2001). Several studies have demonstrated that NTBI is a good index of iron overload in TM (al-Refaie *et al*, 1992; Cabantchik *et al*, 2005); however, data in patients with TI are limited. The current study evaluated whether NTBI is a useful index of iron overload in non-transfused or minimally transfused patients with TI by assessing the relationship between NTBI and serum ferritin, LIC and disease-related parameters.

Materials and methods

This was a cross-sectional study of patients with TI treated at the Chronic Care Centre in Hazmieh, Lebanon. A simple random sample was obtained from 120 patients with TI aged ≥2 years. A total of 74 patients agreed to be included in the study and written informed consent was provided by all patients. Patient charts were reviewed and a medical history compiled, which included details of drug history, comorbid

illnesses and transfusional history. Direct determination of LIC was performed by R2 MRI using an established methodology (St Pierre *et al*, 2005). Blood samples were obtained for assessment of pretransfusion haemoglobin, steady-state serum ferritin levels and NTBI (stored at −20°C).

Serum NTBI content was assayed by high performance liquid chromatography (HPLC) according to the methods of Porter *et al* (1996), with minor modifications. Briefly, 450 µl of serum was added to 50 µl of nitrilotriacetic acid (NTA) 800 mmol/l (pH 7·0) and was allowed to stand for 20 min to remove iron (Fe) non-specifically bound to serum proteins and low-molecular-weight ligands by the excess of NTA; in principle, the scavenged NTBI was quantitatively converted to Fe–NTA complex. The solution was then ultrafiltered using an Amicon Centricon 30 microconcentrator (Amicon Corporation, Lexington, MA, USA), and the ultrafiltrate (20 µl), which contained the Fe–NTA complex, was injected directly into the HPLC system (PerkinElmer series 200 IC titanium pump module; PerkinElmer Life Science, Boston, MA, USA). Chromatographic conditions were as follows: flow rate, 1·5 ml/min; mobile phase, isocratic containing 20% acetonitrile and 3 mmol/l CP22 in 5 mmol/l sodium phosphate buffer, pH 7·0; visible detection, 450 nm. A standard curve was generated by injecting different concentrations of iron (from 0 to 100 µmol/l in steps of 10 µmol/l) prepared in a 100-fold excess of NTA. Standards were run routinely from 0 to 10 µmol/l (in 1 µmol/l steps). Under these conditions, the 0 µmol/l standard corresponded to 80 mmol/l of NTA.

In accordance with previous reports (Aruoma *et al*, 1988; Porter *et al*, 1996), all our normal controls had values of ≤0 (–0·72 ± 0·70 µmol/l) because transferrin captures iron from the Fe–NTA complex. Normal individuals always have negative NTBI values because samples are measured in parallel with a corresponding blank formed by water and NTA. Water *per se* contains small amounts of iron that are not bound by transferrin, whereas in samples, transferrin that is not completely saturated captures some iron from the Fe–NTA complex (Gosriwatana *et al*, 1999). Therefore, the blank subtraction makes the NTBI value in some samples negative.

Descriptive statistics are expressed as means ± standard deviation (SD) or percentages where appropriate. Bivariate correlations between study variables were performed using independent-samples *t*-test for categorical variables and Pearson correlation for continuous variables. A multivariate stepwise regression analysis was done to determine significant correlations where needed. All *P*-values are two sided with the level of significance set at <0·05.

Results and discussion

Data from 74 patients were included in this analysis (Table Ia). All patients were chelation naïve and none of the patients had evidence of hepatitis B or C infection. The mean NTBI, serum ferritin and LIC were above the reference-range levels in this chelation-naïve population, highlighting that many patients with TI will be at risk of significant iron-related morbidity and mortality; and reflecting the haematological heterogeneity of TI patients.

Among study variables, NTBI levels were only significantly correlated to splenectomy status and transfusion history (Table Ib). On multivariate analysis, only splenectomy

Table Ia. Patients' characteristics.

Parameter	Value
Number of patients (*n*)	74
Mean age ± SD, years (range)	26·5 ± 11·5 (8–54)
Male:female	33:41
Splenectomized, *n* (%)	59 (80)
Transfusion history, *n* (%)	
Naïve	20 (27)
Infrequent (few transfusions received in the past)	45 (61)
Regular (2–4 times/year)	9 (12)
Mean haemoglobin ± SD, g/l (range)	84·3 ± 18·6 (49–131)
Mean NTBI ± SD, µmol/l (range)	2·92 ± 3·43 (−3·71–8·5)
Mean serum ferritin ± SD, µg/l (range)	1023 ± 780 (29–3158)
Mean LIC ± SD, mg Fe/g dry weight (range)	9·0 ± 7·4 (0·5–32·1)

NTBI, non-transferrin-bound iron; LIC, liver iron concentration; SD, standard deviation.

Table Ib. Bivariate analysis showing correlations between study variables and iron overload parameters.

Variable	NTBI (µmol/l)	Ferritin (µg/l)	LIC (mg Fe/g dry weight)
*Categorical**			
Gender			
Male (*n* = 33)	2·24 ± 3·50	1027·2 ± 640·6	10·3 ± 8·4
Female (*n* = 41)	2·84 ± 3·41	879·0 ± 648·6	7·9 ± 6·4
P-value	0·868	0·329	0·169
Splenectomy			
Yes (*n* = 59)	4·10 ± 2·87	1116·7 ± 604·9	10·5 ± 6·8
No (*n* = 15)	−1·04 ± 1·65	369·7 ± 400·2	3·9 ± 7·4
P-value	**<0·001**	**0·001**	**<0·001**
Transfusion history			
Naïve (*n* = 20)	1·38 ± 3·84	567·8 ± 455·2	4·0 ± 3·3
Infrequent (*n* = 45)	3·10 ± 3·14	1184·0 ± 869·6	10·5 ± 7·8
Regular (*n* = 9)	5·41 ± 2·28	1116·7 ± 604·9	12·6 ± 7·0
P-value	**0·010**	**<0·001**	**0·001**
Continuous†			
Age (years)	0·117	0·367	0·350
P-value	0·323	**0·001**	**0·002**
Haemoglobin (g/l)	0·017	−0·187	−0·180
P-value	0·888	0·110	0·125

NTBI, non-transferrin-bound iron; LIC, liver iron concentration.
*Statistical correlation evaluated by independent-samples *t*-test; data presented as mean ± SD.
†Statistical correlation evaluated by Pearson Correlation; data presented as Pearson correlation co-efficient (*r*).

remained independently correlated with NTBI ($P < 0{\cdot}001$). Splenectomised patients had higher serum NTBI levels than non-splenectomised patients, which is consistent with previous observations in patients with TI (Fiorelli *et al*, 1990; Cappellini *et al*, 2000). This observation suggests that the intact spleen may be a reservoir of excess iron and may have a possible scavenging effect on iron free fractions including NTBI (Tavazzi *et al*, 2001). Although increasing age was associated with higher levels of serum ferritin and LIC, no direct correlation was observed with NTBI. Serum ferritin and LIC are a cumulative index of iron accumulation over time. By contrast, transferrin saturation and the increased NTBI of patients with saturated transferrin is an acute phenomenon. In TI, increased transferrin saturation is the consequence of ineffective erythropoiesis with an outpouring of catabolic iron at a rate which is 10–15 times normal. Thus, NTBI would emerge at an earlier age when total iron accumulation in the liver and serum ferritin are still at a relatively early stage. This is not the case in TM where blood transfusions almost completely suppress the ineffective erythropoiesis and free iron appears when transferrin is completely saturated and in presence of severe iron burden.

Nevertheless, there was a significant correlation between NTBI and LIC (Pearson correlation 0·36, $P = 0{\cdot}002$; Fig 1A) and NTBI and serum ferritin (Pearson correlation 0·421, $P < 0{\cdot}0001$; Fig 1B). This confirms previous findings in which NTBI has been shown to be a good index of iron overload (as assessed by serum ferritin and total serum iron) in regularly transfused TM patients (al-Refaie *et al*, 1992; Cabantchik *et al*, 2005). However, a recent study showed no correlation between NTBI and LIC or serum ferritin in patients with TM or TI (Piga *et al*, 2009). Regular chelation therapy in these patients may have modified the relationship of serum ferritin and LIC with NTBI (Piga *et al*, 2009). In contrast, patients enrolled in the current study were chelation naïve and had iron levels above the reference range. The large number of patients with high NTBI yet serum ferritin levels below 500 μg/l, signifies that free iron could be present with less total iron burden (low serum ferritin). This is again explained by the aforementioned pathophysiology of iron loading in TI and echoes studies showing that serum ferritin underestimates iron overload in TI patients (Taher *et al*, 2008). These observations have important implications for patient management, as assessment of 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 morbidity and mortality risks. In these patients we were unable to evaluate T2* to estimate myocardial iron accumulation. A recent study has shown that the presence of heart disease is associated with significantly higher NTBI values in patients with TI or TM, which supports the concept that increased severity of myocardial siderosis is caused by a critical expansion of the toxic chelatable iron pool (Piga *et al*, 2009).

In conclusion, this study demonstrated that in non-transfused or minimally transfused patients with TI, NTBI was detectable and above reference-range levels and was significantly correlated with LIC and serum ferritin in the overall population. Despite some patients having low serum ferritin levels, NTBI was increased, therefore suggesting that the assessment of NTBI levels has potential as a simple and reliable approach to determine the iron status of patients with TI.

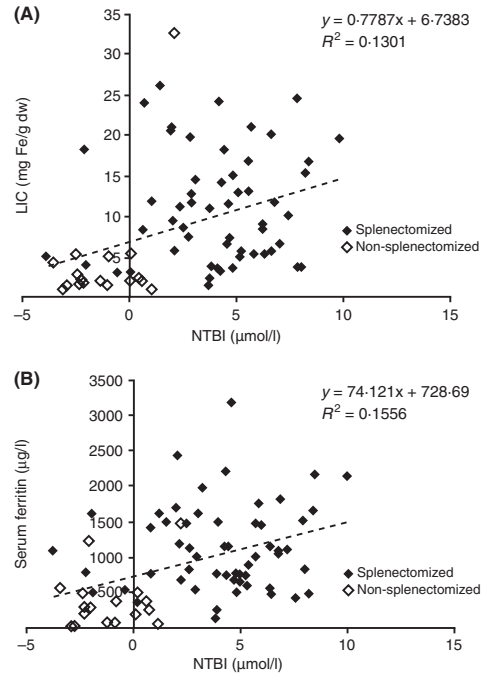

Fig 1. Correlation between non-transferrin-bound iron (NTBI) and (A) liver iron concentration (LIC) and (B) serum ferritin.

Conflict of interest disclosures

Dr Taher has received research grants and lecture fees from Novartis pharmaceuticals. Dr Musallam has no relevant conflicts of interest to disclose. Dr El Rassi has no relevant conflicts of interest to disclose. Dr Duca has no relevant conflicts of interest to disclose. Dr Inati has received lecture fees from Novartis Pharmaceuticals. Dr Koussa has no relevant conflicts of interest to disclose. Dr Cappellini has received lecture fees from Novartis Pharmaceuticals.

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Elevated Liver Iron Concentration Is A Marker Of Increased Morbidity In Patients With β Thalassemia Intermedia

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Elevated liver iron concentration is a marker of increased morbidity in patients with β thalassemia intermedia

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The online version of this article has a Supplementary Appendix.

ABSTRACT

Background
Patients with β thalassemia intermedia can have substantial iron overload, irrespectively of their transfusion status, secondary to increased intestinal iron absorption. This study evaluates whether iron overload in patients with β thalassemia intermedia is associated with morbidity.

Design and Methods
This was a cross-sectional study of 168 patients with β thalassemia intermedia treated at two centers in Lebanon and Italy. Data on demographics, splenectomy status, transfusion status, and presence of co-morbidities were retrieved. Laboratory values of serum ferritin, fetal and total hemoglobin levels, as well as platelet and nucleated red blood cell counts were also obtained. Iron burden was determined directly by measuring liver iron concentration using magnetic resonance imaging. Patients were subdivided according to transfusion and splenectomy status into groups with phenotypes of different severity.

Results
The mean age of the patients was 35.2±12.6 years and 42.9% of them were male. The mean liver iron concentration was 8.4±6.7 mg Fe/g dry weight. On multivariate logistic regression analysis, after adjusting for age, gender, splenectomy status, transfusion status, and laboratory indices, an increase in 1 mg Fe/g dry weight liver iron concentration was independently and significantly associated with higher odds of thrombosis, pulmonary hypertension, hypothyroidism, osteoporosis, and hypogonadism. A liver iron concentration of at least 7 and at least 6 mg Fe/g dry weight were the best thresholds for discriminating the presence and absence of vascular and endocrine/bone morbidities, respectively (area under the receiver-operating characteristic curve: 0.72, $P<0.001$). Elevated liver iron concentration was associated with an increased rate of morbidity in patients with phenotypes of all severity, with a steeper increase in the rate of vascular morbidity being attributed to aging, and an earlier appearance of endocrine and bone disease.

Conclusions
Elevated liver iron concentration in patients with β thalassemia intermedia is a marker of increased vascular, endocrine, and bone disease.

Key words: thalassemia intermedia, liver iron concentration, iron overload, vascular disease, endocrine disease, osteoporosis.

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Introduction

There is diversity in the severity of the phenotype of β thalassemia syndromes. The term β thalassemia intermedia was first suggested to describe patients who have milder anemia than patients with β thalassemia major, who usually present to medical attention later in childhood, and who remain largely transfusion-independent.[1] However, it is now established that the diagnosis of β thalassemia intermedia spans a wide spectrum of severity and carries higher morbidity than previously recognized.[2-3] Three main factors dominate the disease process in β thalassemia intermedia: ineffective erythropoiesis, chronic hemolytic anemia, and iron overload.[3] The combination of ineffective erythropoiesis and chronic anemia leads to hepcidin suppression, increased iron absorption from the gut, and increased release of recycled iron from the reticuloendothelial system. This results in depletion of macrophage iron, relatively low levels of serum ferritin, and preferential portal and hepatocyte iron storage. This, in turn, leads to considerable hepatic iron overload and release of toxic iron species, such as non-transferrin-bound iron (NTBI), into the circulation.[4-7] Iron overload can also be the consequence of transfusion therapy, which despite traditionally being an uncommon practice in β thalassemia intermedia, is now undertaken for many patients with severe disease after showing a potential role in ameliorating some disease complications.[2,8,9] Moreover, age-related changes in adaptation to anemia by the bone marrow, alongside difficulty in maintaining a high output with normal vascular aging, cause many transfusion-independent patients with β thalassemia intermedia to become transfusion-dependent as they age.[10,11] Several studies in patients with β thalassemia major have proven that uncontrolled iron overload is associated with significant morbidity and mortality, especially cardiac, highlighting the essential role of iron chelation therapy for survival.[12] Studies on the morbidity or mortality from iron overload in patients with β thalassemia intermedia are lacking. Cardiac siderosis seems to be uncommon in β thalassemia intermedia, even in patients with severe iron overload.[13-15] It does, therefore, remain essential to determine whether iron overload results in other clinical sequelae, before chelation therapy can be advised.

Liver iron concentration (LIC) has been regarded as the reference standard for estimating body iron load and has been shown to predict total body iron stores accurately.[16] R2 and R2* magnetic resonance imaging (MRI) relaxation time techniques allow for non-invasive estimation of LIC in patients with hemoglobinopathies.[17-19] The LIC cut-off points of 7 and 15 mg Fe/g dry weight (dw) have been used for the past two decades to categorize iron overload status, predict morbidity and mortality, and tailor iron chelation therapy in patients with β thalassemia major. However, these cut-off points were extrapolated from data on patients with hereditary hemochromatosis,[20] and were only linked to liver pathology and cardiac disease in a few small studies on patients with β thalassemia major utilizing liver biopsy.[21-24] There are no studies linking LIC or its cut-offs to morbidity or mortality in patients with β thalassemia intermedia.

The aim of this study was to evaluate the association between iron overload, as determined by LIC, and morbidity in a large cohort of patients with β thalassemia intermedia.

Design and Methods

This was a cross-sectional study of all patients with β thalassemia intermedia treated at two centers in Beirut, Lebanon and Milan, Italy, for whom LIC measurements were available (74/127 from Lebanon and 94/153 from Italy). The main criteria to define the β thalassemia intermedia phenotype on presentation in both centers was age more than 2 years at diagnosis and hemoglobin values maintained between 7 and 9 g/dL without the need for a regular transfusion regimen (at diagnosis) in patients with or without splenomegaly.[25] Patients with Hb S, C, E/β or δβ thalassemia; or those who had co-inheritance of α thalassemia [α^+ (-$\alpha^{3.7}$ and -$\alpha^{4.2}$) or α^0 (--^{Med} and --^{SEA})] or determinants associated with increased γ chain production [*Xmn*-I +/+ genotype at position –158 of *Hβ*G2] were excluded. All extracted data reflected the period of LIC measurement. Patients' charts were reviewed to retrieve data on demographics (age and gender), splenectomy status, and transfusion history. None of the patients was receiving iron chelation therapy or any fetal hemoglobin-inducing agents at the time of LIC measurement. The data for transfusion history were categorized as follows: regularly transfused (patients transfused at regular intervals every 1-3 months), occasionally transfused (patients who required occasional transfusions for transient severe anemia secondary to infections, surgery, or pregnancy); and non-transfused. Laboratory data were retrieved and recorded as a mean of all measurements undertaken during the year of LIC measurement; the parameters of interest were serum ferritin level, fetal and total hemoglobin levels (before the scheduled transfusion in patients who were given transfusions), platelet count and nucleated red blood cell (NRBC) count. The iron burden in the liver (LIC) was determined directly by R2 MRI in Beirut and R2* MRI in Milan using established methodologies, calibrated to mg/g of iron by dry weight in fresh liver biopsy specimens.[17-18] The study received Institutional Review Board approval.

Data were also obtained on morbid conditions known to be common in patients with β thalassemia intermedia[3] or that could be relevant in a state of iron overload. Complications were defined according to Table 1.[26-31] The prevalence of other elements that could modify the rate of morbidities (family history of cardiovascular or endocrine disease, acquired or inherited thrombophilia, anticoagulant or antiplatelet use for reasons other than overt thrombosis, malignancy, orthopedic surgery, hepatitis C or B virus infection) was low and these elements were not, therefore, included in further analysis.

Statistical analysis

Descriptive statistics are expressed as means (standard deviation, SD), medians (interquartile range, IQR) or percentages. Bivariate analysis was performed to determine the correlation between LIC and study variables using the independent samples t-test or the ANOVA test (for categorical variables) and the Pearson's correlation coefficient (for continuous variables). Bivariate correlations between study variables and morbidities were evaluated by the independent samples t-test and the χ^2 test except for heart failure and diabetes mellitus for which correlations were evaluated by the Mann-Whitney U test and the Fisher's exact test. For bivariate analysis including LIC, we also double-checked and confirmed that statistical significance was maintained when geometric means or medians were compared instead of arithmetic means. Multivariate logistic regression analysis, using forward-stepwise selection, was used to determine which variables were independently associated with each morbidity. Transfusion history was categorized as transfused or non-transfused. A *P* value of 0.1 or less was used as the criterion for inclusion into the model to allow for correction of most confounders.

Table 1. Definitions of morbidities.

Morbidity	Definition
Extramedullary hematopoiesis	Radiological evidence of extramedullary hematopoietic foci with or without symptoms
Leg ulcers	An ischemic or necrotic skin lesion on the lower extremity found by general visual inspection
Thrombosis	Compression ultrasonography, contrast venography or angiography evidence of thrombus
Pulmonary hypertension	A systolic pulmonary artery pressure greater than 35 mm Hg, which corresponds to a tricuspid regurgitant velocity on Doppler echocardiography of >2.8 m/sec[26] + exertional dyspnea without evidence of left heart disease.
Heart failure	Modified Framingham criteria [27]
Abnormal liver function	Alanine aminotransferase >50 U/L
Diabetes mellitus	A fasting blood sugar ≥126 mg/dL, or 2-hour post prandial blood sugar ≥200 mg/dL, or symptoms of hyperglycemia and a casual (random) plasma glucose ≥200 mg/dL[28]
Hypothyroidism	Thyroid stimulating hormone >4.7 μU/L and a free T4 <0.8 ng/dL[29]
Osteoporosis	Bone densitometry T-score – 2.5 SD[30]
Hypogonadism	Females: >13 years, not yet Tanner B2 (*i.e.* prepubertal breast development) or >14 years requiring estrogen replacement therapy or >15 years with primary amenorrhoea Males: >14 years, not yet Tanner G2 (*i.e.* prepubertal genital development) or on androgen replacement therapy or >17 years, not yet Tanner G4 (*i.e.* midpubertal genital development)[31]

Multicolinearity between variables in the model was evaluated using the variation inflation factor. All variation inflation factors were 3 or less (acceptable limit <10) indicating absence of multicolinearity. To determine the best LIC cut-offs for discriminating the presence and absence of morbidity, the maximum sum of sensitivity and specificity was calculated from receiver-operating characteristic (ROC) curve analysis. Retrieved cut-offs were also tested using the same multivariate logistic regression model. The effects of splenectomy and transfusion history on the association between LIC and morbidities was explored by grouping patients according to phenotypic severity: mild (neither splenectomized nor transfused), moderate (either splenectomized or transfused) and severe (both splenectomized and transfused). Logarithmic regression curves were used to determine the effect of age on the observed association between LIC and morbidities, as stratified for disease severity groups. All *P*-values are two-sided with values less than 0.05 considered statistically significant.

Results

Patients' characteristics

A total of 168 patients with β thalassemia intermedia were included in this analysis (Table 2). The mean LIC was 8.4±6.7 mg Fe/g dw (range, 0.5-32.1 mg Fe/g dw). Mean LIC was higher in splenectomized patients than in non-splenectomized ones (9.4±6.5 *versus* 5.8±6.6 mg Fe/g dw, respectively; *P*=0.001) and was higher in regularly (9.7±6.7 mg Fe/g dw) or occasionally (9.9±7.2 mg Fe/g dw) transfused patients than in non-transfused patients (4.3±3.1 mg Fe/g dw) (*P*<0.001). There was a weak positive correlation between LIC and serum ferritin level (r=0.53, *P*<0.001) as well as fetal hemoglobin level (r=0.22, *P*=0.008). There were no statistically significant correlations between LIC and age, gender, total hemoglobin level, platelet count or NRBC count.

Liver iron concentration and morbidities

Mean LIC values were significantly higher in patients with leg ulcers, thrombosis, pulmonary hypertension, abnormal liver function, hypothyroidism, osteoporosis, and hypogonadism than in patients without these morbidities (Figure 1). Bivariate correlations between other study parameters and morbidities are summarized in *Online Supplementary Table S1*. On multivariate logistic regression analysis, and after adjusting for all study variables significant at the 0.1 level on bivariate analysis, a 1 mg Fe/g dw increase in LIC was significantly and independently associated with higher odds of thrombosis, pulmonary hypertension, hypothyroidism, osteoporosis, and hypogonadism (*Online Supplementary Table S2*).

Table 2. Patients' characteristics (n=168).

Parameter	Value
Age (years), mean (SD)	35.2 (12.6)
Male, n. (%)	73 (42.9)
Splenectomized, n (%)	121 (72.0)
Transfusion history, n (%)	
None	44 (26.2)
Occasional	80 (47.6)
Regular	44 (26.2)
Total hemoglobin (g/dL), mean (SD)	8.8 (1.6)
Fetal hemoglobin (%), mean (SD)	44.5 (31.1)
Platelet count (×10⁹/L), mean (SD)	609.4 (346.0)
NRBC count (×10⁹/L), median (IQR)	422.5 (11653)
Serum ferritin (ng/mL), median (IQR)	773.3 (938.5)
LIC (mg Fe/g dw), mean (SD)	8.4 (6.7)
Morbidity, n (%)	
Osteoporosis	77 (45.8)
Pulmonary hypertension	56 (33.3)
Abnormal liver function	54 (32.1)
Thrombosis	44 (26.2)
Extramedullary hematopoiesis	43 (25.6)
Leg ulcers	41 (24.4)
Hypothyroidism	30 (17.9)
Hypogonadism	28 (16.7)
Heart failure	9 (5.4)
Diabetes mellitus	6 (3.6)

SD: standard deviation; IQR: interquartile range; NRBC: nucleated red blood cell; LIC: liver iron concentration; dw: dry weight.

Liver iron concentration cut-offs

Using ROC curve analysis, a LIC of at least 7 mg Fe/g dw was found to be the best threshold for discriminating the presence and absence of vascular morbidity (thrombosis or pulmonary hypertension) with an area under the curve (AUC) of 0.723 (P<0.001). Patients with a LIC of at least 7 mg Fe/g dw were 3.76 times more likely to have vascular morbidity compared with patients with a LIC less than 7 mg Fe/g dw (Table 3). Similarly, a LIC of at least 6 mg Fe/g dw was found to be the best threshold for discriminating the presence and absence of endocrine or bone morbidity (hypothyroidism, osteoporosis, or hypogonadism) with an AUC of 0.724 (P<0.001). Patients with a LIC of at least 6 mg Fe/g dw were 4.05 times more likely to have endocrine morbidity than were patients with a LIC less than 6 mg Fe/g dw (Table 3).

Effects of splenectomy and transfusion (phenotype severity)

Patients with a LIC of at least 7 mg Fe/g dw had a significantly higher rate of vascular morbidity than did patients with a LIC less than 7 mg Fe/g dw, in all groups of phenotype severity. Moreover, among the patients with a LIC of at least 7 mg Fe/g dw, the rate of vascular morbidity was significantly higher in those with a severe phenotype than in those with a moderate or mild phenotype (Figure 2A). Patients with a LIC of at least 6 mg Fe/g dw had a significantly higher rate of endocrine or bone morbidity than did patients with a LIC less than 6 mg Fe/g dw, in all phenotype severity groups. Moreover, among patients with a LIC of at least 6 mg Fe/g dw, the rate of endocrine or bone morbidity was significantly higher in those with a severe phenotype than in those with a moderate or mild phenotype (Figure 2B).

Effect of age

The probability of vascular morbidity significantly increased with age irrespectively of LIC, although reaching significantly higher values more steeply in patients with a LIC of at least 7 mg Fe/g dw than in those with a LIC less than 7 mg Fe/g dw (Figure 3A, left panel). When patients were stratified according to phenotype severity, the latter trend was maintained (Figure 3A, right panel). Moreover, the probability of endocrine or bone morbidity increased significantly with age in patients with LIC values less than 6 mg Fe/g dw; however, it showed a flat behavior starting with a high probability at young age in patients with values of at least 6 mg Fe/g dw (Figure 3B, left panel). When patients were stratified according to phenotype severity, the latter trend was maintained in patients with a severe phenotype (Figure 3B, right panel).

Discussion

Our study is the first to associate iron overload, reflected by LIC measurement, with vascular, endocrine, and bone morbidity in patients with β thalassemia intermedia. Elevated LIC was associated with an increased rate of vas-

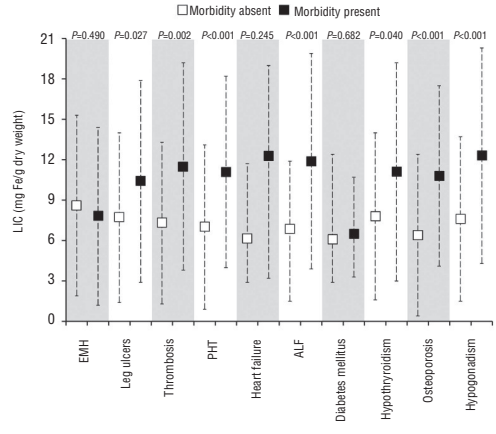

Figure 1. Comparison of LIC values in patients with and without morbidities. Data presented as means (squares) and standard deviations (whiskers), except for heart failure and diabetes mellitus for which data are presented as medians (square), 25th and 75th percentiles (whiskers). The *P* value was calculated using the independent samples t-test, except for heart failure and diabetes mellitus for which it was calculated using the Mann-Whitney U test. LIC: liver iron concentration; EMH: extramedullary hematopoiesis; PHT:pulmonary hypertension; ALF: abnormal liver function.

Table 3. Receiver operating characteristic (ROC) curve analysis to determine best LIC cut-offs for discriminating the presence and absence of morbidity.

Morbidity	LIC cut-off (mg Fe/g dw)	AUC	95% CI	*P* value	Sensitivity	Specificity	AOR (95% CI)[a]
Thrombosis	≥7	0.669±0.049	0.573-0.765	0.001	70.5%	61.3%	2.86 (1.22-5.91)
Pulmonary hypertension	≥6	0.684±0.042	0.601-0.767	<0.001	75%	58%	3.30 (1.54-7.08)
Vascular[b]	≥7	0.723±0.039	0.647-0.800	<0.001	66.3%	71.8%	3.76 (1.81-7.81)
Hypothyroidism	≥6	0.630±0.056	0.521-0.739	0.025	76.7%	52.2%	2.65 (1.03-6.77)
Osteoporosis	≥9	0.796±0.041	0.624-0.787	<0.001	58.4%	81.3%	5.13 (2.46-10.71)
Hypogonadism	≥6	0.689±0.053	0.585-0.793	0.002	78.6%	52.1%	3.35 (1.21-9.26)
Endocrine/bone[c]	≥6	0.724±0.039	0.647-0.801	<0.001	71.3%	70.3%	4.05 (1.96-8.35)

LIC: liver iron concentration; dw: dry weight; AUC: area under the curve; CI: confidence interval; AOR: adjusted odds ratio; CI: confidence interval. [a]Adjusted for age, gender, splenectomy status, transfusion history, total hemoglobin level, fetal hemoglobin level, platelet count, nucleated red blood cell count, and serum ferritin level. The model was built using forward-stepwise selection. P≤0.1 was used as the criterion for inclusion. Multicolinearity was absent in the model as demonstrated by a variation inflation factor ≤3 (acceptable limit up to 10). [b]Patients with pulmonary hypertension or thrombosis. [c]Patients with hypothyroidism, osteoporosis, or hypogonadism.

cular, endocrine, and bone morbidity in patients with phenotypes of all severity. Moreover, elevated LIC was associated with a steeper increase in the rate of vascular morbidity attributed to aging, and permitted endocrine and bone disease to appear at a younger age than in patients with low LIC. These novel findings have important clinical implications, although they need to be interpreted with caution.

A causal relationship between LIC and morbidity cannot yet be established. This is not because our study is cross-sectional in nature. Even if such an association were to be prospectively observed, the complexity of the disease process in β thalassemia intermedia makes it hard to determine whether elevated LIC is only a marker of disease severity (hence the increased morbidity) or a causative, modifiable risk factor. The definition and evaluation of severity in β thalassemia intermedia are challenging, especially given that hemoglobin level does not corre-

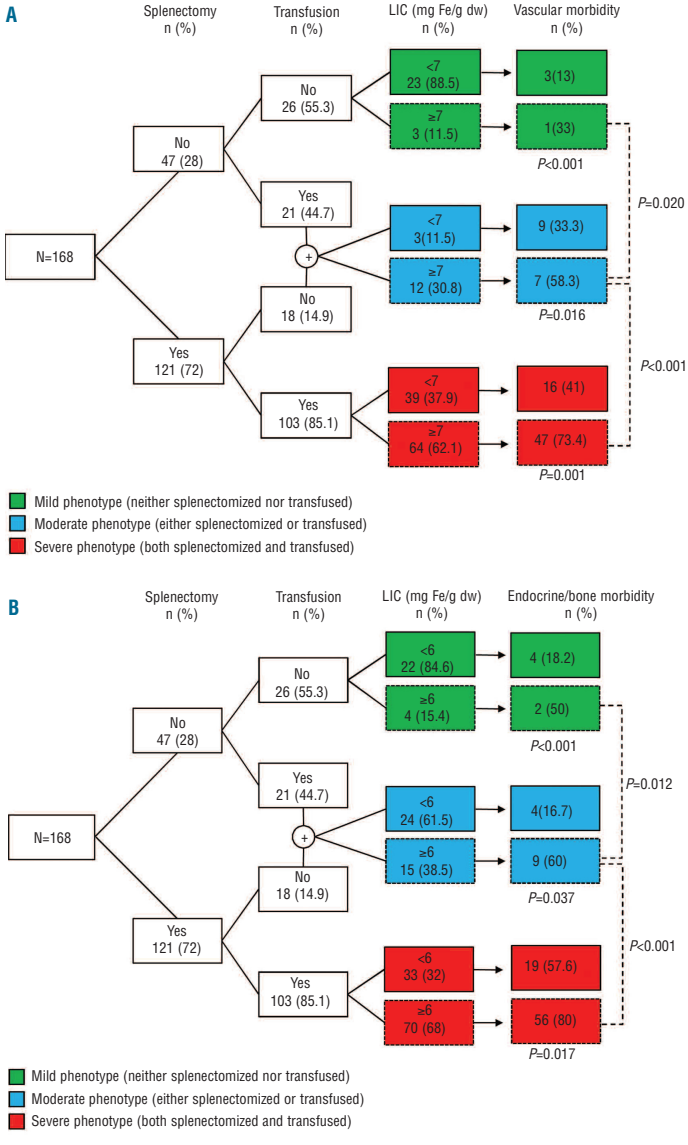

Figure 2. **Flow diagram showing the interplay between splenectomy, transfusion history, and elevated LIC and its effect on the rate of (A) vascular and (B) endocrine/bone morbidity. LIC, liver iron concentration; dw, dry weight. Data analyzed using the χ^2 and Fisher's exact tests.**

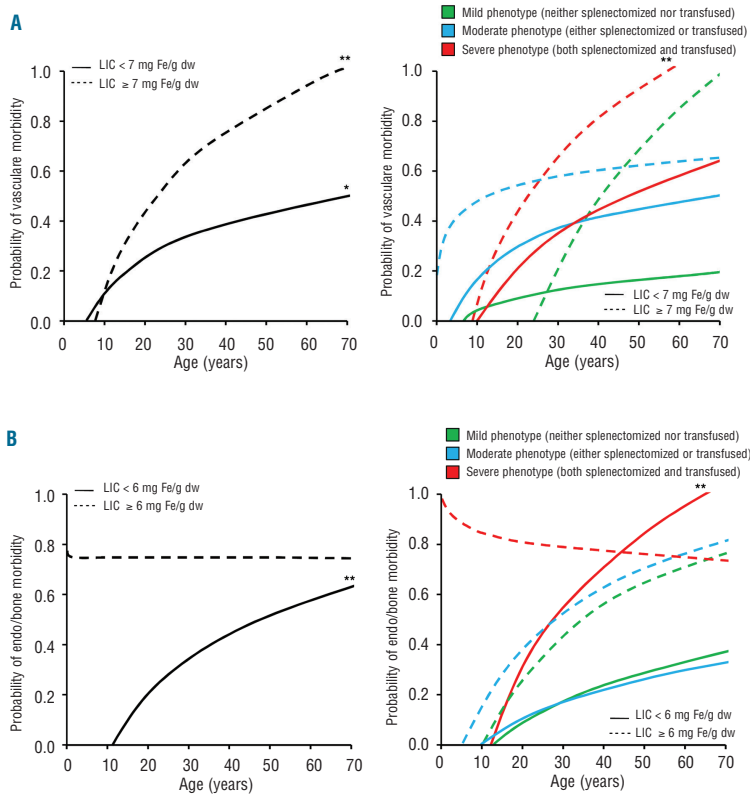

Figure 3. Logarithmic regression curves demonstrating the effect of advancing age on (A) vascular and (B) endocrine/bone morbidity, in different subgroups of patients according to LIC and phenotype severity. LIC, liver iron concentration; dw, dry weight. *P<0.05; **P<0.01.

late with most morbidities,[2] and markers of the severity of ineffective erythropoiesis have not been extensively evaluated. We undertook a practical approach and assumed that the need for splenectomy and transfusion therapy reflects a more severe phenotype. In such cases, elevated LIC was associated with an increased risk of complications in all severity groups, indicating that iron overload may be adding to any other causative factors attributed to a more severe disease. Moreover, elevated LIC worsened the observed effect of aging on complications, again indicating an additive role of iron overload to the established role of advancing age.[11] Nevertheless, true evidence of target-organ iron toxicity can only be confirmed through radio-pathological studies, or through the observation of a beneficial effect of iron chelation therapy. In fact, evidence already exists regarding a protective role of iron chelation therapy, presumably necessitated in some of the severe cases, against several clinical complications in β thalassemia intermedia.[2]

If causation is hypothesized, how could iron toxicity be linked to the observed complications, especially vascular disease? Hypercoagulability in β thalassemia intermedia is attributed to several factors including ineffective erythropoiesis and secondary procoagulant activity of hemolysed circulating red blood cells, microparticles, increased platelet activation, thrombocytosis, coagulation factor defects, depletion of antithrombotic factors, and endothelial inflammation.[32] Hypercoagulability leads to a high rate of thromboembolic events and probably pulmonary hypertension through multiple microthrombi in the pulmonary vasculature; especially in splenectomized and older patients with β thalassemia intermedia.[2,11,33-36] Hemolysis and erythroid hyperplasia have also been linked to increased release of placental growth factor, endothelin-1, and pulmonary hypertension.[37] Iron may contribute directly to hemolysis, or endothelial damage and vasculopathy. Iron-derived reactive oxygen species are implicated in the pathogenesis of several vascular disorders including atherosclerosis, microangiopathic hemolytic anemia, vasculitis, and reperfusion injury.[38] Moreover, the relationship between iron overload and the severity of ineffective erythropoiesis seems to be bidirectional. Recent evidence suggests that managing iron overload with iron chelators or more novel therapeutics could improve the efficiency of erythropoiesis and the survival of the resulting reticulocytes and erythrocytes.[39-42] Thus, iron overload may aggravate ineffective erythropoiesis and the secondary release into the circulation of damaged red blood cells with thrombogenic potential.[43-44]

The need for iron chelation therapy in patients with β thalassemia intermedia who have never been transfused or

have received only occasional transfusions has just recently started to emerge after documenting substantially high LIC and NTBI values in such patients.[4,5] As for other aspects of the management of β thalassemia intermedia, clear guidelines on initiation of chelation therapy are not available. Current recommendations are based on expert opinion or are extrapolated from data on β thalassemia major.[7] If the evidence of iron toxicity suggested here is confirmed, chelation therapy would be recommended to decrease toxic iron species such as NTBI. LIC measurements could be used to flag the hyperabsorption and increased labile iron and to avoid overchelation. Iron chelation therapy in patients with β thalassemia intermedia may not necessarily be life-long. Intermittent periods of iron chelation with careful assessment of LIC throughout the course of the disease could be sufficient in many cases. When LIC is lowered to desirable levels, low dose oral chelation may be of value in preventing further iron loading. Serum ferritin levels could not predict most morbidities in our study, and correlated weakly with LIC. The serum ferritin to LIC ratio was also shown to be lower relative to that in patients with β thalassemia major.[5-6] Thus, reliance on serum ferritin to guide chelation therapy in β thalassemia intermedia may lead to delay in initiating treatment.

The main limitation of our study was the use of echocardiography instead of cardiac catheterization for the diagnosis of pulmonary hypertension which may increase the rate of false positive findings. However, our patients were mainly screened for pulmonary hypertension after presenting with exertional dyspnea with no evidence of left heart disease. Moreover, echocardiography is still the modality of choice used in many studies on thalassemia and sickle cell anemia, both because of the invasiveness and cost of cardiac catheterization, and because of the reports of good relationships between Doppler estimates and invasive measurements of pulmonary arterial pressure at baseline and after treatment.[45-47] Moreover, we could not directly assess liver pathology in this study and relied on alanine aminotransferase levels to reflect liver abnormality. Although serum ferritin correlated better than LIC with liver enzyme levels in this study, associations with fibrosis and carcinoma through biopsy data should be evaluated. In our study, both R2 and R2* MRI techniques were used for the measurement of LIC. In a study of 384 observations in more than 200 patients, LIC measurements using R2* MRI were unbiased with respect to those using R2 MRI.[48]

In conclusion, our study demonstrated that elevated LIC in patients with β thalassemia intermedia is associated with significant vascular, endocrine, and bone morbidity. Further studies are needed to confirm the causative role of iron toxicity and evaluate the role of iron chelation therapy in preventing or reversing morbidity in β thalassemia intermedia. This may require collaborative efforts between international centers to ensure that a large sample from this heterogeneous population is evaluated.

Authorship and Disclosures

The information provided by the authors about contributions from persons listed as authors and in acknowledgments is available with the full text of this paper at www.haematologica.org.

Financial and other disclosures provided by the authors using the ICMJE (www.icmje.org) Uniform Format for Disclosure of Competing Interests are also available at www.haematologica.org.

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SUPPLEMENTARY APPENDIX

Elevated liver iron concentration is a marker of increased morbidity in patients with β thalassemia intermedia

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Online Supplementary Table S1. Bivariate correlations between study parameters and morbidities (Part 1).

Variable	Extramedullary hematopoiesis		Leg ulcers		Morbidity Thrombosis		Pulmonary hypertension		Heart failure	
	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes
LIC (mg Fe/g dw)[a]	8.6 (6.7)	7.8 (6.6)	7.7 (6.3)	10.4 (7.5)*	7.3 (6)	11.5 (7.7)**	7 (6.1)	11.1 (7.1)***	6.1 (8.8)	12.3 (15.8)
Age (years)[a]	34.9 (12.2)	36.1 (13.7)	34.6 (12.3)	37 (13.3)	34.2 (12.6)†	38 (12.1)	33 (13.1)	39.7 (10.2)***	35 (18)	45 (9)*
Gender[b]										
Female (n=96)	71 (74)	25 (26)	70 (72.9)	26 (27.1)	64 (66.7)	32 (33.3)	63 (65.6)	33 (34.4)	90 (93.7)	6 (6.3)
Male (n=72)	54 (75)	18 (25)	57 (73.7)	15 (26.3)	60 (83.3)	12 (16.7)*	49 (68.1)	23 (31.9)	69 (95.8)	3 (4.2)
Splenectomized[b]										
No (n=47)	38 (80.9)	9 (19.1)	39 (83)	8 (17)	43 (91.5)	4 (8.5)	39 (83)	8 (17)	44 (93.6)	3 (6.4)
Yes (n=121)	87 (71.9)	34 (28.1)	88 (72.7)	33 (27.3)	81 (66.9)	40 (33.1)**	73 (60.3)	48 (39.7)**	115 (95)	6 (5)
Transfusion[b]										
None (n=44)	37 (84.1)	7 (15.9)	42 (95.5)	2 (4.5)	39 (88.6)	5 (11.4)	35 (79.5)	9 (20.5)	43 (97.7)	1 (2.3)
Occasional (n=80)	57 (71.2)	23 (28.8)	55 (68.7)	25 (31.3)	55 (68.7)	25 (31.3)	53 (66.2)	27 (33.8)	76 (95)	4 (5)
Regular (n=44)	31 (70.5)	13 (29.5)	30 (31.8)	14 (31.8)**	30 (68.2)	14 (31.8)*	24 (54.5)	20 (45.5)*	40 (90.9)	4 (9.1)
Total hemoglobin (g/dL)[a]	8.8 (1.6)	8.8 (1.6)	8.9 (1.6)	8.4 (1.5)†	8.9 (1.7)	8.3 (1.4)*	8.8 (1.8)	8.7 (1.3)	8.6 (1.9)	9.2 (2.4)
Fetal hemoglobin (%)[a]	42.7 (31.7)	49.4 (29.1)	42.8 (32.5)	50.1 (25.7)	43.3 (30.9)	51.6 (30.9)	40.6 (31.4)	44.8 (28.6)*	37.1 (57.9)	55.5 (37)
Platelet count ($x10^9$/L)[a]	591.9 (341.4)	657.8 (358)	589.8 (341.9)	667.1 (355.9)	582.2 (362.5)	684.5 (286.1)	594.1 (361.3)	641.5 (312.4)	613 (520)	351 (267)*
NRBC count ($x10^9$/L)[c]	349.5 (4745)	865 (16385)	359 (4751)	857 (16380)	325 (7947)	900 (18832)	353 (907)	6680 (25087)**	411 (12300)	567 (8860)
Serum ferritin (ng/mL)[c]	807.5 (919)	746.5 (1104)*	747 (751)	1095 (1142)†	740.3 (754.3)	1106.3 (916.8)	641.3 (777)	1006.5 (902.3)**	765 (855)	1403 (1297)†

*LIC: liver iron concentration; dw: dry weight; NRBC: nucleated red blood cell. Data presented as [a]mean (SD) [except for heart failure and diabetes mellitus for which the median (IQR) was used], [b]n (%), or [c]median (IQR). All correlations evaluated by the independent samples t-test and the χ^2 test except for heart failure and diabetes mellitus for which correlations were evaluated by the Mann-Whitney U test and the Fisher's exact test. †P<0.1; *P<0.05; **P<0.01; ***P<0.001.*

Online Supplementary Table S1. Bivariate correlations between study parameters and morbidities (Part 2).

Variable	Morbidity: Abnormal liver function		Diabetes mellitus		Hypothyrodism		Osteoporosis		Hypogonadism	
	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes
LIC (mg Fe/g dw)[a]	6.7 (5.2)	11.9 (8)***	6.1 (9.5)	6.5 (7.4)	7.8 (6.2)	11.1 (8.1)*	6.4 (6)	10.8 (6.7)***	7.6 (6.1)	12.3 (8)***
Age (years)[a]	32 (12.6)	42 (9.6)***	35 (19)	42 (9)	35.1 (13.1)	35.6 (10.1)	32.2 (13)	38.8 (11)***	35.5 (13.1)	33.8 (9.8)
Gender[b]										
Female (n=96)	67 (69.8)	29 (30.2)	91 (94.8)	5 (5.2)	78 (81.2)	18 (18.8)	51 (53.1)	45 (46.9)	77 (80.2)	19 (19.8)
Male (n=72)	47 (65.3)	25 (34.7)	71 (98.6)	1 (1.4)	60 (83.3)	12 (16.7)	40 (55.6)	32 (44.4)	63 (87.5)	9 (12.5)
Splenectomized[b]										
No (n=47)	39 (83)	8 (17)	46 (97.9)	1 (2.1)	43 (91.5)	4 (8.5)	36 (76.6)	11 (23.4)	45 (95.7)	2 (4.3)
Yes (n=121)	75 (62)	46 (38)**	116 (95.9)	5 (4.1)	95 (78.5)	26 (21.5)*	55 (45.5)	66 (54.5)***	95 (78.5)	26 (21.5)**
Transfusion[b]										
None (n=44)	39 (88.6)	5 (11.4)	44 (100)	0 (0)	43 (97.7)	1 (2.3)	36 (81.8)	8 (18.2)	42 (95.5)	2 (4.5)
Occasional (n=80)	52 (65)	28 (35)	78 (97.5)	2 (2.5)	64 (80)	16 (20)	37 (46.2)	43 (53.8)	67 (83.7)	13 (16.3)
Regular (n=44)	23 (52.3)	21 (47.7)**	40 (90.9)	4 (9.1)†	31 (70.5)	13 (29.5)**	18 (40.9)	26 (59.1)***	31 (70.5)	13 (29.5)**
Total hemoglobin (g/dL)[a]	8.8 (1.7)	8.7 (1.3)	8.7 (2)	8.7 (1.3)	8.8 (1.7)	8.6 (1.3)	8.9 (1.7)	8.6 (1.5)	8.8 (1.6)	8.8 (1.5)
Fetal hemoglobin (%)[a]	38.3 (31.3)	35.4 (31)	40 (58.9)	35 (44.2)	42.8 (31.2)	53.5 (29.5)	40 (30.1)	50.1 (30.9)†	42.9 (31.5)	54.5 (26.7)
Platelet count (x10⁹/L)[a]	602.8 (370.2)	625 (283.9)	608 (542.5)	520 (295)	611.5 (360.2)	600 (279.4)	571.1 (369.4)	652.9 (314.1)	588 (348.9)	713.3 (317.1)†
NRBC count (x10⁹/L)[c]	325 (817)	11130 (25004)**	395 (9170)	9030 (16830)	342 (9073)	900 (13786)	310 (1668)	548.5 (14295)	400 (12300)	570 (8980)
Serum ferritin (ng/mL)[c]	617.5 (670)	1465 (1265)***	773 (982)	831 (548)	749.3 (961)	999.5 (850)	596.5 (724)	1019 (973.5)*	747.8 (889)	978 (1004)

*LIC: liver iron concentration; dw: dry weight; Hb; NRBC, nucleated red blood cell. Data presented as [a]mean (SD) [except for heart failure and diabetes mellitus for which the median (IQR) was used], [b]n (%), or [c]median (IQR). All correlations evaluated by the independent samples t-test and the χ^2 test except for heart failure and diabetes mellitus where correlations were evaluated by the Mann-Whitney U test and the Fisher's exact test. †,P<0.1; *,P<0.05; **P<0.01; ***P<0.001.*

Online Supplementary Table S2. Multivariate logistic regression to determine independent risk factors for morbidities (Part 1).

Variable	Morbidity: Extramedullary hematopoiesis		Leg ulcers		Thrombosis		Pulmonary hypertension		Heart failure	
	AOR	95% CI	AOR	95% CI	AOR	95% CI	AOR	95% CI	AOR	95% CI
LIC, 1 mg Fe/g dw increase	1.01	0.94-1.08	1.04	0.99-1.10	1.12	1.05-1.20	1.08	1.02-1.14	1.06	0.97-1.16
Age, 1 year increase	--	--	--	--	1.04	1.01-1.07	1.05	1.02-1.09	--	--
Gender										
Female	1.00	Referent	1.00	Referent	1.00	Referent	1.00	Referent	1.00	Referent
Male	--	--	--	--	0.35	0.16-0.81	--	--	--	--
Splenectomized										
No	1.00	Referent	1.00	Referent	1.00	Referent	1.00	Referent	1.00	Referent
Yes	--	--	--	--	5.82	1.77-19.19	2.99	1.20-7.44	--	--
Transfusion										
No	1.00	Referent	1.00	Referent	1.00	Referent	1.00	Referent	1.00	Referent
Yes	1.81	1.07-3.08	2.01	1.17-3.47	--	--	--	--	--	--
Total Hb, 1 g/dL increase	--	--	--	--	--	--	--	--	--	--
Fetal Hb, 1% increase	--	--	--	--	--	--	--	--	--	--
Platelet count, x10⁹/L increase	--	--	--	--	--	--	--	--	0.992	0.986-0.998
NRBC count, x10⁹/L increase	--	--	--	--	--	--	--	--	--	--
Ferritin, 100 ng/mL increase	--	--	--	--	--	--	--	--	--	--

AOR: adjusted odds ratio; CI: confidence interval; LIC: liver iron concentration; dw: dry weight; Hb: hemoglobin; NRBC: nucleated red blood cell. The model was built using forward-stepwise selection. P≤0.1 was used as the criterion for inclusion. Multicolinearity was absent in the model as demonstrated by a variation inflation factor ≤3 (acceptable limit up to 10).

Online Supplementary Table S2. Multivariate logistic regression to determine independent risk factors for morbidities (Part 2).

Variable	Morbidity									
	Abnormal liver function		Diabetes mellitus		Hypothyroidism		Osteoporosis		Hypogonadism	
	AOR	95% CI	AOR	95% CI	AOR	95% CI	AOR	95% CI	AOR	95% CI
LIC, 1 mg Fe/g dw increase	1.05	0.97-1.13	0.92	0.78-1.07	1.05	1.01-1.11	1.10	1.04-1.16	1.10	1.03-1.16
Age, 1 year increase	1.09	1.05-1.14	--	--	--	--	1.05	1.02-1.08	--	--
Gender										
Female	1.00	Referent	1.00	Referent	1.00	Referent	1.00	Referent	1.00	Referent
Male	--	--	--	--	--	--	--	--	--	--
Splenectomized										
No	1.00	Referent	1.00	Referent	1.00	Referent	1.00	Referent	1.00	Referent
Yes	--	--	--	--	--	--	3.67	1.57-8.55	--	--
Transfusion										
No	1.00	Referent	1.00	Referent	1.00	Referent	1.00	Referent	1.00	Referent
Yes	--	--	5.49	1.21-24.85	2.54	1.34-4.84	--	--	2.97	1.39-6.35
Total Hb, 1 g/dL increase	--	--	--	--	--	--	--	--	--	--
Fetal Hb, 1% increase	--	--	--	--	--	--	--	--	--	--
Platelet count, x10^9/L increase	--	--	--	--	--	--	--	--	--	--
NRBC count, x10^6/L increase	--	--	--	--	--	--	--	--	--	--
Ferritin, 100 ng/mL increase	1.14	1.06-1.23	--	--	--	--	--	--	--	--

AOR: adjusted odds ratio; CI: confidence interval; LIC: liver iron concentration; dw: dry weight; Hb: hemoglobin; NRBC: nucleated red blood cell. The model was built using forward-stepwise selection. P≤0.1 was used as the criterion for inclusion. Multicolinearity was absent in the model as demonstrated by a variation inflation factor ≤3 (acceptable limit up to 10).

Magnetic Resonance Evaluation Of Hepatic And Myocardial Iron Deposition In Transfusion-independent Thalassemia Intermedia Compared To Regularly Transfused Thalassemia Major Patients

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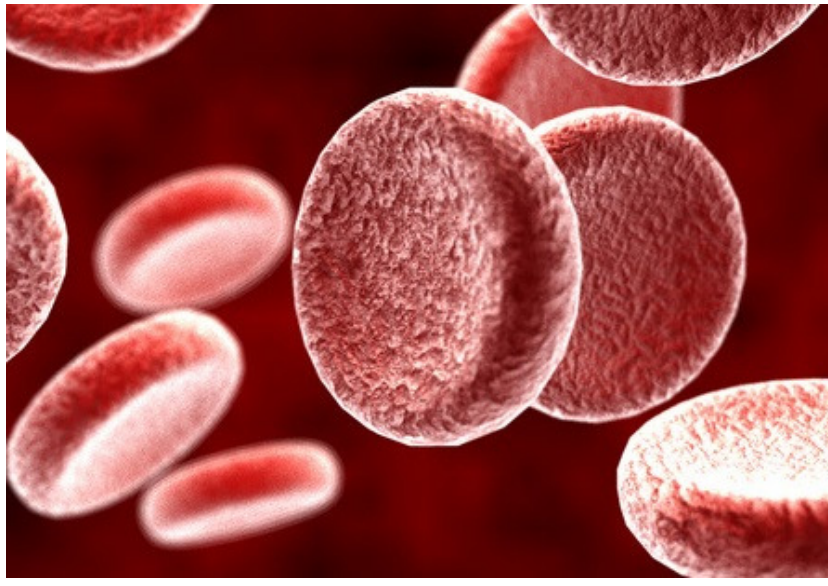

Magnetic resonance evaluation of hepatic and myocardial iron deposition in transfusion-independent thalassemia intermedia compared to regularly transfused thalassemia major patients

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Extremely diverse phenotypes exist within the homozygous and compound heterozygote states for β-thalassemia. The terms thalassemia major (TM) and intermedia (TI) lack specific molecular correlates, but encompass a wide spectrum of clinical and laboratory abnormalities [1]. At the severe end of the spectrum are patients whose clinical course is characterized by profound anemia, who present to medical attention in the first year of life, and who subsequently require regular transfusions for survival, the condition known as TM. But many patients with inheritance of two mutant beta alleles have a milder illness, with a broad range of severity including, at least in early childhood, a virtually asymptomatic state. Patients in this group who present to medical attention in later childhood and remain largely transfusion free are said to have TI [1]. The pathophysiology, clinical consequences, and treatment of iron overload in regularly transfused patients with TM have been extensively studied; however, in transfusion-independent patients with TI data remain limited. Recent advances in the assessment of organ-specific iron deposition using magnetic resonance imaging (MRI) are promising and could potentially aid understanding the pathophysiology of iron in patients with TI.

We evaluated 19 TI and 19 age- and sex-matched TM patients attending to the Chronic Care Center, Hazmieh, Lebanon. All patients were splenectomized. None of the patients had clinical signs of heart failure according to Framingham's modified criteria [2], and none had any history or echocardiographic evidence of cardiopulmonary disease including pulmonary hypertension. Moreover, none of the patients had a history of hepatitis B or C infection (by HB_s Ag and HCV RNA-PCR testing, respectively) or abnormal liver function (defined as alanine transaminase serum

TABLE I. Comparison of the Studied Parameters Between Thalassemia Intermedia and Major Patients (Independent Samples *t*-Test)

Parameter	Thalassemia intermedia, $n = 19$	Thalassemia major, $n = 19$	*P*-value
Mean age ± SD, years (range)	32.8 ± 7.9 (18–51)	33.0 ± 7.4 (17–49)	0.861
Male/Female	11/8	11/8	–
Mean Hb ± SD, g/dl (range)	8.9 ± 2.3 (4.9–13.1)	9.9 ± 1.6 (7.1–12.2)	0.241
Mean SF ± SD, ng/ml (range)	1316.8 ± 652.3 (460–3,157)	3723.8 ± 2568.8 (827–10,214)	0.001
Mean LIC ± SD, mg Fe/g dw (range)	15.0 ± 7.4 (3.4–32.1)	15.7 ± 9.9 (1.7–32.6)	0.095
Mean cardiac T2* ± SD, msec (range)	47.3 ± 7.1 (35.0–66.9)	21.5 ± 15.2 (5.1–50.7)	<0.001

Hb, hemoglobin; SF, serum ferritin; LIC, liver iron concentration; dw, dry weight.

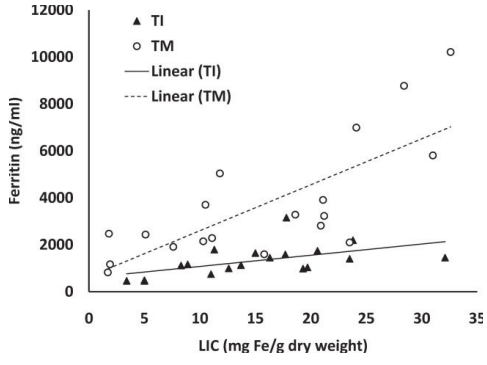

Figure 1. Linear regression analysis of serum ferritin (SF) versus liver iron concentration (LIC) in both thalassemia intermedia (TI) and major (TM) patients. In TI: SF = 583 + 42.6 × LIC; $R^2 = 0.43$; $P = 0.003$; standard error for the intercept is 202 and for the slope is 12.2 (there was one outlier excluded). In TM: SF = 642 + 196 × LIC; $R^2 = 0.57$; $P = 0.0002$; standard error for the intercept is 765 and for the slope is 41.5 (no outliers). The 95% confidence intervals for slope TI are [17.0–68.2] and for slope TM are [109–283]. As the error bars do not overlap, the slope differences are statistically significant. There are no differences in the intercepts.

level > 50 IU/l). All TI patients were transfusion and iron chelation naive, whereas all TM patients were regularly transfused (every 2–3 weeks) and iron chelated with desferrioxamine (started before the age of 7 years, in a daily dose of 30–50 mg/kg, given 5–6 times weekly). For all patients, blood samples were obtained for the assessment of hemoglobin (Hb) (pretransfusion in case of TM) and steady-state serum ferritin (SF) levels. Direct determination of liver iron concentration (LIC) was performed by R2 MRI using established methodology [3]. This technique has been widely validated in multicenter trials and independently verified [4]. Cardiac iron levels were measured by MRI T2*. Patients were scanned with MRI 1.5 T Magnetom Avanto Siemens using a multiecho breath-hold sequence [echo times (TE) 5·6–17·6 msec] as described by Wood [4]. In this study, cardiac T2* > 20 msec was considered normal. The study received institutional review board approval, and all patients signed written informed consents.

Despite having comparable LICs, TI patients showed a statistically significant lower SF and higher cardiac T2* values (all TI patients had normal cardiac T2*) than patients with TM (Table I). Although the predictive power of SF for LIC was low in both TI and TM patients, SF had a statistically significant steeper (nearly fivefold) relationship with LIC in TM compared with TI patients (see Fig. 1). Extrapolating from the equations of the plots of LIC versus SF, for a similar SF, $LIC_{TI} = (4.1 \times LIC_{TM}) + 0.9$ mg Fe/g dry weight. Cardiac R2* (1000/T2*) values did not correlate with SF or LIC in both TM and TI patients.

Our study demonstrates that in transfusion-independent and nonchelated TI patients, LIC may be comparable to that of regularly transfused TM patients and surpass the recommended levels, highlighting that many patients with TI will be at risk of significant iron-related morbidity and mortality without the introduction of adequate chelation therapy. Data from this study show that isolated SF measurement has sufficient variability and hence poor predictability of LIC in both TI and TM patients. However, it appears particularly easy to underappreciate the severity of underlying iron overload when using SF in TI patients because the relationship between SF and LIC is so weak relative to background fluctuations in SF. Studies on patients with TI have consistently shown that SF levels are significantly lower than in patients with TM despite comparable levels of liver iron, which has significant implications on patient management and follow-up [5–7]. In TI, the combination of ineffective erythropoiesis and chronic anemia/hypoxia results in hepcidin suppression, increased intestinal iron absorption, and increased release of recycled iron from the reticuloendothelial (RE) system. This results in depletion of macrophage iron, relatively low levels of SF, and preferential portal and hepatocyte iron loading [5,7,8]. The situation in TI is similar to that seen in patients with hereditary hemochromatosis syndromes, which is characterized by impaired hepcidin production. By contrast, in transfused TM patients, iron is preferentially distributed to the macrophages in the RE system including the liver, stimulating ferritin synthesis and its release to the circulation, leading to high SF levels [7–9].

Despite significant hepatic siderosis, none of the TI patients showed evidence of cardiac iron loading in contrast to patients with TM. These data, which show cardiac T2* within the normal range in all patients, support other recent findings in which cardiac T2* was >20 msec in never or minimally transfused TI patients despite elevated LIC [10–12]. However, absence of evidence does not necessarily mean evidence of absence. The number of TI patients recruited in our study and previous reports remains small. In fact, cardiac iron deposition has been documented in small subgroups of older TI patients both through MRI evaluation [13,14] and as determined by endomyocardial biopsy [15]. Thus, similar to patients with hereditary hemochromatosis, untreated TI patients would most likely develop cardiac siderosis in middle age or as senior citizens. Moreover, accumulation of toxic iron species within myocytes is not necessary to induce cardiac dysfunction, and only initial exposure to nontransferrin-bound iron may be enough to cause damage to cardiac tissue. The latter entails that even without evidence of cardiac siderosis, TI patients may still be at risk of iron-related organ dysfunction secondary to hepatic iron overload with subsequent release of toxic species, thus highlighting the need for effective iron chelation therapy in this patient population [16–18].

In fact, the relationship between cardiac T2* values and iron balance is quite complicated because the mechanisms and kinetics of cardiac iron uptake and clearance differ from the liver [19]. The lack of correlation between cardiac T2* and LIC or SF in this study is consistent with data from a previous studies on TI patients in which the authors found no correlation between cardiac T2* and SF values [10–14]. Studies on patients with TM and sickle cell disease confirm that cardiac T2* values do not correlate with SF concentration and LIC in cross-sectional analysis, while longitudinal studies continue to imply a causal relationship [19]. It was demonstrated that there is a significant latency to cardiac T2* changes, relative to liver accumulation, suggesting a long delay between poor iron control and detectable cardiac iron deposition [20,21]. Other MRI work suggests that a "critical" liver saturation is necessary to achieve positive cardiac iron balance [22]. This may explain absence of cardiac iron overload even in TI patients who had LIC > 15 mg Fe/g dry weight in this study.

In conclusion, in patients with TI who had not received previous transfusion or iron chelation therapy, we found no evidence of cardiac iron overload although hepatic iron accumulation was significant. However, patients with TI, especially the elderly, should still be screened for evidence of cardiac siderosis until further research helps to better understand if (and when) detectable cardiac iron deposition can occur in patients with TI. Data also confirm that isolated SF levels do not accurately reflect the level of hepatic iron overload in TI or TM patients and can lead to significant underappreciation of the true burden of iron overload in transfusion-independent patients with TI. Thus, recommendations for the management of patients with TI should include regular assess-

ment of LIC via biopsy or noninvasive imaging methods, with iron chelation therapy being initiated in patients with LIC levels indicating liver iron overload.

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Absence of cardiac siderosis despite hepatic iron overload in Italian patients with thalassemia intermedia: an MRI T2* study

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Abstract Cardiac involvement in patients with thalassemia intermedia (TI) is characterized by a high-output state and pulmonary hypertension, with systolic left ventricle function usually being preserved. Myocardial iron overload in patients with TI has not been extensively studied. We conducted a cross-sectional study of 49 Italian patients with TI. Patient charts were reviewed and data collected for transfusion and iron chelation history, status of the spleen, and comorbid illnesses or infections. Blood samples were obtained for assessment of hemoglobin, serum ferritin, and liver enzyme levels. Doppler echocardiography was done for all patients. Cardiac and hepatic iron levels were measured by magnetic resonance imaging T2*. The mean age was 40.5±8.3 years, with a male to female ratio of 29:20. A total of 34 (69.4%) patients were splenectomized, and four patients had evidence of hepatitis C infection. Around 45% of patients were transfusion naïve while the rest received infrequent (47%) or regular (8%) transfusions. A total of 31 (63.3%) patients were maintained on iron chelation therapy. None of the patients had evidence of heart failure. Mean serum ferritin and liver iron concentration were 1,060.2 ng/ml and 8.2 mg Fe per gram dry weight, respectively. None of the patients had evidence of cardiac iron overload (mean cardiac T2*=38.7±11.0 ms). There were no statistically significant correlations between cardiac T2* values and liver iron concentration, serum ferritin, or any patient, disease, or treatment-related parameters. Patients with TI show absence of cardiac iron overload even if hepatic iron accumulation is significant.

Keywords Thalassemia intermedia · Iron overload · Heart · Liver · Magnetic resonance imaging

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Introduction

In contrast to thalassemia major (TM), patients with thalassemia intermedia (TI) often have mild anemia that generally does not require regular blood transfusion therapy until later in life. However, TI patients remain at risk of the clinical sequelae of iron overload, primarily due to increased intestinal iron absorption and ineffective erythropoiesis [1]. Heart disease is the primary cause of death in TM, with myocardial iron loading observed in approximately two thirds of patients receiving iron chelation therapy [2]. In TI, cardiac involvement is mainly characterized by a high-output state and pulmonary hypertension, with systolic left ventricle function usually being preserved

[3, 4]. Myocardial iron overload in patients with TI has not been extensively studied; however, a recent report has shown no evidence of cardiac iron in a small group (n=20) of patients [5]. The current study aims to further evaluate cardiac iron overload in a larger group of TI patients and explore its correlation with liver iron concentration (LIC) and serum ferritin (SF) levels among other patient and disease characteristics.

Materials and methods

This was a cross-sectional study of patients with TI treated at the Centro Anemie Congenite, Ospedale Maggiore Policlinico, IRCCS, University of Milan, Milan, Italy. All patients were diagnosed with TI based on criteria previously described [6, 7]. This study recruited adult patients only to avoid the need for sedation to complete the magnetic resonance imaging (MRI) in children. Out of a cohort of 124 patients registered at the center, 49 adults (≥18 years) were identified. Patient charts were reviewed for data on patient's demographics (age and gender), splenectomy status, presence of comorbid illnesses or infections, and history of transfusion and iron chelation therapy. For transfusion status, patients were divided as follows: regularly transfused (two to four times per year), infrequently transfused (few transfusions received in the past), and transfusion naïve. Iron chelation therapy had to be administered for at least 1 year or else the patient was considered nonchelated. Written informed consent was provided by all patients, and approval was obtained from the ethical committee at the center.

Laboratory tests

Blood samples were obtained for assessment of pretransfusion hemoglobin (Hb), steady-state SF, and alanine transaminase (ALT) levels.

Echocardiography

Doppler echocardiography to assess left ventricular ejection fraction (LVEF) was done.

Magnetic resonance imaging

Cardiac iron levels were measured by MRI T2*. Patients were scanned with MRI 1.5 T Magnetom Avanto Siemens using a multiecho breath-hold sequence (echo times (TE) 2.58–18·9 ms) as described by Wood [8]. In this study, cardiac T2* >20 ms was considered normal. LIC was calculated from liver T2* images (TE 0.99–16.50 ms) according to the formula $[1/(\mathrm{T2}^*/1{,}000)] \times 0.0254 + 0.202]$ [9]. All T2* images were analyzed using postprocessing software (CMR Tools, Imperial College, London).

Statistical analysis

Data are expressed as means ± standard deviation (SD) or percentages where appropriate. All univariate comparisons between iron overload parameters (SF, LIC, and cardiac T2*) and continuous variables (age, Hb, LVEF, ALT) were evaluated using Spearman's (r_s) correlation coefficients. Comparisons with categorical variables (gender, splenectomy, transfusion, and chelation status) were evaluated using the independent samples t test and analysis of variance (ANOVA) test. A multivariate stepwise regression analysis was done to determine significant correlations. Linear regression analysis was performed to study correlations between SF, LIC, ALT, and cardiac R2* values ($\mathrm{R2}^* = 1{,}000/\mathrm{T2}^*$). All P values are two sided with the level of significance set at <0.05.

Results

Data from 49 patients were included in this analysis (Table 1). A total of four patients had evidence of hepatitis C virus (HCV) infection confirmed by polymerase chain reaction RNA testing. Among patients who received iron chelation therapy, 29 (93.5%) were maintained on subcutaneous deferoxamine regimens (20–60 mg/kg for 12 h a day, 3 days a week) while two (6.5%) received the oral iron chelator deferasirox (15–20 mg/kg/day). No patient had evidence of heart failure by modified Framingham criteria [10].

The mean ± SD of SF levels and LIC were 1,060.2± 1,090.3 ng/ml (range: 165–6,361 ng/ml) and 8.2±6.0 mg Fe per gram dry weight (dw; range: 1.1–26.4 mg Fe per gram dw),

Table 1 Patients' characteristics

Parameter	Value
Mean age ± SD, years (range)	40.5±8.3 (23–64)
Male/Female	29/20
Splenectomized, *n* (%)	34 (69.4)
Transfusion status, *n* (%)	
Naïve	22 (44.9)
Infrequent	23 (46.9)
Regular	4 (8.2)
Chelation received, *n* (%)	31 (63.3)
Mean ALT ± SD, IU/L (range)	36.2±23.5 (9–91)
Mean Hb ± SD, g/dL (range)	8.8±1.5 (5.3–13.1)
Mean LVEF ± SD, % (range)	65.8±4.9 (57–79)

ALT alanine transaminase, *Hb* hemoglobin, *LVEF* left ventricular ejection fraction

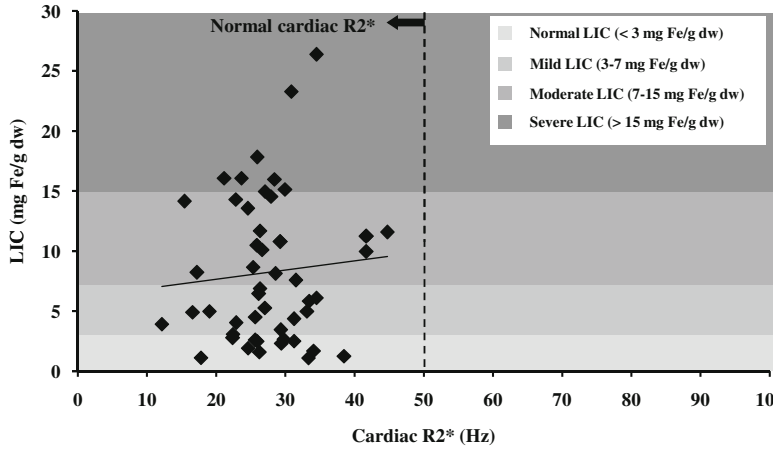

Fig. 1 Correlation between liver iron concentration (*LIC*) and cardiac R2* values

respectively. None of the patients had evidence of cardiac iron overload (mean cardiac T2* = 38.7 ± 11.0 ms; range: 22.4–82.4 ms). On linear regression analysis, there were no statistically significant correlations between cardiac R2* and LIC (R^2=0.007; P=0.569; Fig. 1) or SF (R^2=0.003; P=0.716). There was a statistically significant positive correlation between SF and LIC but with poor linearity (R^2=0.435; P=<0.001).

Table 2 Univariate analysis showing correlations between study variables and iron overload parameters

Variable	SF (ng/mL)	LIC (mg Fe per gram dry weight)	Cardiac T2* (ms)
Categorical[a]			
Gender			
Male (n=29)	1,132.9±1,285.9	7.6±5.8	40.8±11.8
Female (n=20)	943±685.3	9.2±6.4	35.6±9.3
P value	0.567	0.375	0.107
Splenectomy			
Yes (n=34)	1,264.1±1235.4	8.5±5.9	40.2±12.5
No (n=15)	579.4±308.2	7.7±6.6	35.3±5.9
P value	0.048	0.691	0.157
Transfusion status			
Naïve (n=22)	642.3±685.3	6.3±5.0	37.4±5.9
Infrequent (n=23)	1,341.7±1,265.0	10.0±6.7	41.1±14.5
Regular (n=4)	1,826.3±1191.0	9.1±5.8	32.4±6.7
P value	0.044	0.115	0.270
Iron chelation			
Yes (n=31)	1313.8±1233.8	9.1±6.5	38.9±11.8
No (n=18)	612.5±570.0	7.7±6.6	38.4±10.0
P value	0.033	0.220	0.895
Continuous[b]			
Age (years)	0.2	−0.023	−0.196
P value	0.178	0.878	0.177
Hb (g/dL)	−0.103	−0.129	0.003
P value	0.489	0.377	0.986
ALT[c] (IU/L)	0.600	0.421	−0.039
P value	<0.001	0.004	0.801
LVEF (%)	0.109	−0.129	0.003
P value	0.464	0.377	0.986

SF serum ferritin, *LIC* liver iron concentration, *Hb* hemoglobin, *ALT* alanine transaminase, *LVEF* left ventricular ejection fraction

[a] Statistical correlation evaluated by the independent samples t test and ANOVA test; data presented as mean ± SD

[b] Statistical correlation evaluated by Spearman's correlation; data presented as Spearman's correlation coefficient (r_s)

[c] Excluding the four patients with hepatitis C infection

SF levels were significantly higher in splenectomized, regularly transfused, and chelated patients and correlated positively with ALT levels (Table 2). On multivariate analysis, only ALT levels retained correlation with SF levels ($P<0.001$). Similarly, LIC values were only significantly positively correlated with ALT levels (Table 2). However, ALT levels did not rise linearly with SF ($R^2=0.371$) or LIC ($R^2=0.127$). There was no statistically significant correlation between cardiac T2* values and any of the study parameters (Table 2).

Of note, there was no statistically significant differences between HCV-positive and HCV-negative patients with regards to SF ($P=522$), LIC ($P=603$), or cardiac T2* ($P=534$); however, HCV-positive patients had higher mean ALT levels than HCV-negative patients (58.8 vs. 34.1 IU/L; $P=0.043$).

Discussion

Data from this cross-sectional study show absence of cardiac iron overload in patients with TI, despite significant hepatic iron accumulation (>7 mg Fe per gram dw), thus providing additional insight to aid our understanding of iron deposition in this patient group.

These data, which show cardiac T2* within the normal range in all patients, support other recent findings in which cardiac T2* was >20 ms in 20 never or minimally transfused TI patients despite elevated LIC [5]. Furthermore, they are consistent with studies showing that patients with TI are generally less prone to the cardiac iron overload associated with morbidity and mortality than patients with TM [4]. However, they contrast with other reports indicating moderate cardiac iron overload in subgroups of TI patients [11, 12]. The varying results might be explained by differences in baseline characteristics (e.g., previous transfusion history and copathology) between patient populations.

In TI, the combination of ineffective erythropoiesis and chronic anemia/hypoxia results in hepcidin suppression, increased intestinal iron absorption, and increased release of recycled iron from the reticuloendothelial (RE) system (macrophages) to the hepatocytes [1, 13, 14]. This explains the relatively low levels of SF yet high LIC (regardless of transfusion history) and confirms that SF underestimates iron burden in TI with poor correlation with LIC [15, 16]. By contrast, in transfused TM patients, iron is preferentially distributed to the RE system, stimulating SF synthesis and its release to the circulation [15]. The effect of this disparity in the pathophysiology of iron overload on cardiac sparing in TI patients and the probability of eventual cardiac iron overload with continuous transfusion therapy merits consideration in long-term follow-up studies.

In fact, the relationship between cardiac T2* values and iron balance is quite complicated because the mechanisms and kinetics of cardiac iron uptake and clearance differ from the liver [17, 18]. The lack of correlation between cardiac T2* and LIC or SF in the current study is consistent with data from a previous study on TI patients in which the authors found no correlation between cardiac T2* and SF values [5]. Data from animal models of iron overload in TI also indicate early accumulation of iron in the liver, with accumulation of iron in the heart occurring over longer periods [19]. Studies on patients with TM and sickle cell disease (SCD) confirm that cardiac T2* values do not correlate with SF concentration and LIC in cross-sectional analysis, while longitudinal studies continue to imply a causal relationship [12, 17, 18, 20–22]. Wood and Noetzli [17, 20] demonstrated a significant latency to cardiac T2* changes, relative to liver accumulation, suggesting a long delay between poor iron control and detectable cardiac iron deposition. Other MRI work suggests that a “critical” liver saturation is necessary to achieve positive cardiac iron balance [23]. This may explain absence of cardiac iron overload even in patients who had LIC >15 mg Fe per gram dw in this study.

In conclusion, in patients with TI who had or had not received previous transfusion therapy, we found no evidence of cardiac iron overload although hepatic iron accumulation was significant. Further research is needed to better understand if (and when) detectable cardiac iron deposition can occur in patients with TI. Data also confirm that SF levels do not accurately reflect the level of iron overload. Thus, recommendations for the management of patients with TI should include regular assessment of LIC via biopsy or noninvasive imaging methods, with iron chelation therapy being initiated in patients with LIC levels indicating liver iron overload.

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Glomerular Hyperfiltration And Proteinuria In Transfusion-independent Patients With β-thalassemia Intermedia

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ABSTRACT

Background: Renal manifestations have been described in β-thalassemia major and were attributed to transfusional iron overload and chelation therapy. Patients with the milder phenotype, β-thalassemia intermedia (TI), remain largely transfusion- and iron chelation-independent while enduring a chronic hemolytic anemia and primary iron overload. Data on renal function in patients with TI are lacking. **Methods:** In this cross-sectional study of 50 TI patients, we evaluated the association of estimated glomerular filtration rate (eGFR) and urinary protein to creatinine (UPr/UCr) ratio with relevant patient, disease, and laboratory indices. **Results:** The median age of patients was 28 years (44% males). The eGFR was >90 ml/min/1.73 m^2 in all patients with a median value of 142.3 ml/min/1.73 m^2. The median UPr/UCr ratio was 213.2 mg/g. There was a negative correlation between age and eGFR, while the UPr/UCr ratio correlated positively with markers of anemia, hemolysis, and iron overload. A total of 24 (48%) patients had evidence of glomerular hyperfiltration while seven (14%) had proteinuria (UPr/UCr ratio >500 mg/g). Patients with proteinuria were characterized by elevated liver iron concentration (>7 mg Fe/g dry weight), non-transferrin-bound iron levels, and nucleated red blood cell counts. **Conclusions:** A considerable proportion of TI patients show evidence of abnormally elevated eGFR, with a

declining trend towards advancing age. The occurrence of proteinuria is associated with anemia, hemolysis, and iron toxicity.

Keywords: anemia; glomerular filtration rate; iron overload; proteinuria thalassemia; tubule cells.

INTRODUCTION

The study of renal manifestations in patients with β-thalassemia has primarily revolved around the potential nephrotoxic effects of iron chelators used to treat transfusional iron overload in β-thalassemia major (TM) patients [1]. Data in transfusion-independent patients with β-thalassemia intermedia (TI) are scarce [2-4]. Three main pathophysiologic mechanisms dominate the disease process in this latter population: chronic anemia and hypoxia, intra- and extra-vascular hemolysis, and primary iron overload due to ineffective erythropoiesis and secondary increase in intestinal iron absorption [5]. In this study we aim to investigate key renal manifestations in a cohort of transfusion-independent patients with TI, and evaluate their association with the underlying disease pathophysiology.

METHODS

This was a cross-sectional study of TI patients attending the Chronic Care Centers in Hazmieh, Lebanon. The current study utilized a completely de-identified dataset. Data were collected as part of now completed clinical studies, and which were approved by the Institutional Review Board. All patients had signed an informed consent form for participating in the original studies in accordance with the Declaration of Helsinki. An age of diagnosis beyond two years, hemoglobin values maintained between 7 and 9 g/dl without

the need for regular transfusional regimen, with or without splenomegaly, were the main criteria to define the TI phenotype on presentation [6]. After excluding patients who were eventually placed on regular transfusion regimens or iron chelation therapy for disease deterioration as well as patients receiving fetal hemoglobin inducers, 50 patients were available for analysis. Some of the patients received occasional transfusions prior to surgery, during infection or pregnancy (see Table 1). By review of genetic records, none of the patients had co-inheritance of α thalassemia [α^{+} ($-\alpha^{3.7}$ and $-\alpha^{4.2}$) or α^{0} ($--^{Med}$ and $--^{SEA}$)] or determinants associated with increased γ chain production [*Xmn*-I +/+ genotype at position –158 of *HβG2*]. Moreover, none of the patients had hemoglobin S, C, E/β or δβ thalassemia. Medical charts were reviewed to retrieve data on demographics (age and sex), splenectomy status, receipt of any occasional transfusions, history of hepatitis B or C or HIV infection, and history of cardiovascular disease.

Laboratory studies were performed for the measurement of serum ferritin level, fetal and total hemoglobin levels, as well as platelet and nucleated red blood cell (NRBC) counts. For liver iron concentration (LIC), direct determination of iron burden was performed with R2 magnetic resonance imaging (MRI) using established methodology, calibrated to mg of iron per g of dry weight in fresh liver biopsy specimens [7]. Measurement of non-transferrin-bound iron (NTBI) was also performed as previously

described [8]. NTBI is a low-molecular-weight form of iron that is detected in conditions of iron overload when transferrin becomes fully saturated and is unable to bind excess iron. Moreover, serum creatinine (SCr) levels were obtained. We estimated glomerular filtration rate (eGFR) using the isotope dilution mass spectrometry (IDMS)-traceable Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [9]: eGFR (ml/min/1.73 m^2) = 141 X $\min(SCr/\kappa,1)^{\alpha}$ X $\max(SCr/\kappa,1)^{-1.209}$ X 0.993^{Age} X 1.018 (if female) X 1.159 (if black) [Where SCr is in mg/dl, κ is 0.7 for females and 0.9 for males, α is –0.329 for females and –0.411 for males, min indicates the minimum of SCr/κ or 1, and max indicates the maximum of SCr/κ or 1]. For children <15 years (6 patients, median height 150.5 cm), we used the IDMS-traceable Bedside Schwartz equation [10-11]: eGFR (ml/min/1.73 m^2) = 0.413 x Height in cm/SCr. Urinary protein (UPr) and urinary creatinine (UCr) levels were also obtained to calculate the UPr/UCr ratio. UPr was measured using a turbidimetric method (COBAS, Roche Diagnostics GmbH, Mannheim, Germany) with the normal range for the assay being <140 mg/l. Accordingly, the normal UPr/UCr ratio was determined as <200 mg/g.

Statistical analysis

Descriptive statistics are presented as medians (interquartile range [IQR]), and percentages. Comparisons were made using the Mann

Whitney U test for continuous variables and the Fisher's exact test for categorical variables. Bivariate correlations were evaluated using the Spearman's correlation coefficient (r_s). Logistic regression analysis was used to determine the odds ratio (95% confidence interval [CI]) of developing outcomes according to categorized study variables. Receiver operating characteristic (ROC) curve analysis was used to determine the maximum sum of specificity and sensitivity and establish thresholds that discriminate occurrence of outcomes for continuous study variables. All p-values are two-sided with the level of significance set at 0.05.

RESULTS

Patients characteristics are described in Table 1. The median age of the 50 patients in this study was 28 (IQR: 15) years (range, 8-63 years), with 22 patients (44%) being males. The median SCr level was 0.5 (IQR: 0.2) mg/dl (range, 0.2-0.9 mg/dl) and the median eGFR was 142.3 (IQR: 24.4) ml/min/1.73 m^2 (range, 90.6-301.5 ml/min/1.73 m^2) (Figure 1). None of the patients had eGFR less than 90 ml/min/1.73 m^2. The median UPr/UCr ratio was 213.2 (IQR: 225.8) mg/g (range, 93.3-1538.9 mg/g).

A total of 24 (48%) patients had evidence of glomerular hyperfiltration relying on previously described definitions in a similarly aged, non-diabetic population (>149 and >134 ml/min/1.73

m^2 for women and men, respectively [12]). There was a significant and negative correlation between age and eGFR (Table 2A and Figure 2).

A total of 30 (60%) patients had abnormal UPr/UCr ratio (≥200 mg/g), and seven (14%) patients had a UPr/UCr ratio >500 mg/g indicating proteinuria. The UPr/UCr ratio correlated positively with serum ferritin level, NTBI level, LIC, and NRBC count; yet correlated negatively with hemoglobin level (Table 2A) (Figure 3). Splenectomized patients had a higher median UPr/UCr ratio compared with nonsplenectomized patients (Table 2B). The median eGFR and UPr/UCr ratio were comparable between both sexes and in patients with a history of occasional transfusion, pulmonary hypertension, or thromboembolic disease compared with those without (Table 2B).

The median age was significantly lower in patients with glomerular hyperfiltration than in patients without glomerular hyperfiltration (p=0.002) (Table 3). Each 1-year increase in age was associated with a 0.90 (95% CI: 0.84 to 0.97) decreased odds of having glomerular hyperfiltration. The decline in eGFR with age followed the linear formula: eGFR (ml/min/1.73 m^2) = (-1.600 x Age in years) + 189.593 (see Figure 2).

Four of the seven patients with proteinuria (UPr/UCr ratio >500 mg/g) also had glomerular hyperfiltration (Figure 4). Patients with proteinuria had a higher median LIC compared with patients with normal UPr/UCr ratio (p=0.041) (Table 4) as well as those with an abnormal UPr/UCr ratio of ≥200 to 500 mg/g (p=0.047). On ROC curve analysis, a LIC threshold of >7 mg Fe/g dry weight discriminated patients who had from those who did not have proteinuria (area under the curve: 0.76, p=0.030, sensitivity 100%, specificity 51.2%). Patients with proteinuria also had higher median NTBI levels and NRBC counts compared with patients with normal UPr/UCr ratio (Table 4).

DISCUSSION

A spectrum of renal involvement has been described in some hemoglobinopathies, most typically in sickle cell disease. Sickle cell anemia is a vasoocclusive entity that has been implicated in causing tubulomedullary lesions and dysfunction, glomerulopathy with proteinuria, and progressive kidney failure that leads to end-stage renal disease (ESRD) [13-14]. Thalassemias have not been associated with well documented renal effects.

Our study demonstrates that renal abnormalities are common in transfusion-independent patients with TI who also never received iron chelation therapy. A considerable proportion of patients showed

elevated eGFR and probable evidence of glomerular hyperfiltration and some patients had glomerular permeability abnormalities or substantial renal tubular cell damage manifesting as overt proteinuria.

There was a positive correlation between UPr/UCr ratio and elevated iron overload indices, with increasing LIC as a risk factor for the occurrence of proteinuria. This finding is in agreement with studies that attributed increased urinary excretion of several markers of proximal tubular damage to iron overload in TM patients [1, 15-16]. Reversal of these tubular defects following iron chelation therapy has also been documented [16-17]. Preliminary studies show that rats subjected to chronic iron-loading develop iron deposits that are clearly evident in glomeruli, proximal tubules, and interstitium together with signs of significant glomerulosclerosis, tubular atrophy, and interstitial fibrosis [18]. Autopsy series of patients with TM have also shown hemosiderin deposits in both the terminal portion of proximal tubules and in distal tubules [19]. Iron accumulation can result in the production of reactive oxygen species and subsequent cellular injury [20-22]. The mechanism of injury is mediated by mitochondrial stress as evident from increased cytochrome c efflux, lactate deyhdrogenase release, and reduction in adenosine triphosphate [23-24]. Oxidants also increase the glomerular basement membrane damage by increasing its susceptibility to proteolytic damage and decreasing the synthesis of

glomerular proteoglycans which are essential to its integrity [25]. The UrPr/UCr ratio also correlated negatively with total hemoglobin level in our study, which is in agreement with studies showing a good correlation between the severity of anemia and markers of tubular abnormalities in TM patients [26]. Chronic anemia and hypoxia are also associated with oxidative stress, lipid peroxidation, and functional abnormalities in tubular cells [27-28].

Although most patients had high eGFR, levels decreased with advancing age. The average decline in eGFR in the patients appeared to be greater than that expected due to physiological aging in the normal adult population [29]. The causes of high GFR and the decreased levels with increasing age are unclear. Creatinine clearance and GFR are increased in children with TM [2]. Anemia may reduce systemic vascular resistance, leading to a hyperdynamic circulation that can increase the renal plasma flow and GFR [30]. This, however, may eventually lead to stretching of the glomerular capillary wall and subsequent endothelial and epithelial injury together with transudation of macromolecules into the mesangium leading to dysfunction [31]. In the long-term, these changes may cause a progressive decline in GFR. Moreover, chronic hypoxia of tubular cells with increased metabolic demand may cause apoptosis or epithelial-mesenchymal transdifferentiation leading to the development of tubulointerstitial injury and consequent glomerular sclerosis and kidney fibrosis [1, 32]. Moreover, tubular cell damage

from heavy iron-overload allows the injured cells to release cytokines and growth factors into the interstitium that can cause tubulointerstitial scarring and glomerular sclerosis, leading to further decrease in GFR [33].

The question thus arises as to whether TI patients are at increased risk for worsening proteinuria and progressive loss of GFR over a period of years, similar to the experience with sickle cell disease [13-14]. In this regard it is noteworthy that some patients with TI develop ESRD requiring dialysis. In the total cohort of 120 patients with TI registered at the Chronic Care Center in Lebanon, we encountered six patients on chronic hemodialysis, two of whom had been proteinuric when kidney biopsy showed mesangial cell proliferation and evidence for focal/segmental glomerulosclerosis (unpublished data).

How splenectomy contributes to renal abnormalities in TI patients is unclear. Splenectomy has been associated with several complications in TI patients, especially thrombotic disease [34-35] attributed to an increase in the number of hemolyzed red blood cells with thrombogenic potential [36]. A positive correlation between NRBC counts and UPr/UCr ratio was evident in our study, with an increased likelihood of developing proteinuria at higher NRBC counts. The mechanisms of renal injury due to these prothrombotic RBCs merits further evaluation, although an association between

hemolysis and renal abnormalities in patients with sickle cell anemia has been established [13, 37-38]. Moreover, 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 NTBI, thus protecting from iron-related end-organ damage [8, 39]. High NTBI levels were associated with the occurrence of proteinuria in our cohort.

Early detection and treatment of chronic anemia/hypoxia and iron overload in TI patients may be useful to prevent progression of kidney disease. This is in line with recent evidence suggesting the benefits of regular transfusion and iron chelation therapy in TI patients [5]. Iron chelation therapy, however, has also been linked to nonprogressive increases in SCr levels, especially with the use of the novel iron chelator defersairox [1]. It may be questionable whether this would be considered an adverse event in TI patients or rather a useful defense mechanism against the potential damage of persistent glomerular hyperfiltration. Attempts to introduce screening tools that identify which patients are at highest risk for subsequent renal damage should also be considered. The BOLD MRI, a non-invasive technique that detects changes in oxygenation level, can be considered for such purpose [40].

One limitation of our study is estimation rather than direct measurement of GFR. The currently available formulas for

estimation of GFR from SCr values in children have not been validated for high GFR values. A 12-year-old boy in this report had extremely high eGFR (~300 ml/min/1.73 m^2); when we excluded the patient from further analyses results remained the same (data not shown). In adults, however, the CKD-EPI equation used herein offers a better precision of GFR measurement at higher levels than the Modification of Diet in Renal Disease (MDRD) equation. In all cases, when we conducted the same analyses using SCr instead of eGFR (while defining glomerular hyperfiltration as a SCr value of <0.4 mg/dl), results remained essentially unchanged (data not shown).

In conclusion, renal abnormalities may be a common finding in patients with TI. Close monitoring and thorough follow-up of at-risk patients are recommended. Nonetheless, longitudinal studies are necessary to confirm whether these renal abnormalities may have long-term effects and to truly establish the deleterious effects of TI on the nephron.

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Table 1. Characteristics of the 50 patients.

Parameter	**Value**
Age in years, median (IQR: range)	28 (15: 8-63)
Male, n (%)	22 (44)
Splenectomized, n (%)	39 (78)
Occasional transfusions, n (%)*	15 (30)
Hepatitis B or C or HIV, n (%)	0 (0)
Systemic hypertension, n (%)	0 (0)
Pulmonary hypertension, n (%)	28 (56)
Thromboembolic disease, n (%)	14 (28)
Heart failure, n (%)	0 (0)
Diabetes mellitus, n (%)	0 (0)
Laboratory measurements	
Hemoglobin level in g/dl, median (IQR: range)	8.2 (2.4: 4.9-13.1)
Fetal hemoglobin level in %, median (IQR: range)	33.8 (58.9: 8.7-100.0)
Platelet count $x10^9$/l, median (IQR: range)	762.5 (655.0: 135.0-1733.0)
NRBC count x $10^3/mm^3$, median (IQR: range)	313.0 (441.8: 0.0-1622.0)
Serum ferritin level in µg/l, median (IQR: range)	835.5 (1022.1: 18.0-3157.5)
LIC in mg Fe/g dry weight, median (IQR: range)	8.1 (10.6: 0.6-32.1)
NTBI level in µmol/l, median (IQR: range)	3.2 (5.5: -3.7-10)

*Prior to surgery, during infection or pregnancy.
NRBC = nucleated red blood cell; LIC = liver iron concentration; NTBI = non-transferrin-bound iron; IQR = interquartile range.

Table 2. Bivariate correlations between study parameters, eGFR, and UPr/UCr ratio.

(A)

	Age (years)	Hemoglobin level (g/dl)	Fetal hemoglobin level (%)	Platelet count ($x10^9$/l)	NRBC count ($x10^3$/l)	Serum ferritin level (µg/l)	LIC (mg Fe/g dry weight)	NTBI (µmol/l)
eGFR (ml/min/1.73 m^2)								
Spearman's correlation coefficient	-0.705	0.082	-0.040	0.217	0.199	-0.260	-0.100	0.132
p-value	<0.001	0.571	0.787	0.131	0.166	0.069	0.491	0.361
UPr/UCr ratio (mg/g)								
Spearman's correlation coefficient	0.073	-0.252	0.190	0.241	0.237	0.282	0.357	0.444
p-value	0.614	0.008	0.195	0.092	0.048	0.048	0.011	0.001

eGFR = estimated glomerular filtration rate; UPr/UCr = urinary protein/creatinine ratio; NRBC = nucleated red blood cell; LIC = liver iron concentration; NTBI = non-transferrin-bound iron.

(B)

	Sex		Splenectomized		Occasionally transfused		PHT		VTE	
	Male	**Female**	**Yes**	**No**	**Yes**	**No**	**Yes**	**No**	**Yes**	**No**
eGFR (ml/min/1.73 m^2)										
Median	142.4	142.0	142.3	134.5	140.8	142.3	141.0	150.5	133.5	145.4
(IQR)	(28.9)	(23.0)	(23.0)	(32.9)	(32.0)	(27.5)	(20.5)	(28.9)	(28.9)	(27.9)
p-value	0.769		0.393		0.597		0.287		0.054	
UPr/UCr ratio (mg/g)										
Median	208.3	223.4	240.2	158.8	240.2	211.6	189.6	240.2	220.8	213.2
(IQR)	(277.9)	(199.2)	(276.6)	(78.0)	(635.4)	(180.9)	(223.103)	(240.0)	(319.2)	(199.7)
p-value	0.953		0.011		0.204		0.113		0.940	

eGFR = estimated glomerular filtration rate; UPr/UCr = urinary protein/creatinine ratio; PHT = pulmonary hypertension; VTE = venous thromboembolism; IQR = interquartile range.

Table 3. Comparison of study parameters between patients with and without glomerular hyperfiltration.

Parameter	No glomerular hyperfiltration n = 26	Glomerular hyperfiltration* n = 24	p-value
Age in years, median (IQR)	31.5 (13.8)	18.5 (14.8)	0.002
Male, n (%)	9 (34.6)	13 (54.2)	0.254
Splenectomized, n (%)	20 (76.9)	19 (79.2)	1.000
Occasional transfusions, n (%)	9 (34.6)	6 (25)	0.545
Pulmonary hypertension, n (%)	16 (61.5)	12 (50)	0.569
Thromboembolic disease, n (%)	12 (46.2)	2 (8.3)	0.051
Hemoglobin level in g/dl, median (IQR)	8.3 (2.4)	8.2 (2.7)	0.634
Fetal hemoglobin level in %, median (IQR)	37 (57)	33.1 (59.2)	0.710
Platelet count $x10^9$/l, median (IQR)	722 (539)	872.5 (658.8)	0.281
NRBC count x $10^3/mm^3$, median (IQR)	309.0 (409.5)	313.0 (501.5)	0.690
Serum ferritin level in µg/l, median (IQR)	1145 (1155)	659.5 (684.5)	0.071
LIC in mg Fe/g dry weight, median (IQR)	9.9 (13.5)	7 (9)	0.332
NTBI level in µmol/l, median (IQR)	3.0 (6.4)	3.6 (6.0)	0.698

*eGFR >149 and >134 ml/min/1.73 m^2 for women and men, respectively.

NRBC = nucleated red blood cell; LIC = liver iron concentration; NTBI = non-transferrin-bound iron; IQR = interquartile range.

Table 4. Comparison of study parameters between patients with and without proteinuria.

Parameter	UPr/UCr <200 mg/g n = 20	UPr/UCr ≥200-500 mg/g n = 23	p-value*	UPr/UCr >500 mg/g n = 7	p-value*
Age in years, median (IQR)	25.5 (14.8)	29 (16)	0.435	25 (12)	0.912
Male, n (%)	8 (40)	11 (47.8)	0.760	3 (42.9)	1.000
Splenectomized, n (%)	12 (60)	20 (87)	0.078	7 (100)	0.068
Occasional transfusions, n (%)	5 (25)	5 (21.7)	1.000	5 (71.4)	0.065
Pulmonary hypertension, n (%)	15 (75)	10 (43.5)	0.063	3 (42.9)	0.175
Thromboembolic disease, n (%)	6 (30)	6 (26.1)	1.000	2 (28.6)	1.000
Hemoglobin level in g/dl, median (IQR)	8.6 (2.0)	8.1 (2.3)	0.262	7.7 (1.8)	0.361
Fetal hemoglobin level in %, median (IQR)	28.4 (36.6)	34.5 (57.0)	0.225	48 (50.1)	0.167
Platelet count $x10^9$/l, median (IQR)	690.5 (699.5)	851 (513)	0.197	856 (509.5)	0.086
NRBC count x $10^3/mm^3$, median (IQR)	165.5 (390.0)	334 (467)	0.071	525 (285.5)	0.027
Serum ferritin level in µg/l, median (IQR)	760.5 (1088.8)	863.5 (946.0)	0.450	1590 (1046.5)	0.121
LIC in mg Fe/g dry weight, median (IQR)	4.7 (9.4)	8 (8)	0.205	14.1 (5.4)	0.041
NTBI level in µmol/l, median (IQR)	0.7 (6.6)	4.0 (4.1)	0.005	4.9 (2.8)	0.013

*Compared with the UPr/UCr <200 mg/g group.

UPr/UCr = urinary protein/creatinine ratio; NRBC = nucleated red blood cell; LIC = liver iron concentration; NTBI = non-transferrin-bound iron; IQR = interquartile range.

Figure 1. Scatter plot of serum creatinine (SCr) and estimated glomerular filtration rate (eGFR).

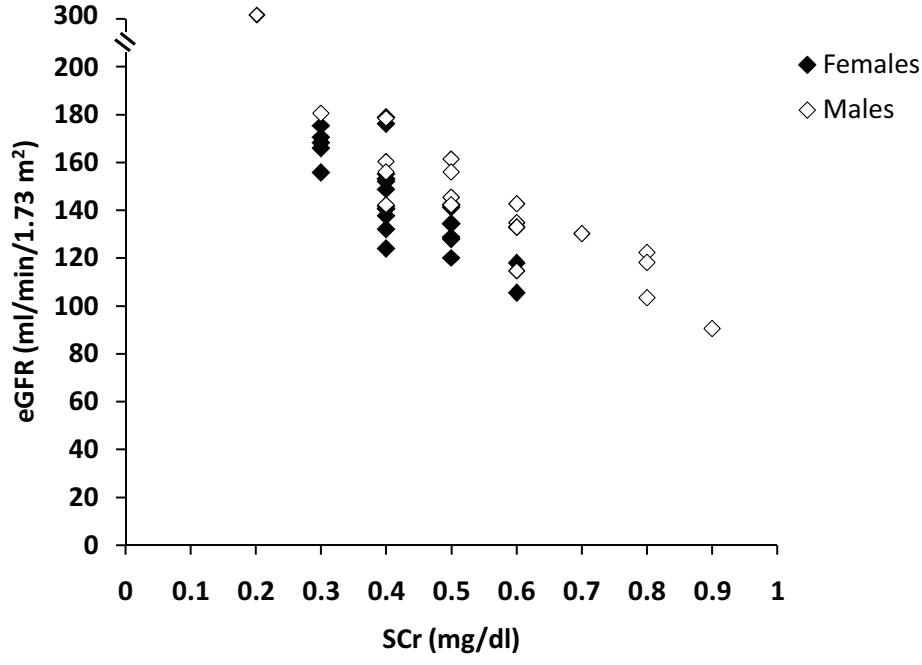


Figure 2. Linear regression analysis of age and estimated glomerular filtration rate (eGFR).

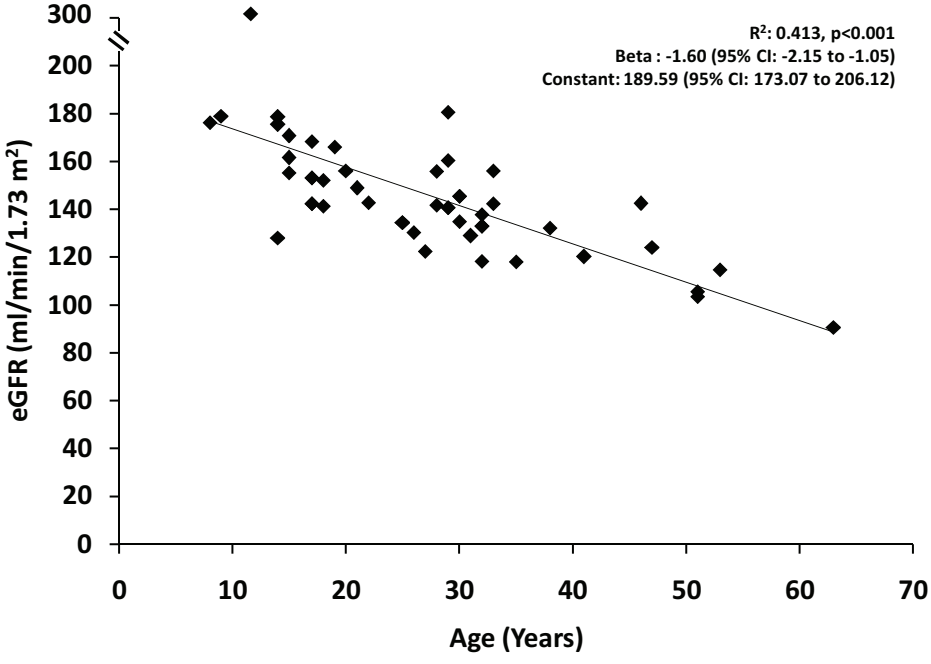


Figure 3. Correlation between urinary protein/urinary creatinine (UPr/UCr) ratio and (A) liver iron concentration (LIC), (B) non-transferrin-bound iron (NTBI), (C) nucleated red blood cell (NRBC) count, and (D) total hemoglobin (Hb) level.

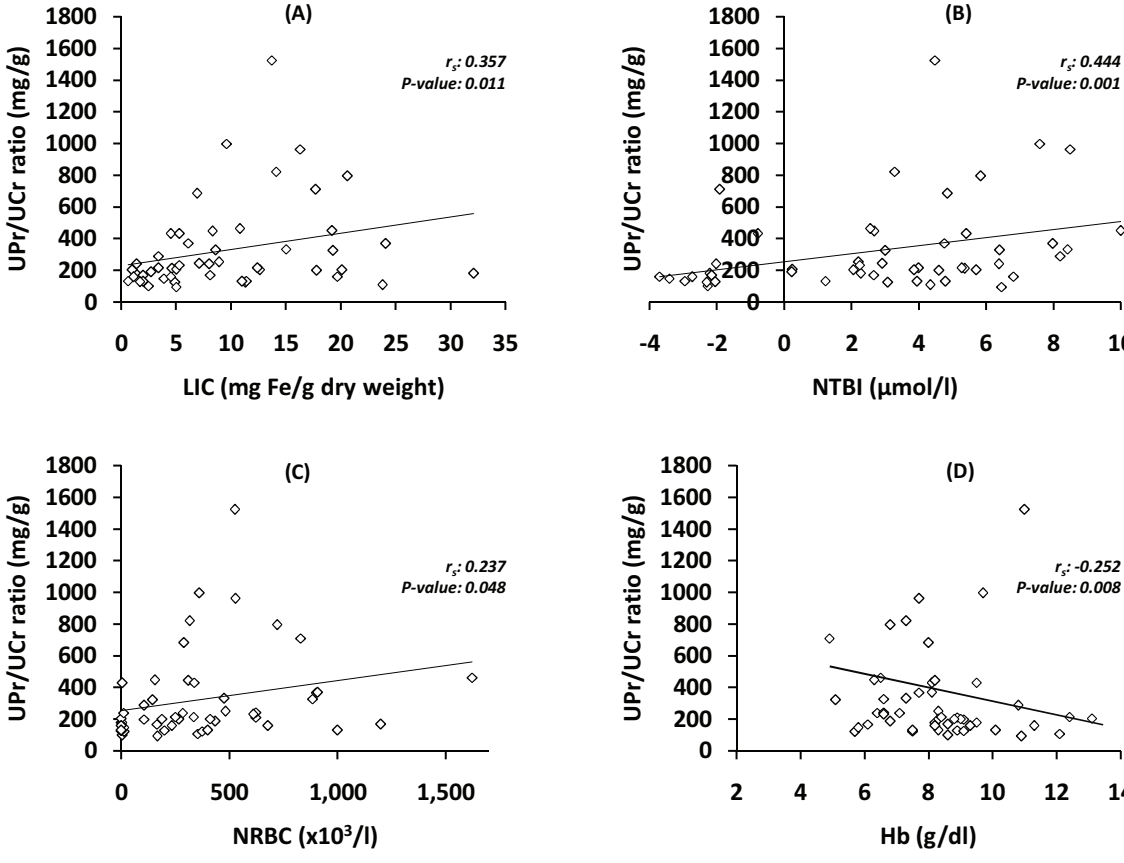
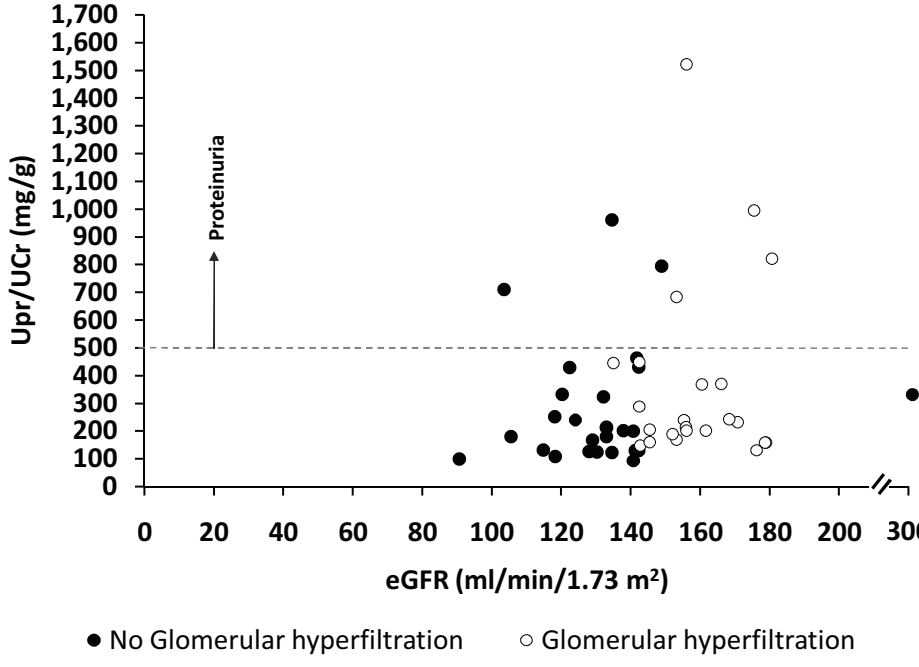


Figure 4. Scatter plot of estimated glomerular filtration rate (eGFR) and urinary protein/urinary creatinine (UPr/UCr) ratio. Hollow circles indicate patients with glomerular hyperfiltration which was defined as >149 ml/min/1.73 m2 for females and >134 ml/min/1.73 m2 for males.



Chapter 4

Vascular Disease

Splenectomy And Thrombosis: The Case Of Thalassemia Intermedia

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Splenectomy and thrombosis: the case of thalassemia intermedia

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See also Mannucci PM. Red cells playing as activated platelets in thalassemia intermedia. This issue, pp 2149–51.

Summary. *Background:* Hypercoagulability in splenectomized patients with thalassemia intermedia (TI) has been extensively evaluated. However, clinical and laboratory characteristics of patients who eventually develop overt thromboembolic events (TEE) are poorly studied. *Patients/Methods:* Three Groups of TI patients ($n = 73$ each) were retrospectively identified from a registry involving six centers across the Middle East and Italy: Group I, all splenectomized patients with a documented TEE; Group II, age- and sex-matched splenectomized patients without TEE; and Group III, age- and sex-matched non-splenectomized patients without TEE. Retrieved data included demographics, laboratory parameters, clinical complications, and received treatments that may influence TEE development, and reflected the period prior to TEE occurrence in Group I. *Results:* The mean age of Group I patients at development of TEE was 33.1 ± 11.7 years, with a male to female ratio of 33:40. TEE were predominantly venous (95%) while four patients (5%) had documented stroke. Among studied parameters, Group I patients were more likely to have a nucleated red blood cell (NRBC) count $\geq 300 \times 10^6$ L^{-1}, a platelet count $\geq 500 \times 10^9$ L^{-1} and evidence of pulmonary hypertension (PHT), or be transfusion naïve. The median time to thrombosis following splenectomy was 8 years. Patients with an NRBC count $\geq 300 \times 10^6$ L^{-1}, a platelet count $\geq 500 \times 10^9$ L^{-1}, or who were transfusion naive also had a shorter time to thrombosis following splenectomy. *Conclusion:* Splenectomized TI patients who will develop TEE may be identified early on by high NRBC and platelet counts, evidence of PHT, and transfusion naivety.

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Introduction

The thalassemias, a group of inherited disorders of hemoglobin synthesis, are the most common monogenetic disease worldwide [1]. Extremely diverse phenotypes exist within the thalassemia syndromes. At one end of the spectrum is thalassemia minor, a clinically silent, mildly hypochromic and microcytic anemia. At the other end is thalassemia major (TM), which refers to those patients whose clinical course is characterized by profound anemia, who are presented for medical attention in the first year of life, and who subsequently require regular blood transfusions for survival [2]. The term thalassemia intermedia (TI) was first suggested to describe patients who had clinical manifestations that were too severe to be termed minor yet too mild to be termed major, although there remains substantial overlap between the three conditions [3]. Our understanding of the molecular and pathophysiological mechanisms underlying the disease process in patients with TI has substantially increased over the past decade [4]. Three main factors highlight the pathophysiology of TI: ineffective erythropoiesis, chronic anemia/hemolysis, and iron overload secondary to increased intestinal absorption [4]. However, the extreme diversity in phenotypic expression in TI patients led to a wide variation in observed clinical complications and management practises, which remain solely based on physician preferences rather than evidence-based guidelines [5].

Among the clinical complications of TI that were found to occur at a higher rate than in patients with TM are

thromboembolic events (TEE) [6,7]. The largest epidemiological study to date analyzed data from 8860 thalassemia patients (6670 TM and 2190 TI) and demonstrated that TEE occurred 4.38 times more frequently in TI than TM patients [7]. The hypercoagulability in TI has been attributed to several factors, including a procoagulant activity of hemolyzed circulating red blood cells (RBCs), increased platelet activation, coagulation factor defects, depletion of antithrombotic factors and endothelial inflammation, among others [8]. These factors have been observed at a higher rate in splenectomized patients [8]. Clinical studies also confirmed that splenectomized TI patients have a higher incidence of TEE than non-splenectomized controls [5,7,9,10]. In the OPTIMAL CARE study, 73/325 (22.5%) splenectomized patients developed TEE compared with 9/259 (3.5%) non-splenectomized patients ($P < 0.001$) [5]. However, characteristics of those splenectomized TI patients that eventually develop TEE have never been evaluated.

In this study, we aim to demonstrate clinical and laboratory identifiers that characterize splenectomized TI patients who develop TEE, in an effort to highlight those patients that should be considered for preventive strategies through optimal intervention.

Patients/Methods

This was a retrospective review of data from TI patients currently registered at six comprehensive care centers in Lebanon, Italy, Iran, Egypt, the United Arab Emirates and Oman. Institutional review boards (IRBs) at each center approved the study protocol. All patients were diagnosed with TI based on criteria previously described [11]. All patients had pure β globin gene mutations [IVS-I-6 (T → C), IVS-I-5 (G → C), IVS-II-1 (G → A), or Codon 39 (C → T)]. Three groups of patients were assigned: Group I, splenectomized patients with a documented TEE; Group II, age- and sex-matched splenectomized patients without TEE; and Group III, age- and sex-matched non-splenectomized patients without TEE. For matching, we used the age at development of TEE in Group I, and matched patients from Group II and III accordingly. That age was considered the last patient follow-up and all retrieved data reflected the preceding period. In Group I and II patients, retrieved data also reflected the period after splenectomy. Data included: demographics; type of TEE; duration since splenectomy; evidence of pulmonary hypertension (PHT) (defined as a systolic pulmonary artery pressure > 35 mmHg, which corresponds to a tricuspid regurgitant velocity on Doppler echocardiography of > 2.8 m sec^{-1} + exertional dyspnea without evidence of left heart disease [12]), heart failure (HF) (modified Framingham criteria [13]), diabetes mellitus (DM) (American Diabetes Association criteria [14]), abnormal liver function (alanine aminotransferase level > 50 IU L^{-1}), family history of TEE, thrombophilia (factor V Leiden, factor II [prothrombin] *G20210A*, or methylenetetrahydrofolate reductase *C677T* mutations; antithrombin III, protein C, or protein S deficiency), or malignancy; use of blood transfusions, hydroxyurea (none of the patients received any other fetal hemoglobin [HbF] inducers or erythropoietin), antiplatelets or anticoagulants; and mean total hemoglobin (Hb) level, HbF level, nucleated RBC (NRBC) count, and platelet count of all available laboratory records for each patient.

Statistical analysis

Descriptive statistics are expressed as medians, means ± standard deviation (SD), or percentages. Univariate analysis was performed to determine differences in study parameters between the three groups using the ANOVA test for continuous variables and the chi-square and Fisher's exact tests for categorical variables. Multivariate logistic regression analysis was carried out to determine the independent effect of study parameters, where all significant variables on univariate analysis were entered into the model. In the multivariate model, NRBC counts were categorized as < or ≥ 300×10^6 L^{-1} and platelet counts were categorized as < or ≥ 500×10^9 L^{-1}, as these represented rounded median values. Comparisons of median time to thrombosis (TTT) following splenectomy were carried out by Kaplan–Meier analysis, and *P*-values from the log rank test were reported. All *P*-values are two sided with the level of significance set at < 0.05.

Results

Thromboembolic events

A total of 73 splenectomized TI patients with documented TEE (Group I) were identified. The mean age of patients at development of TEE was 33.1 ± 11.7 years (range, 6–76 years), with a male to female ratio of 33:40. Thromboembolic events were predominantly venous (95%) while four patients (5%) had evidence of stroke (Table 1). None of the patients had recurrent TEE. The diagnosis of deep vein thrombosis (DVT), superficial thrombophlebitis and portal vein thrombosis was based on ultrasonography or venography in all patients. All patients with pulmonary embolism had evidence of DVT, and diagnosis was based on lung ventilation/perfusion scan (23%) or computed tomography pulmonary angiography (77%). The diagnosis of stroke was based on both clinical and radiological grounds in all cases.

Table 1 Type of thromboembolic event in splenectomized TI patients (Group I)

Type of thromboembolic event	*n* (%)
DVT, *n* (%)	46 (63.0)
PE*, *n* (%)	13 (17.8)
STP, *n* (%)	12 (16.4)
PVT, *n* (%)	11 (15.1)
Stroke, *n* (%)	4 (5.5)

DVT, deep vein thrombosis; PE, pulmonary embolism; STP, superficial thrombophlebitis; PVT, portal vein thrombosis. *All patients who had PE had confirmed DVT.

Characteristics of splenectomized TI patients who developed TEE

Group I patients were compared with 73 age- and sex-matched patients from each of Groups II and III (Table 2). There were no statistically significant differences in mean Hb or HbF levels between the three groups. However, mean NRBC counts were significantly higher in Group I, followed by Group II, then Group III ($P < 0.001$). Similarly, mean platelet counts were highest among Group I patients, followed by Group II, then Group III ($P < 0.001$). There was no statistically significant difference in the proportion of patients with HF, DM, abnormal liver function, family history of TEE, thrombophilia or malignancy between the three groups. However, a higher proportion of patients had pulmonary hypertension in Group I as compared with Groups II and III ($P < 0.001$). The highest proportion of patients receiving transfusion therapy was in Group III, followed by Group II, then Group I ($P = 0.001$). There was no statistically significant difference in the proportion of patients receiving antiplatelets, anticoagulants or hydroxyurea between the three groups. Moreover, there was no statistically significant difference between groups in the proportion of patients with co-inheritance of α thalassemia [α^+ ($-\alpha^{3.7}$ and $-\alpha^{4.2}$) or α^0 ($^{-Med}$ and $^{-SEA}$)] or determinants associated with increased γ-chain production (*Xmn*-I +/+ genotype at position −158 of *HBG2*) (data not shown in Table 2).

On multivariate logistic regression analysis, an NRBC count $\geq 300 \times 10^6$ L^{-1}, a platelet count $\geq 500 \times 10^9$ L^{-1}, PHT and transfusion naivety were all independent and significant factors that differentiated the three groups. Group I patients were 11.11 times and 76.92 times more likely to have an NRBC count $\geq 300 \times 10^6$ L^{-1} and a platelet count $\geq 500 \times 10^9$ L^{-1}, respectively. Moreover, Group I patients were 7.30 times and

Table 2 Comparison of study parameters between Group I, II and III patients

Parameter	Group I Splenectomized with TEE $n = 73$	Group II Splenectomized without TEE $n = 73$	Group III Non-splenectomized $n = 73$	*P*-value
Mean age ± SD, years	33.1 ± 11.7	33.3 ± 11.9	33.4 ± 13.1	0.991
Male: female	33:40	35:38	34:39	0.946
Mean Hb ± SD, g dL^{-1}	9.0 ± 1.3	8.8 ± 1.2	8.7 ± 1.3	0.174
Mean HbF ± SD, %	45.9 ± 28.0	54.4 ± 32.8	44.2 ± 27.2	0.429
Mean NRBC count ± SD, × 10^6 L^{-1}	436.5 ± 205.5	279.0 ± 105.2	239.5 ± 128.7	< 0.001
Mean platelet count ± SD, × 10^9 L^{-1}	712.6 ± 192.5	506.3 ± 142.1	319.2 ± 122.0	< 0.001
PHT, *n* (%)	25 (34.2)	17 (23.3)	3 (4.1)	< 0.001
HF, *n* (%)	7 (9.6)	5 (6.8)	1 (1.4)	0.101
DM, *n* (%)	4 (5.5)	5 (6.8)	1 (1.4)	0.256
Abnormal liver function, *n* (%)	2 (2.7)	2 (2.7)	3 (4.1)	0.863
Family history of TEE	3 (4.7)	1 (1.4)	3 (4.7)	0.554
Thrombophilia, *n* (%)	3 (4.7)	2 (2.7)	2 (2.7)	0.863
Malignancy, *n* (%)	1 (1.4)	2 (2.7)	0 (0)	0.363
Transfused, *n* (%)	32 (43.8)	48 (65.8)	54 (74.0)	0.001
Antiplatelet or anticoagulant use, *n* (%)	1 (1.4)	3 (4.1)	2 (2.7)	0.598
Hydroxyurea use, *n* (%)	13 (17.8)	17 (23.3)	29 (27.4)	0.383

TEE, thromboembolic events; Hb, total hemoglobin; NRBC, nucleated red blood cell; HbF, fetal hemoglobin; PHT, pulmonary hypertension; HF, heart failure; DM, diabetes mellitus.

Table 3 Multivariate analysis of parameters that differentiate Group I, II and III patients

Parameter	Group	OR	95% CI	*P*-value
NRBC count $\geq 300 \times 10^6$ L^{-1}	Group III	1.00	Referent	< 0.001
	Group II	5.35	2.31–12.35	
	Group I	11.11	3.85–32.26	
Platelet count $\geq 500 \times 10^9$ L^{-1}	Group III	1.00	Referent	< 0.001
	Group II	8.70	3.14–23.81	
	Group I	76.92	22.22–250.00	
PHT	Group III	1.00	Referent	0.020
	Group II	4.00	0.99–16.13	
	Group I	7.30	1.60–33.33	
Transfusion naivety	Group III	1.00	Referent	0.001
	Group II	1.67	0.82–3.38	
	Group I	3.64	1.82–7.30	

NRBC, nucleated red blood cell; PHT, pulmonary hypertension; OR, adjusted odds ratio; CI, confidence interval.

3.64 times more likely to have PHT or be transfusion naïve, respectively (Table 3).

Duration since splenectomy and development of TEE

In Group I patients, the median TTT following splenectomy was 8 years (range, 1–33 years) (Fig. 1). The median TTT was significantly shorter in patients with an NRBC count $\geq 300 \times 10^6$ L^{-1} compared with those with $< 300 \times 10^6$ L^{-1} (8 vs. 15 years, $P = 0.002$; Fig. 2A). Similarly, the median TTT was significantly shorter in patients with a platelet count $\geq 500 \times 10^9$ L^{-1} compared with $< 500 \times 10^9$ L^{-1} (8 vs. 22 years, $P = 0.008$; Fig. 2B). The median TTT was also significantly shorter in transfusion naïve compared with transfused patients (7 vs. 13 years, $P = 0.009$; Fig. 2C). There was no statistically significant difference in the median TTT between patients with and without PHT (9 vs. 8 years, $P = 0.703$; Fig. 2D). For patients who had an NRBC count $\geq 300 \times 10^6$ L^{-1}, a platelet count $\geq 500 \times 10^9$ L^{-1} and who were transfusion naïve the median TTT was 6 years (range, 2–15 years).

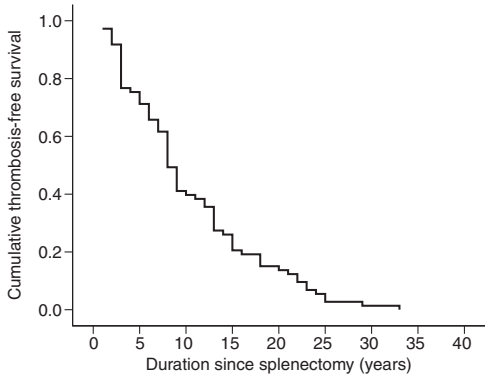

Fig. 1. Thrombosis-free survival in Group I patients.

Discussion

Our study indicates that splenectomized TI patients who develop TEE are characterized by high NRBC and platelet counts, and are more likely to have evidence of PHT and be transfusion naive. Moreover, high NRBC and platelet counts as well as transfusion naivety are associated with earlier development of TEE following splenectomy. The main indications for splenectomy in patients with TI include growth retardation or poor health, leucopenia, thrombocytopenia,

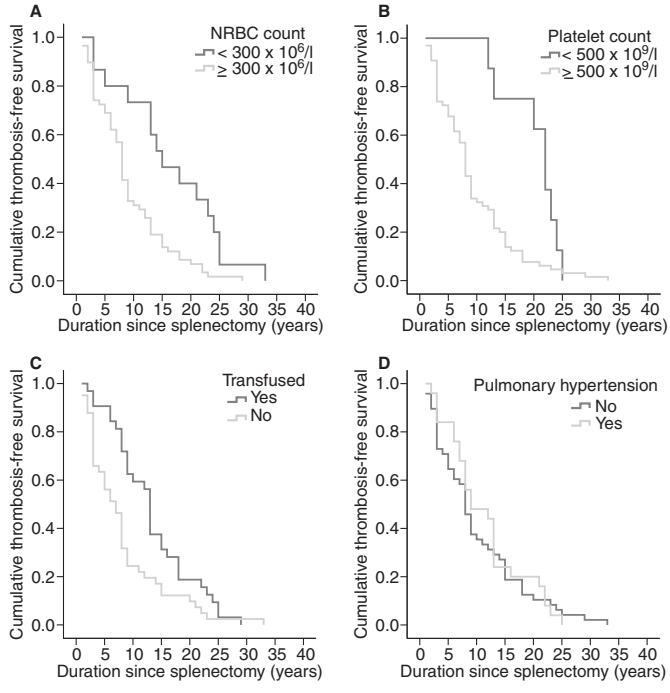

Fig. 2. Thrombosis-free survival in Group I patients according to (A) nucleated red blood cell (NRBC) count ($< 300 \times 10^6$ L^{-1}, $n = 15$; $\geq 300 \times 10^6$ L^{-1}, $n = 58$), (B) platelet count ($< 500 \times 10^9$ L^{-1}, $n = 8$; $\geq 500 \times 10^9$ L^{-1}, $n = 65$), (C) transfusion status (transfused, $n = 32$; non-transfused, $n = 41$), and (D) pulmonary hypertension (yes, $n = 25$; no, $n = 48$).

increased transfusion demand, or symptomatic splenomegaly [4]. A substantial amount of evidence, however, continues to support harmful effects of splenectomy in both normal individuals and patients with hematological disorders [5,9,15]. Complications such as an increased susceptibility to infections and TEE remain the most commonly reported and worrisome [5,7,9,10,15]. However, until a replacement for splenectomy is recommended through evidence-based guidelines, a large number of TI patients will continue to be splenectomized. These, alongside patients who had already undergone splenectomy, constitute a large proportion of TI patients at risk of TEE. In our study, we aimed to identify the characteristics of those splenectomized TI patients who will eventually develop TEE, which should help in undertaking appropriate measures and aid in the design of prospective trials that evaluate the efficacy and safety of intervention. We decided to investigate those characteristics that should be easily identified through careful patient monitoring with history taking and physical examination alongside routine and simple laboratory tests, which are part of regular patient follow-up. Despite the fact that more specific markers and tests of hypercoagulability merit evaluation for such an aim [8], these may not be available, practical or affordable in developing countries where TI is prevalent [16].

The hemolytic anemia implicated in patients with TI causes iron-dependent oxidation of membrane proteins and formation of red-cell senescence antigens such as phosphatidylserine that cause thalassemic RBCs to be rigid and deformed and to aggregate, resulting in premature cell removal [17–20]. Studies have shown that thalassemic RBCs may be a source of negatively charged phospholipids, which can eventually increase thrombin generation [21,22]. An even higher number of circulating RBCs with negatively charged phospholipids was found in splenectomized patients [23]. Moreover, a study evaluating circulating RBC microparticles (submicrometric membrane fragments with procoagulant potential) found significantly higher levels in patients with TI compared with controls, especially in splenectomized patients [24]. These abnormalities have been reduced to normal range after the patients received a blood transfusion that decreases the number of circulating damaged RBCs [25]. These findings may partly explain why patients who had high NRBC counts or were transfusion naïve had a higher occurrence of TEE in our cohort. In TI, 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 arise. Consequently, unlike TM, evaluation of the role of transfusions in the management of TI has been limited. Similar to our findings, few observational studies have also confirmed that transfused TI patients suffer fewer TEE, PHT and silent brain infarcts as compared with transfusion naïve patients [5,7,26,27]. This may be attributed to correction of the underlying ineffective erythropoiesis and the resulting damaged RBCs with thrombogenic potential. As such, earlier introduction of transfusion therapy aiming to prevent the consequences of chronic hemolytic anemia may benefit TI patients by prevention, rather than palliation of late and irreversible hemolysis-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. Although earlier introduction of blood transfusions will increase the rate of iron accumulation, effective methods of iron chelation are available for patients with TI [5,28–31], and the benefits of transfusion therapy may greatly outweigh the cost and inconvenience of iron chelation therapy.

The medical literature is rich in evidence suggesting that patients with thalassemia have activated platelets. Moreover, flow cytometric studies have also confirmed the chronic platelet activation status. In thalassemia, there is evidence of increased platelet aggregation [32], and an increased proportion of platelets expressing CD62P (P-selectin) and CD63 [33,34]. Further evidence of chronic platelet activation in patients with thalassemia was provided by the measurement of urinary metabolites of prostacyclin (PGI2) and thromboxane A2 (TXA2), where a significant increase (4–10-fold) in the urinary excretion of the stable hydrolysis products of TXA2 and PGI2 was found in thalassemia patients compared with controls [35]. The thrombocytosis observed after splenectomy may thus imply increased hypercoagulability and TEE risk in these patients [36]. In our study, higher platelet counts were found in those splenectomized patients who developed TEE. As such, consideration of antiplatelet aggregants (e.g. aspirin) for the prevention of TEE in these patients remains logical. The use of anticoagulant or antiplatelet agents in thalassemia patients has never been prospectively evaluated in large well-designed trials, although patients who were placed on aspirin were found to have lower recurrence rates of TEE than those who were not [7].

The higher occurrence of PHT in splenectomized patients who develop TEE may suggest a common underlying etiology between the two conditions. Unlike patients with sickle cell disease [37], the pathophysiology of PHT in patients with thalassemia has not been extensively studied and is mainly attributed to a state of chronic anemia [38]. PHT has been linked to the intensity of hemolysis, nitric oxide metabolic dysregulation, and hypercoagulability in patients with sickle cell disease; whether the same mechanisms contribute to PHT in TI is not yet known and needs to be investigated. Of note, autopsies of a large series of patients with TI revealed thrombotic lesions in the pulmonary arteries, which may have been due to circulating platelet aggregates [39]. Similar findings of multiple microthrombi, which were composed mainly of platelets, were seen in the pulmonary arterioles and microcirculation in autopsies of two splenectomized patients with thalassemia [40]. Whether these microthrombi contribute to PHT in TI is not well understood. However, the effect of intervention (initiated to prevent TEE) on PHT in TI patients merits evaluation.

The delayed type of TEE (median 8 years) observed in splenectomized TI patients has an important clinical implica-

tion. First, it highlights that TEE in this population is not an acute postoperative complication but rather a late manifestation of a progressive underlying pathology. In fact, a negative impact of time (aging) on physiological adaptation of thalassemia patients to their underlying disease has been documented [41]. Second, it requires that any modality considered for prevention of TEE has to be evaluated for long-term efficacy and safety, as patients may be placed on such interventions for long durations to ensure effective control of hypercoagulability and TEE prevention. The aforementioned suggestions in this report, namely tailored transfusion programs and antiplatelet/anticoagulant therapy, have documented long-term experience in several patient populations and are worthwhile examining for TI.

One limitation in our study is the use of echocardiography instead of cardiac catheterization for the diagnosis of PHT, which may increase the rate of false positive findings. However, our patients were mainly screened for PHT after presenting with exertional dyspnea with no evidence of left heart disease. Moreover, echocardiography is still the modality of choice used in many studies on thalassemia and sickle cell anemia due to financial/practical reasons and reliance on reports of good relationship between Doppler estimates and invasive measurements of pulmonary arterial pressure at baseline and after treatment, despite the variable echocardiographic cut-off values used to label patients with PHT [42–44].

In conclusion, our study demonstrated that splenectomized TI patients who will develop TEE may be identified early on by high NRBC and platelet counts, evidence of PHT, and transfusion naivety. It also calls for prospective clinical trials that evaluate the efficacy, safety and cost effectiveness of transfusion and antiplatelet/anticoagulant therapy in preventing TEE in this patient population. Such studies are expected to evaluate the optimal timing, dose and duration of intervention, and the added advantage of multimodal therapy.

Addendum

A. T. Taher, K. M. Musallam and M. D. Cappellini were responsible for conception and design, data analysis and interpretation, and manuscript writing; K. M. Musallam performed statistical analysis; M. Karimi, A. El-Beshlawy, K. Belhoul, S. Daar and M. Saned gave administrative support and helped in provision of study material or patients; C. Cesaretti helped in collection and assembly of data. All authors gave final approval of the manuscript for submission.

Disclosure of Conflict of Interests

The authors state that they have no conflict of interest.

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Risk Factors For Pulmonary Hypertension In Patients With β Thalassemia Intermedia

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Risk factors for pulmonary hypertension in patients with β thalassemia intermedia

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ABSTRACT

Background: Pulmonary hypertension (PHT) is a common yet poorly understood complication of β thalassemia intermedia (TI).
Methods: We herein evaluated risk factors for PHT in TI, through comparing 64 TI patients with evidence of PHT by symptomatology and echocardiography (Group I) to age- and sex-matched TI patients without PHT (Group II). Retrieved data included demographics, laboratory parameters, clinical characteristics, and received treatments that may influence PHT development; and reflected the period prior to PHT occurrence in Group I.
Results: The mean age of Group I patients at development of PHT was 37.3 ± 10.6 years; with 44% being males. Among studied parameters, Group I patients were more likely to be splenectomized (4.9-times), transfusion-naive (3.5-times); hydroxyurea-naive (2.6-times), or iron chelation-naive (2.3-times); and have nucleated red blood cell count $\geq 300 \times 10^6$/l (2.59-times) or a previous history of thromboembolic events (3.69-times).
Conclusion: TI patients who eventually develop PHT may be identified early on by being splenectomized, having high nucleated red blood cell counts and a previous history of thromboembolism. Prospective clinical trials that evaluate the efficacy, safety, and cost effectiveness of transfusion, iron chelation, and hydroxyurea therapy in preventing PHT in TI are invited.

1. Introduction

The term β thalassemia intermedia (TI) was first suggested to describe patients who have a milder anemia compared to patients with β thalassemia major, and who usually present to medical attention later in childhood and remain largely transfusion independent. However, it is now recognized that the diagnosis of TI carries higher morbidity than previously recognized [1]. Three main factors lead to the clinical sequelae of TI, ineffective erythropoiesis, chronic anemia/hemolysis, and iron overload secondary to increased intestinal absorption [2,3]. The extreme diversity in phenotypic expression in TI patients results in a wide variation in observed clinical complications [1]. Among the clinical complications of TI that was found to occur at a relatively high frequency, especially compared to patients with thalassemia major, is pulmonary hypertension (PHT) [1,3–7]. Significant advances have been made toward understanding the pathophysiology, diagnostic challenges, morbidity, mortality, and optimal management of PHT in patients with other hemoglobinopathies, namely sickle cell anemia [8]; however, data on patients with TI is limited. Chronic anemia and hypoxia [7], iron overload [9,10], splenectomy [11,12], hypercoagulability [13], and chronic hemolysis [4] have all been implicated in the pathophysiology of PHT in TI. PHT is neither associated with myocardial siderosis [14,15] nor left ventricular dysfunction in TI, but is a leading cause of right-sided heart failure and thus warrants attention [6,9,16].

In this study, we aim to demonstrate risk factors for PHT in patients with TI, in an effort to further understand the mechanism behind this potentially disabling complication, and to highlight those patients that carry a high-risk of developing PHT and deserve consideration for preventive strategies.

2. Materials and methods

This was a retrospective review of data collected on 584 patients with TI currently registered at six comprehensive care centers in

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Egypt, Iran, Italy, Lebanon, Oman, and the United Arab Emirates. Institutional review boards at each center approved the study protocol. An age of diagnosis beyond 2 years, hemoglobin values maintained between 7 and 9 g/dl without the need for regular transfusional regimen, with or without splenomegaly, were the main criteria to define the TI phenotype on presentation [17]. It should be mentioned, however, that transfusion may be later undertaken for many patients with TI when if the disease worsens as they grow older or after developing complications. Patients had the following β globin gene mutations: IVS-I-6 (T→C), IVS-I-5 (G→C), IVS-II-1 (G→A), or Codon 39 (C→T). None of the patients had Hb S, C, E/β or δβ thalassemia. Two Groups of patients were assigned: Group I (n = 64), TI patients with documented PHT defined as a systolic pulmonary artery pressure greater than 35 mm Hg, which corresponds to a tricuspid regurgitant velocity on Doppler echocardiography of >2.8 m/s + exertional dyspnea without evidence of left heart disease [18]; and Group II (n = 64), age- and sex-matched TI patients without PHT. For matching, we used the age at development of PHT in Group I, and matched patients from Group II. That age was considered the last patient follow-up and all retrieved data reflected the preceding period. Retrieved data included: demographics (age and gender); splenectomy status; history of heart failure (modified Framingham criteria [19]); history of previous thromboembolic events; history of thrombophilia (factor V Leiden, factor II [prothrombin] *G20210A*, or methylenetetrahydrofolate reductase *C677T* mutations, antithrombin III, protein C, or protein S deficiency); use of blood transfusions, iron chelation, hydroxyurea, antiplatelet or anticoagulant therapy; mean total hemoglobin level (pre-transfusion in transfused patients), fetal hemoglobin level, serum ferritin level, nucleated red blood cell count, and platelet count of all available laboratory records for each patient.

2.1. Statistical analysis

Descriptive statistics are expressed as means ± standard deviation (SD) or percentages where appropriate. Bivariate analysis was performed to determine differences in study parameters between the two Groups using the independent sample *t*-test for continuous variables and the Chi-square and Fisher's exact tests for categorical variables. Multivariate logistic regression analysis, using forward-stepwise selection, was done to determine the independent effect of study parameters. $P \leq 0.1$ was used as the criterion for inclusion into the model and a $P \geq 0.05$ was used for exclusion, where all significant variables on univariate analysis were entered into the model. In the multivariate model, nucleated red blood cell counts were categorized as < or $\geq 300 \times 10^6$/l and platelet counts were categorized as < or $\geq 500 \times 10^9$/l. All *P*-values are two sided with the level of significance set at <0.05.

3. Results

A total of 64 patients with documented PHT (Group I) were identified. The mean age at PHT diagnosis was 37.3 ± 10.6 years; with 44% being males. The mean age of matched patients without PHT (Group II, n = 64) was 37.9 ± 11.4; with 44% being males.

The mean serum ferritin level was higher in Group I compared to Group II (1233.2 ± 499.2 vs. 654.7 ± 234.5 ng/ml; $P = 0.01$). Moreover, the mean nucleated red blood cell count was higher in Group I compared to Group II patients (354.2 ± 199.2 vs. 214.7 ± 94.5 × 10^6/l; $P = 0.03$). A higher proportion of patients was splenectomized (84.4% vs. 46.9%; $P<0.001$) or had a previous history of thromboembolic events (40.6% vs. 7.8%; $P<0.001$) in Group I compared to Group II patients. Conversely, a higher proportion of patients received transfusion (78.1% vs. 56.2%; $P<0.001$), iron chelation (62.5% vs. 37.5%; $P<0.001$), and hydroxyurea (34.4% vs. 17.2%; $P<0.001$) therapy in Group II compared to Group I patients. There were no statistically significant differences between both groups with regards to other parameters (Table 1). Moreover, there was no statistically significant difference between Groups I and II in the proportion of patients with co-inheritance of α thalassemia [α^+ (-$\alpha^{3.7}$ and -$\alpha^{4.2}$) or α^0 (–^{Med} and –^{SEA})] or determinants associated with increased γ-chain production [*Xmn*-I +/+ genotype at position −158 of *HBG2*] (data not shown in Table 1).

Multivariate logistic regression analysis revealed that patients in Group I are more likely to be splenectomized (adjusted odds ratio [AOR]: 4.9, 95% confidence interval [CI]: 1.9–8.5); transfusion-naive (AOR: 3.5, 95% CI: 2.1–6.25); hydroxyurea-naive (AOR: 2.6, 95% CI: 1.1–5.25) or iron chelation-naive (AOR: 2.3, 95% CI: 1.2–4.25); and have NRBC count $\geq 300 \times 10^6$/l (AOR: 2.59, 95% CI: 1.69–6.05) or a previous history of thromboembolic events (AOR: 3.69, 95% CI: 2.38–7.05) (Table 2).

4. Discussion

Our study indicates that TI patients who develop PHT are characterized by being splenectomized, having high nucleated red blood cell counts and a previous history of thromboembolic events. Moreover, it suggests a potential role for transfusion, iron chelation, or hydroxyurea therapy in lowering the risk of PHT in patients with TI. These findings have important implications in understanding the pathophysiology of PHT in patients with TI, and pave the way towards the development of preventive and management strategies.

Table 1
Comparison of study parameters between Group I and Group II patients.

Parameter	Group I Pulmonary hypertension n = 64	Group II No pulmonary hypertension n = 64	*P*-value
Mean age ± SD, years	37.3 ± 10.6	37.9 ± 11.4	NS
Male, n (%)	28 (44)	28 (44)	NS
Mean total hemoglobin ± SD, g/dl	9.0 ± 1.3	8.8 ± 1.2	NS
Mean fetal hemoglobin ± SD,%	46.9 ± 27.0	52.4 ± 31.4	NS
Mean serum ferritin ± SD, ng/ml	1233.2 ± 499.2	654.7 ± 234.5	0.01
Mean nucleated red blood cell count ± SD, × 10^6/l	354.2 ± 199.2	214.7 ± 95.4	0.03
Mean platelet count ± SD, × 10^9/l	616.6 ± 197.4	556.3 ± 144.7	NS
Splenectomized, n (%)	54 (84.4)	30 (46.9)	<0.001
Heart failure, n (%)	3 (4.7)	2 (3.1)	NS
Previous thromboembolic events, n (%)	26 (40.6)	5 (7.8)	<0.001
Thrombophilia, n (%)	3 (4.7)	2 (3.1)	NS
Transfused, n (%)	36 (56.2)	50 (78.1)	<0.001
Iron chelation therapy, n (%)	24 (37.5)	40 (62.5)	<0.001
Antiplatelet or anticoagulant use, n (%)	3 (4.7)	2 (3.1)	NS
Hydroxyurea use, n (%)	11 (17.2)	22 (34.4)	<0.001

NS = non significant ($P \geq 0.05$).

Table 2
Multivariate analysis of parameters that differentiate patients with (Group I) and without pulmonary hypertension (Group II).

Parameter	Group II Referent	Group I AOR	95% CI	*P*-value
Splenectomized	1.00	4.90	1.90–8.50	<0.001
Nucleated red blood cell count $\geq 300\times10^6$/l	1.00	2.59	1.69–6.05	0.010
Splenectomized	1.00	3.21	1.29–6.55	0.007
Non-splenectomized	1.00	1.13	1.09–2.05	0.047
Previous thromboembolic events	1.00	3.69	2.38–7.05	0.020
Splenectomized	1.00	4.20	1.78–9.16	0.011
Non-splenectomized	1.00	2.11	1.03–7.44	0.049
Transfusion naivety	1.00	3.50	2.10–6.25	0.001
Splenectomized	1.00	4.00	1.18–8.45	0.004
Non-splenectomized	1.00	1.37	1.04–1.99	0.049
Iron chelation naivety	1.00	2.30	1.20–4.25	0.001
Splenectomized	1.00	1.12	1.01–3.32	0.038
Non-splenectomized	1.00	2.26	1.33–3.67	0.001
Hydroxyurea naivety	1.00	2.60	1.10–5.25	<0.001
Splenectomized	1.00	2.20	1.35–2.98	0.003
Non-splenectomized	1.00	1.22	1.02–1.99	0.044

AOR = adjusted odds ratio; CI = confidence interval.

A hypercoagulable state in patients with thalassemia has been established [20]. Ineffective erythropoiesis, in combination with premature intra- and extravascular hemolysis, lead to the emergence of damaged, prothrombotic red blood cells in the circulation of TI patients. Hemolysis causes iron-dependent oxidation of membrane proteins and formation of red-cell senescence antigens such as phosphatidylserine that cause thalassemic red blood cells to be rigid and deformed and to aggregate, resulting in premature cell removal [21–24]. Studies have shown that thalassemic red blood cells, through being a source of negatively charged phospholipids, can eventually increase thrombin generation [25,26]. An even higher number of circulating red blood cells with prothrombotic potential were found in splenectomized patients [27]. This justifies the high rate of thromboembolic events, especially in splenectomized patients with TI [1,28–32]. It should be noted that prothrombotic mutations do not play a role in the hypercoagulability of thalassemia [33,34]. Hypercoagulability, secondary to the aforementioned hemolysis and other established risk factors [35], and subsequent pulmonary vasculature thrombosis or embolism may explain the occurrence of PHT in TI [13], but need further evaluation. Of note, autopsies of a large series of patients with TI revealed thrombotic lesions in the pulmonary arteries, which may have been due to circulating platelet aggregates [36]. Similar findings of multiple microthrombi, which were composed mainly of platelets, were seen in the pulmonary arterioles and microcirculation in autopsies of two splenectomized patients with thalassemia [37]. A high rate of PHT in splenectomized TI patients has been documented and attributed to a chronic thromboembolic state [12,38]. Moreover, elevated levels of circulating red blood cell-derived microparticles were detected in splenectomized patients with TI [39]. Whether they contribute to the development of PHT in this patient population merits an evaluation.

Transfusion therapy was associated with a lower rate of PHT. In previous years, the prevailing approach has been avoidance of early blood transfusions and the concomitant requirement for chelation therapy in patients with TI through splenectomy, reserving the introduction of transfusion until later in the disease course when complications manifest. However, collective evidence now recommends avoidance or delay of splenectomy because of a multitude of associated complications, and an increasing evidence for a beneficial role of transfusions [1]. Observational studies continue to document a lower occurrence of thromboembolic events and PHT in transfused compared to transfusion-independent patients with TI [1,30,31,40]. Our results confirm these findings, which may be attributed to the potential role of transfusions in correcting the underlying ineffective erythropoiesis and the resulting damaged red blood cells with thrombogenic potential [41]. Although the suggestion of earlier introduction of blood transfusions will increase the rate of iron accumulation in TI patients, effective methods of iron chelation are now available [42]. In fact, iron chelation therapy was independently associated with a lower rate of PHT in our study. How iron overload can contribute to vascular disease in patients with TI is not completely understood. One previous study demonstrated a correlation between elevated liver iron concentration and PHT in TI; suggesting that PHT is strongly affected by iron overload and is not merely a consequence of the chronic hypoxic damage that progresses with age [9].

Fetal hemoglobin inducing agents like decitabine and hydroxyurea were shown to lower plasma markers of thrombin generation [35]. Hydroxyurea may modulate hypercoagulability in several ways, it may reduce phospholipid expression on the surface of red blood cells and platelets, and decrease red blood cell adhesion to thrombospondin, a thrombin sensitive protein [35]. It may also decrease leukocyte count, particularly monocytes expressing tissue factor, in addition to being a nitric oxide donor [37]. Thus, it is likely that these mechanisms explain the lower risk of PHT attributed to hydroxyurea use in our patients, as well as in those reported in other studies [43].

One limitation in our study is the use of echocardiography instead of cardiac catheterization for the diagnosis of PHT which may increase the rate of false positive findings. However, our patients were mainly screened and diagnosed with PHT after presenting with exertional dyspnea with no evidence of left heart disease. Echocardiography is still the modality of choice used in many studies on thalassemia and sickle cell anemia relying on reports of good relationship between Doppler estimates and invasive measurements of pulmonary arterial pressure at baseline and after treatment [4,6,7,44,45].

In conclusion, our study demonstrated that TI patients who eventually develop PHT are most likely to be splenectomized, have high nucleated red blood cell counts and a previous history of thromboembolism. This calls for a review of splenectomy as a procedure of choice, especially with its potential role in increasing TI-related complications aside from the inherent risk of infection associated with the procedure. It also calls for prospective clinical trials that evaluate the efficacy, safety, and cost effectiveness of transfusion, iron chelation, and hydroxyurea therapy in preventing PHT in this patient population. Earlier introduction of transfusion and iron chelation therapy aimed at preventing the consequences of chronic hemolytic anemia may benefit TI patients by prevention, rather than palliation of late and irreversible complications. Rather than enforcing the regular transfusion and iron chelation regimens implemented in thalassemia major, blood transfusion and chelation therapy, if initiated in patients with TI will require closer monitoring and should be individually tailored to meet patient needs.

Learning points

- A history of splenectomy or thromboembolic events predicts future development of pulmonary hypertension in thalassemia intermedia patients.
- High nucleated red blood cell counts also characterize thalassemia intermedia patients who develop pulmonary hypertension.
- Transfusion, iron chelation, and hydroxyurea therapy seem to have a protective role against pulmonary hypertension in thalassemia intermedia, which deserve to be prospectively evaluated.

Conflict of interest statement

The authors have no conflicts of interest to disclose. This study did not receive external funding.

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Asymptomatic Brain Magnetic Resonance Imaging Abnormalities In Splenectomized Adults With Thalassemia Intermedia

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Asymptomatic brain magnetic resonance imaging abnormalities in splenectomized adults with thalassemia intermedia

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Summary. *Background*: A high incidence of thrombotic events in thalassemia intermedia (TI) patients led to the identification of a hypercoagulable state. Brain involvement has not been widely studied in TI, although limited reports confirm a low incidence of overt stroke and high incidence of silent brain infarcts. *Patients/methods*: This was a cross-sectional study conducted on 30 adult, splenectomized TI patients. Patients were screenedforabsence of neurological signs orsymptoms, and stroke-related risk factors. Patient charts were reviewed for demographics, duration since splenectomy, and any history oftransfusion therapy. Blood samples were obtained for complete blood counts and serum ferritin. Direct determination of liver iron concentration (LIC) was performed by R2 magnetic resonance imaging(MRI). Brain MRI was performed on all patients, looking for ischemic lesions and/or atrophy. *Results*: The mean age of patients was 32.1 ± 11 years (range, 18–54 years), with a male to female ratio of 13:17. Eighteen patients (60%) had evidence of one or more white matter lesions (WMLs) on brain MRI, all involving the subcortical white matter. Fourteen patients had evidence of multiple WMLs, with a mean of 5 ± 10 lesions (range, 2 to > 40 lesions). The vast majority of patients (94%) had small (< 0.5 cm) to medium (0.5–1.5cm) WMLs, with only one patient showing evidence of a large (> 1.5 cm) WML. Eleven patients (37%) had mild cerebral atrophy. On multivariate analysisonly age and transfusion history were independently and significantly associated with the occurrence of zero, single or multiple WMLs. *Conclusion*: WMLs and brain atrophy are a common finding in adult, splenectomized, TI patients. Increasing age and transfusion naivety are associated with a higher incidence and multiplicity of lesions.

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Introduction

A hypercoagulable state in the thalassemia syndromes has been established [1]. This is attributed to a number of factors, including the procoagulant activity of damaged circulating red blood cells (RBCs), co-inheritance of coagulation defects, depletion of antithrombotic factors, endothelial inflammation and conditions that increase thrombotic burden [1]. The largest clinical study to date, on 8860 thalassemia patients, demonstrated that thromboembolic events (TEE) occur 4.38 times more frequently in thalassemia intermedia (TI) than thalassemia major (TM) patients [2]. In this and most other studies on TI patients, older age and splenectomy are implicated as significant risk factors for the development of TEE [2,3]. Brain involvement has not been widely studied and the incidence of clinically overt stroke in TI has not been established, although limited reports confirm lower incidence than TM [2]. However, in a study done to assess the rate of brain damage in patients with benign hemoglobinopathies, 37.5% of patients with TI showed asymptomatic brain damage on magnetic resonance imaging (MRI) [4], indicating that further investigation is warranted. This study aims to evaluate the incidence of and risk factors for silent brain abnormality in splenectomized adults with TI utilizing brain MRI.

Patients/methods

This was a cross-sectional studyconducted on all splenectomized TI patientsaged 18 years or older (n = 43) attending the Chronic Care Center (Lebanon) between June and December 2008. Afterscreening patients for exclusion criteria (Table 1), 30 patients were recruited into the study.

Table 1 Exclusion criteria

Criterion	Definition and assessment method
Neurological and/or gross cognitive signs or symptoms	Abnormality detected during medical history taking, neurological exam, or mini mental status exam (MMSE) performed by a qualified neurologist (AB)
Use of anticoagulant or antiplatelet therapy	Any current or previous history of anticoagulant or antiplatelet therapy
Diabetes	Use of antidiabetic drugs or a fasting blood sugar ≥ 126 mg dL^{-1}
Hypertension	Use of antihypertensive drugs or a blood pressure ≥ 140/90 mmHg on two readings 6 weeks apart
Cardiac disease	Any abnormality on electrocardiography or echocardiography including: arrhythmias, valvular disease, dysfunction, presence of atrial or ventricular thrombi, or pulmonary hypertension
Carotid stenosis	Evidence of > 50% narrowing of the carotid(s) on color-flow duplex scanning
Thrombophilia	Evidence of factor V Leiden, prothrombin or MTHFR mutations on genetic studies; or abnormality in protein C, protein S, antithrombin III, Lupus anticoagulant, or cardiolipin antibodies levels.
Smoking	Any current or previous history of smoking

MMSE, mini mental status exam; MTHFR, methyletetrahydrofolate reductase.

Brain MRIs were performed on a 3.0 Tesla, eight channel head coil, Achieva Philips Scanner using axial T1-weighted images (TR/TE, 450/10), T2 gradient-echo weighted images (TR/TE, 731/16), fluid-attenuated inversion recovery (FLAIR) images (TR/TE, 11000/125) and diffusion weighted imaging (TR/TE, 2312/68). Coronal FLAIR images (TR/TE, 11000/125) as well as coronal and sagittal T2 weighted images (TR/TE, 3000/80) were also obtained. No contrast material was administered. Two blinded neuroradiologists reviewed the studies, looking for ischemic lesions and/or atrophy. Infarction or ischemic lesions were defined as areas of abnormally increased signal intensity on the T2 and FLAIR weighted sequences and were classified by anatomic location and size. The size of lesions was classified into small (< 0.5 cm), medium (0.5–1.5 cm) and large (> 1.5 cm). For patients with multiple lesions, the largest lesion was used to define size. Atrophy was visually assessed as a decrease in brain volume greater than that which would be expected in a healthy volunteer of similar age and graded as mild, moderate or severe. This study was approved by the Institutional Review Board and written consents were obtained from all patients.

Patient charts were reviewed for demographics (age and gender), duration since splenectomy, and any history of transfusion therapy. Blood samples were obtained for assessment of total and fetal hemoglobin levels, nucleated RBC counts, platelet counts, and steady-state serum ferritin levels. Direct determination of liver iron concentration (LIC) was performed by R2 MRI using established methodology [5].

Statistical analysis

Descriptive statistics are expressed as means ± standard deviation (SD) or percentages where appropriate. Bivariate correlations between MRI findings and multiple variables were performed using independent-samples *t*-test for continuous variables and chi-square test for categorical variables. Multivariate analysis was conducted for all significant associations at the bivariate level using a generalized linear model. All *P*-values are two sided with the level of significance set at < 0.05.

Results

A total of 30 patients were included in this study (Table 2). Eighteen patients (60%) had evidence of one or more white

Table 2 Patients' characteristics

Parameter	Value
Mean age ± SD, years (range)	32.1 ± 11 (18–54)
Male:female	13:17
Mean duration since splenectomy ± SD, years (range)	17 ± 9.9 (2–36)
Transfusion history, *n* (%)	
None	18 (60)
Occasional	12 (40)
Mean Hb ± SD, g dL^{-1} (range)	8.6 ± 2.1 (4.9–13.1)
Mean HbF ± SD, % (range)	56.1 ± 30.6 (10.5–98.8)
Mean nucleated RBC count ± SD, × 10^3 mm^{-3} (range)	367.4 ± 319.8 (0–1366)
Mean platelet count ± SD, × 10^3 mm^{-3} (range)	791.2 ± 355.3 (189–1602)
Mean serum ferritin ± SD, ng mL^{-1} (range)	1176 ± 641.9 (116–3158)
Mean LIC ± SD, mg Fe per g dw (range)	11.3 ± 7.8 (1–32.1)

Hb, hemoglobin; HbF, fetal hemoglobin; RBC, red blood cell; LIC, liver iron concentration; dw, dry weight.

Table 3 Distribution of number, location and size of identified white matter lesions (WMLs) on brain MRI

Parameter	*n* (%)
Number	
Single	4 (22.2)
Multiple	14 (77.8)
Location	
Frontal	17 (94.4)
Parietal	9 (50)
Temporal	1 (5.6)
Occipital	3 (16.7)
Internal capsule	1 (5.6)
External capsule	5 (27.8)
Size*	
Small (< 0.5 cm)	10 (55.5)
Medium (0.5–1.5 cm)	7 (38.9)
Large (> 1.5 cm)†	1 (5.6)

*For patients with multiple lesions, the largest lesion was used to define size. †The possibility of misreading confluent multiple lesions was excluded radiologically based on lesion shape.

matter lesions (WMLs) on brain MRI, all involving the subcortical white matter (Table 3). Most of those patients (14 patients) had evidence of multiple WMLs, with a mean of 5 ± 10 lesions (range, 2 to > 40 lesions). The frontal subcortical white matter was nearly always involved, followed by the parietal and occipital subcortical white matter. The external capsule was involved in 28% of patients. The vast majority of patients (94%) had evidence of small to medium WMLs, with only one patient showing evidence of a large WML (Fig. 1). Eleven patients (37%) had evidence of mild cerebral atrophy, 10 of whom had associated WMLs.

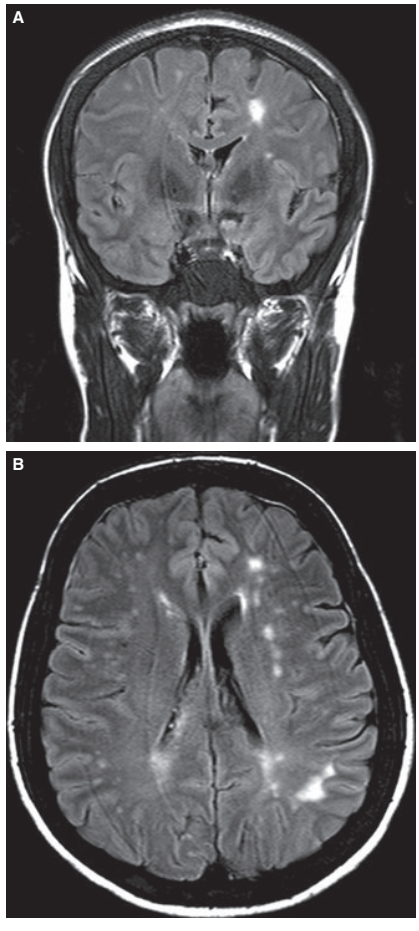

Fig. 1. (A) Coronal and (B) axial FLAIR images showing multiple foci of high signal seen in the subcortical and periventricular white matter with one large lesion (1.7 cm) seen in the left parietal white matter.

Risk factors for white matter lesions

Among all study variables, only age (*t*-test) and transfusion history (chi-square test) were significantly associated with presence of WMLs (Table 4). The mean age was higher in patients who had white matter lesions compared with those who had no lesions (mean age difference of 10 years). A logistic regression analysis to determine the probability of having a WML [Y] at a particular age was performed (Fig. 2) with the following result:

$$[\mathrm{Y}] = \exp(-2.9179 + (0.107846)^{*}\mathrm{age}/1 + \exp(-2.9179 + (0.107846)^{*}\mathrm{age})).$$

This formula had a predictive value of 72.4%.

In addition, in the subgroup of patients with WMLs the mean age of patients who had multiple lesions was higher than those with single lesions (37.1 vs. 30.7 years); although this difference did not reach statistical significance ($P = 0.342$).

Patients who occasionally received transfusions had a significantly lower incidence of WMLs (25%) compared with those who had never received a transfusion (83.3%) ($P = 0.001$). When WMLs were present, patients who were occasionally transfused had a significantly lower incidence of multiple lesions (40%) compared with patients who had never been transfused (92.3%) ($P = 0.017$).

Other variables, including gender, and means of duration since splenectomy, Hb, HbF, nucleated RBC count, platelet count, serum ferritin and LIC, were not significantly associated with the presence or number of WMLs.

On multivariate analysis using a generalized linear model, both age ($P = 0.018$) and transfusion history ($P = 0.008$)

Table 4 Comparison of study variables between patients with and without brain white matter lesions (WMLs)

Parameter	WML neg $n = 12$	WML pos $n = 18$	*P*-value
Mean age (years)	26.1	36.1	**0.011**
Male:female	9:3	8:10	0.098
Mean duration since splenectomy (years)	12.3	18.3	0.156
Transfusion history, *n* (%)			
None	3 (25)	15 (83.3)	**0.001**
Occasional	9 (75)	3 (16.7)	
Mean Hb (g dL^{-1})	7.9	8.8	0.230
Mean HbF (%)	44.8	63.7	0.136
Mean nucleated RBC count ($\times 10^3$ mm^{-3})	413.8	332.7	0.517
Mean platelet count ($\times 10^3$ mm^{-3})	862.7	737.5	0.366
Mean serum ferritin (ng mL^{-1})	1083	1296	0.396
Mean LIC (mg Fe per g dw)	10.3	11.9	0.052

WML, white matter lesion; MRI, magnetic resonance imaging; Hb, hemoglobin; HbF, fetal hemoglobin; RBC, red blood cell; LIC, liver iron concentration; dw, dry weight. Bold values are those that are statistically significant ($P < 0.05$).

Fig. 2. Logistic regression analysis for the probability of having white matter lesions (WMLs) with increasing age.

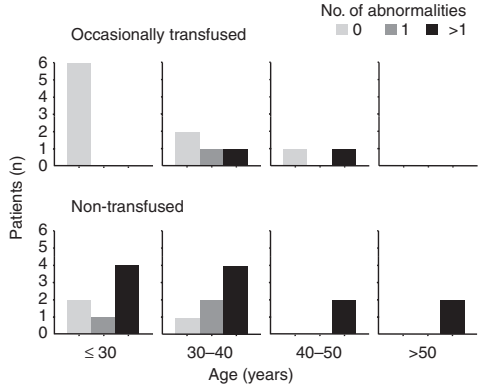

Fig. 3. A histogram showing the number of patients having no, single or multiple white matter lesions (WMLs) according to age and transfusion history.

were independently and significantly associated with the occurrence of zero, single or multiple WMLs (Fig. 3). Among all study variables, only age was significantly associated with presence or absence of brain atrophy. The mean age was higher in patients with (38.5 years) than in those without (28.3 years) brain atrophy ($P = 0.011$).

Discussion

In our study, brain MRI evaluation demonstrates that WMLs and brain atrophy are a common finding in adult, splenectomized, TI patients. Moreover, increasing age and transfusion naivety are associated with a higher incidence and multiplicity of lesions. Our study echoes that of Manfre *et al.* [4] on 16 TI patients, and further brings attention to this previously uninvestigated complication in TI patients. The higher incidence (60% vs. 37%) and multiplicity (77.8% vs. 12.5%) of WMLs in our cohort compared with that of Manfre's may be attributed to higher patient age (mean age 32.1 vs. 29 years) and imaging modality used (3.0 T-MRI vs. 0.5 T-MRI).

Although neither our study nor that of Manfre *et al.* [4] had a control group, the incidence of WMLs in the aging brain of healthy volunteers has been investigated. High incidence of WMLs among healthy elderly populations (e.g. those aged greater than 70) has been reported in many studies [6–11]. However, relatively fewer brain MRI studies recruited populations (or samples) of healthy individuals < 50 years of age (Table 5) [12–18]. The outcome of these studies reveals that the frequency of WMLs and/or cerebral atrophy in healthy young individuals ranges from 0% to 11%. Comparing this figure with the frequency observed in our patients (60%), it seems more likely that the changes described in this report are pathological rather than normal variations. This observation is similar to that reported in patients with sickle cell disease (SCD), where incidence of silent brain ischemia has been well studied and reports document occurrences up to 83% [19].

The presence of WMLs raises the suspicion of several underlying diagnoses. However, in our patients these diagnoses may be ruled out based on the radiological appearance of lesions (e.g. viral encephalopathy or multiple sclerosis) or absence of associated risk factors and clinical symptoms (e.g. vasculitis or Binswanger). Thus, MRI findings in this report most likely represent ischemic lesions. WMLs correspond to increasing severity of ischemic tissue damage, ranging from mild perivascular alterations to large areas with variable loss of fibers, multiple small cavitations, and marked arteriolosclerosis. Microcystic infarcts and patchy rarefaction of myelin are also characteristics of irregular periventricular high signal intensities [20]. These lesions were significantly associated with impaired cognitive skills, suggesting they can be nearly as damaging to cognitive function as overt stroke [21]. In addition to cognitive effects, WMLs have a role in the decline of other functional performances, and this places individuals with higher-grade lesions at increased risk of developing disability [21]. Although patients in this report had no evidence of gross cognitive disabilities, minor and more specific cognitive disability or psychological disease cannot be ruled out.

Table 5 Summary of brain MRI studies that recruited a population (or sample) of healthy individuals < 50 years of age

Reference	*n*	Mean age* (range), years	Abnormality	Frequency (%)
Fazekas 1989 [12]	87	(31–83)	WML	11% for patients in 4th decade
Salonen 1997 [13]	23	(30–53)	WML	0
Katzman 1999 [14]	1000	30.6 (3–83)	WML	0.8
Hopkins 2006 [15]	243	(16–65)	WML	5.3%
Weber 2006 [16]	750	(45–59)	WML	7.2%
Vernooij 2007 [17]	2536	20.5 (17–35)	Atrophy	0.43
Yamada 2008 [18]	16 206	70 (39–90)	WML	0.37

WML, white matter lesions > 0.5 mm. *Where available.

The anemia in TI does not seem to contribute to the pathogenesis of WMLs, as in our analysis there was no statistically significant difference in mean hemoglobin level of patients with and without WMLs. The literature only supports a role for acute anemia in cerebral injury of perioperative and critical care patients [22,23], increased morbidity and mortality in patients with acute anemia and first-ever stroke [24], and a potential role for chronic anemia in cerebral injury of patients with multiple stroke-related risk factors [25]. Thus, it seems less likely that chronic anemia in our patients caused WMLs. However, the hemolytic anemia implicated in patients with TI causes iron-dependent oxidation of membrane proteins and formation of red-cell 'senescence' antigens such as phosphatidylserine that cause thalassemic red cells to be rigid and deformed and to aggregate, resulting in premature cell removal. Studies have shown that, as such, thalassemic RBCs may be a source of negatively charged phospholipids, which can eventually increase thrombin generation [26–32]. These abnormalities have been reduced to normal range after the patients have received a blood transfusion, which decreases the number of circulating damaged RBCs [33]. This could partly explain why patients in our study who were never transfused had a higher incidence of WMLs than patients receiving occasional transfusions. As such, several trials in patients with SCD demonstrated a significantly reduced risk of silent strokes in participants receiving blood transfusions [34,35]. This should sound promising for patients with TI; however, it should be balanced against the iron loading secondary to chronic transfusions, especially in this patient population with high susceptibility to iron overload secondary to endogenously increased intestinal iron absorption [36].

Clinical observations have suggested that splenectomy in TI can contribute to an increased susceptibility to thrombosis [2,3]. The development of these complications has been ascribed to the presence of high platelet counts following splenectomy [37,38] and/or to increased number of nucleated RBCs [39]. In splenectomized TI patients, thrombin generation was significantly higher than in control subjects and patients who had not undergone splenectomy [3]. The high incidence of ischemic lesions in our splenectomized population may be attributable to such pathophysiology. The contribution of splenectomy to hypercoagulability in other hemolytic anemias (excluding SCD where vasculopathy and sickling dominate the picture, and TM where the presence of co-morbidities defines the risk of stroke) may warrant similar brain MRI evaluation, as cerebrovascular events are increasingly being reported [40,41]. However, the lack of association between the duration since splenectomy, nucleated RBC levels, platelet counts and the incidence of brain pathology questions the clinical implication of the proposed mechanism.

The milder course of TI compared with TM has provided patients with survival benefit. However, this increased life expectancy is not without its own side-effects. Our study further adds to the role of age in accumulating complications in TI patients by demonstrating that both WMLs and atrophy may eventually ensue. This brings further attention to our aging TI patient population. It directly calls for earlier intervention to prevent serious long-term sequelae and fortifies the notion that thalassemia is transforming into an adult disease [42]. If the pathogenesis of these lesions is to be understood and potentially other modifiable factors to be identified, the study of individuals in the earliest stages of development of the lesions would be valuable.

Further research is required to examine the use of transfusion in preventing brain ischemia in adult, splenectomized, TI patients. The need to delay or halt the progression of WMLs should lead to further clarification of the role of some risk factors and performance of therapeutic trials where WMLs are used as a surrogate marker for the endpoint of small-vessel disease.

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Disclosure of Conflict of Interests

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Brain Magnetic Resonance Angiography In Splenectomized Adults With β-thalassemia Intermedia

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Brain magnetic resonance angiography in splenectomized adults with β-thalassemia intermedia

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Abstract

Background: Hypercoagulability and venous thromboembolism are common in patients with β-thalassemia intermedia (TI), especially in the splenectomized adult. Although arterial involvement is not commonly reported, we have recently observed a high prevalence (60%) of silent brain infarction on brain MRI in 30 splenectomized adults with TI. The pathophysiology of these white matter lesions remains unknown. *Methods:* In this work, we evaluated magnetic resonance angiography (MRA) scans of the same cohort of 30 patients. Data collected were the presence or absence of vascular lesions, their locations, and severity. Correlations between MRA abnormality and patients/disease characteristics were evaluated. Comparisons between MRA and previous MRI findings were made. *Results:* Of 29 evaluable patients, 8 (27.6%) had evidence of arterial stenosis on MRA. The majority of lesions had mild narrowing and mostly involved the internal carotid artery. Five patients (17.2%) had evidence of aneurysms. Low total hemoglobin and high non-transferrin-bound iron levels independently characterized patients with evidence of stenosis on MRA. Among the 18 patients with silent brain infarction on MRI, three had evidence of stenosis on MRA with only one patient having lesions that could explain the silent infarcts. *Conclusions:* Cerebral vasculopathy is common in splenectomized adults with TI. However, large-vessel disease does not explain the occurrence of silent brain infarction. The combined use of MRA and MRI better identifies splenectomized TI adults with neuroimaging abnormalities.

Key words thalassemia intermedia; splenectomy; brain; silent stroke; magnetic resonance angiography

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Significant advances have been attained toward the understanding and management of stroke in patients with sickle cell disease (SCD) (1). However, data on patients with thalassemia syndromes remain limited. A hypercoagulable state has been recognized in thalassemia (2). It is mainly attributed to the procoagulant activity of hemolyzed red blood cells and activated platelets, especially in splenectomized patients, as well as coagulation factor abnormalities and endothelial inflammation (3). The largest epidemiological study to date examined data from 8860 patients in the Mediterranean area and Iran and showed that thromboembolic events occur 4.38 times more frequently in patients with β-thalassemia intermedia (TI) than in patients with β-thalassemia major (TM) (4). Thromboembolic events in patients with TI are mostly venous, and their occurrence increases with age (4–6). The risk of thromboembolism is also higher in splenectomized and never-transfused patients (4, 6–8).

Strokes, on the other hand, are less frequent in patients with TI compared with TM patients (28% vs. 9%, respectively) (4). This could be attributed to the higher rate of iron overload-mediated morbidity (diabe-

tes mellitus, cardiac dysfunction and arrhythmias) in patients with TM, which could increase stroke risk (9). Nevertheless, one study showed that 37.5% of patients with TI have evidence of silent brain infarction on magnetic resonance imaging (MRI) (10). More recently, we (11) and others (12) conducted brain MRI screening studies on patients with TI who had no history of neurological events. In our 30 splenectomized adults with TI, the rate of silent brain infarction was as high as 60% and involved the subcortical white matter in all patients (11). Such a high rate reflected a pathological finding, as no more than 10% of healthy individuals show incidental ischemic lesions on brain MRI. Moreover, other neurological pathologies causing white matter lesions were excluded (11). However, the mechanisms contributing to silent brain infarction in patients with TI remain unknown. We herein evaluate magnetic resonance angiography (MRA) scans of the same 30 patients to determine whether large-vessel pathology can account for the high rate of silent brain infarction in splenectomized adults with TI.

Patients and methods

Patients

This was a cross-sectional study conducted on all splenectomized adult (≥18 yr) patients with TI attending the Chronic Care Center (Lebanon) over a period of 6 months. All patients were diagnosed with TI based on previously described criteria (13). None of the patients had Hb-S-thalassemia, C-thalassemia, E /β-thalassemia or $\delta\beta$-thalassemia, co-inheritance of α-thalassemia or co-inheritance of determinants associated with increased γ-chain production.

A total of 43 patients were screened for exclusion criteria: neurological and/or gross cognitive signs or symptoms (abnormality detected during medical history taking, neurological exam or mini mental status exam performed by a qualified neurologist); history of anticoagulant or antiplatelet therapy; diabetes (use of antidiabetic drugs or a fasting blood sugar ≥ 126 mg/dL); hypertension (use of antihypertensive drugs or a blood pressure ≥ 140/90 mm Hg on two readings 6 wk apart); cardiac disease (any abnormality on electrocardiography or echocardiography including: arrhythmias, valvular disease, dysfunction, presence of atrial or ventricular thrombi, or pulmonary hypertension); thrombophilia (evidence of factor V Leiden, prothrombin, or methylenetetrahydrofolate reductase gene mutations or abnormality in protein C, protein S, antithrombin III, Lupus anticoagulant, or cardiolipin antibody levels); and smoking (any current or previous history of smoking). Thirty eligible patients were included in the study. The study was approved by the Institutional Review Board of the center, and written consents were obtained from all patients.

Patient charts were reviewed for demographics (age and gender) and any history of transfusion therapy. Blood samples were obtained for assessment of total hemoglobin level, nucleated red blood cell (NRBC) and platelet counts, and steady-state serum ferritin levels. Levels of non-transferrin-bound iron (NTBI) were also measured as previously described (14). Direct determination of liver iron concentration (LIC) was performed by R2 MRI using established methodology (15).

Brain imaging

All patients underwent MRI of the brain followed by MRA of the extracranial and intracranial circulation. Two neuroradiologists blinded to the clinical data reviewed the studies.

Brain MRIs were performed on a 3.0 Tesla, eight channel head coil, Achieva Philips Scanner using axial T1-weighted images (TR/TE, 450/10), T2 gradient-echo weighted images (TR/TE, 731/16), fluid-attenuated inversion recovery (FLAIR) images (TR/TE, 11 000/125) and diffusion-weighted imaging (TR/TE, 2312/68). Coronal FLAIR images (TR/TE, 11 000/125) as well as coronal and sagittal T2-weighted images (TR/TE, 3000/80) were also obtained. No contrast material was administered. Infarction or ischemic lesions were defined as areas of abnormally increased signal intensity on the T2- and FLAIR-weighted sequences and were classified by anatomic location and size. The size of lesions was classified into small (< 0.5 cm), medium (0.5–1.5 cm), and large (> 1.5 cm). For patients with multiple lesions, the largest lesion was used to define size.

Brain MRA was performed using three-dimensional time-of-flight (TOF) angiography of the circle of Willis with maximal intensity projection (MIP) reconstruction. Sequence parameters used were TR = 23 ms, TE = 3.453 ms, 200 slices, 0.8 mm thick and a directional field of view of 20 cm. Both the MRA source images and the MIP derived from the MRA data were evaluated. Data collected were the presence or absence of vascular lesions, their locations, and severity. The internal carotid arteries (ICA), middle cerebral arteries, anterior cerebral arteries (ACA), posterior cerebral arteries (PCA), as well as the vertebral and basilar arteries were rated from normal to occluded. Arterial segments were defined to be normal or to be mildly (≤50%), moderately (51–75%), or severely (> 75%) stenosed, or totally occluded (100%) (16). The presence of aneurysm was also recorded. All MRAs were reviewed by two neuroradiologists who were blind to the patient's clinical history. After that, the two results' forms were compared

and discrepant findings were reviewed until a consensus was reached.

Statistical analysis

Descriptive data are presented as means ± SD or percentages. Bivariate correlations between MRA abnormality and study parameters were evaluated using the independent samples *t*-test, the Chi-square test, and the Fisher's exact test. Multivariate regression models were constructed to determine the variables independently associated with MRA abnormality. All *P*-values were two-sided with the level of significance set at < 0.05.

Results

MRA findings

A total of 30 patients were included in this analysis (Table 1). One patient had a non-evaluable MRA scan. In the remaining 29 patients, 8 (27.6%) had evidence of arterial stenosis. Two patients (25%) had more than one artery involved (one had two and the other had four arteries) (Fig. 1), while the remaining 6 (75%) had a single stenosed cerebral artery. The ICA was the most commonly involved artery (in six of eight patients, 75%). Among the 12 identified stenotic lesions, two were severe (16.7%), one was moderate (8.3%), and the remaining 9 (75%) were mild (Table 2).

Moreover, five patients (17.2%) had evidence of aneurysms. Three patients had a single aneurysm: one in the left ICA (2 mm), one in the right superior cerebellar artery (3 mm), and one fusiform aneurysm of the right PCA. The other two patients had multiple aneurysms: the first patient had two aneurysms in the right ICA (3 mm) and the other patient had four aneurysms. In this last case, three aneurysms were located in the right ICA ranging from 2 mm to 1 cm and one fusiform aneurysm was located in the A1 segment of the right ACA (Fig. 2).

Table 1 Patients' characteristics

Parameter	Value
Mean age ± SD (range), yr	31.9 ± 11 (18–54)
Men : women	13 : 17
Transfusion, *n* (%)[1]	
None	18 (60)
Occasional	12 (40)
Mean total hemoglobin ± SD (range), g/L	86.4 ± 20.8 (49–131)
Mean NRBC count ± SD (range), $\times 10^3/mm^3$	367.4 ± 319.8 (0–1366)
Mean platelet count ± SD (range), $\times 10^9$/L	791.2 ± 355.3 (189–1602)
Mean serum ferritin ± SD (range), μg/L	1176 ± 641.9 (116–3158)
Mean NTBI ± SD (range), μM	3.4 ± 3.4 (–2.1 to 10)
Mean LIC ± SD (range), mg Fe/g dw	11.3 ± 7.8 (1–32.1)

NRBC, nucleated red blood cell; NTBI, non-transferrin-bound iron; LIC, liver iron concentration; dw, dry weight.

[1]Patients were occasionally transfused during infections, surgery, or pregnancy. None of the patients were on iron chelation or hydroxyurea therapy.

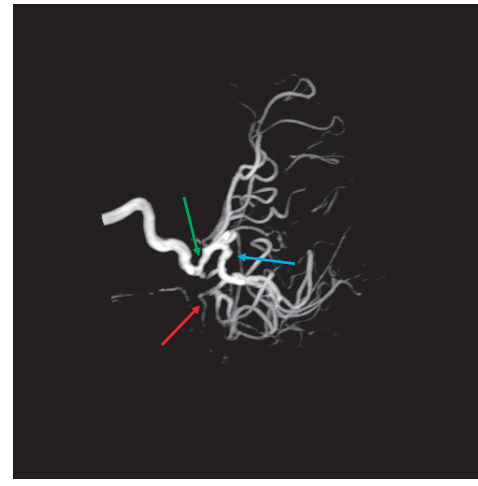

Figure 1 A 37-yr-old female patient. Coronal oblique maximal intensity projection of the magnetic resonance angiography of the anterior circulation demonstrated severe stenosis and almost occlusion of the cavernous segment of the left internal carotid artery (ICA) (red arrow) and mild narrowing of the cavernous portion of the right ICA of <50% (green arrow). There was also irregularity and fusiform enlargement of the A1 segment of the right anterior cerebral artery (blue arrow).

Risk factors for MRA abnormality

There was no statistically significant correlation between evidence of stenosis on MRA and any of age, gender, transfusion history, NRBC count, platelet count, serum ferritin level, or LIC. However, the mean total hemoglobin level was significantly lower in patients with evidence of stenosis on MRA than those without (71.3 vs. 91.1 g/L, $P = 0.018$). Moreover, the mean NTBI level was significantly higher in patients with evidence of stenosis on MRA than those without (6.4 vs.2.3 μM, $P = 0.002$).

Using both total hemoglobin level and NTBI level as independent variables in a generalized linear model, both variables were significant in explaining the dependent categorical variable stenosis on MRA ($P = 0.046$ for total hemoglobin and $P = 0.039$ for NTBI). A logistic

Table 2 Patients with evidence of stenosis on MRA and their corresponding brain MRI findings

Patient	MRA stenosis		MRI infarction		
	Location	Severity	Location	Number	Size (cm)
1	Right ICA	Mild	Bilateral frontal	23	<0.5 (21), 0.5–1.5 (1), >1.5 (1)
	Left ICA	Severe	Bilateral parietal	21	<0.5 (20), >1.5 (1)
	Left MCA-M3	Mild	Bilateral occipital	3	<0.5 (3)
	Left PCA	Severe	Bilateral EC	3	<0.5 (3)
2	Right ICA	Mild	Bilateral frontal	8	<0.5 (8)
			Left occipital	1	<0.5 (1)
			Left EC	1	<0.5 (1)
3	Left ACA	Mild	Bilateral frontal	4	<0.5 (4)
4	Right ICA	Mild	None		
5	Right MCA-M2	Moderate	None		
6	Right ICA	Mild	None		
7	Left ICA	Mild	None		
	Left PCA	Mild			
8	Right ICA	Mild	None		

MRA, magnetic resonance angiography; MRI, magnetic resonance imaging; ICA, internal carotid artery; MCA, middle cerebral artery; ACA, anterior cerebral artery; PCA, posterior cerebral artery; EC, external capsule.

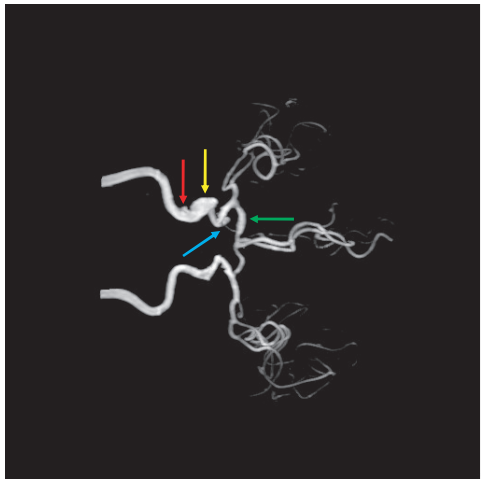

Figure 2 A 45-yr-old female patient. Coronal oblique maximal intensity projection of the magnetic resonance angiography of the anterior circulation demonstrated fusiform aneurysm of the petrous portion of the right internal carotid artery (ICA) (yellow arrow) and a tiny saccular aneurysm of the same segment (red arrow). Also noted were a third 4-mm saccular aneurysm of the cavernous portion of the right ICA (blue arrow) and a fusiform enlargement of the A1 segment of the right anterior cerebral artery (green arrow).

regression model was also performed to estimate the probability of stenosis on MRA using total hemoglobin level and NTBI (Fig. 3). The model was significant ($P < 0.001$) and had a predictive value of 82.76%.

MRA vs. MRI

Among the 30 patients, 18 (60%) had evidence of silent brain infarcts on MRI, all within the subcortical white matter. Fourteen (77.8%) had evidence of multiple white matter lesions (2 to >40). The frontal ($n = 17$, 94.4%) and parietal ($n = 9$, 50%) lobes were the most commonly involved. Patients mostly had small ($n = 10$, 55.5%) or medium ($n = 7$, 38.9%) lesions, with only one patient (5.6%) having a large lesion.

Among the 18 patients with MRI abnormalities, three had evidence of stenosis on MRA (patients 1–3 in Table 2), with only one patient having extensive lesions that could explain the silent infarcts (patient 1 in Table 2). The remaining two patients (patients 2 and 3 in Table 2) had large-vessel involvement that does not geographically explain the silent infarcts. MRA identified five additional TI patients with vascular abnormalities that have normal MRIs (Table 2).

Discussion

This is the first MRA study of patients with TI. It demonstrates that although large-vessel disease is a common finding in splenectomized adults with TI, it does not explain the occurrence of silent brain infarction at high rates. These findings could constitute the first step toward understanding cerebral vasculopathy in this patient population.

Around one-third of patients in this report had evidence of stenosis in at least one major cerebral artery. High levels of NTBI characterized this subgroup of patients. NTBI is a low-molecular-weight form of iron that is directly detected when transferrin becomes fully

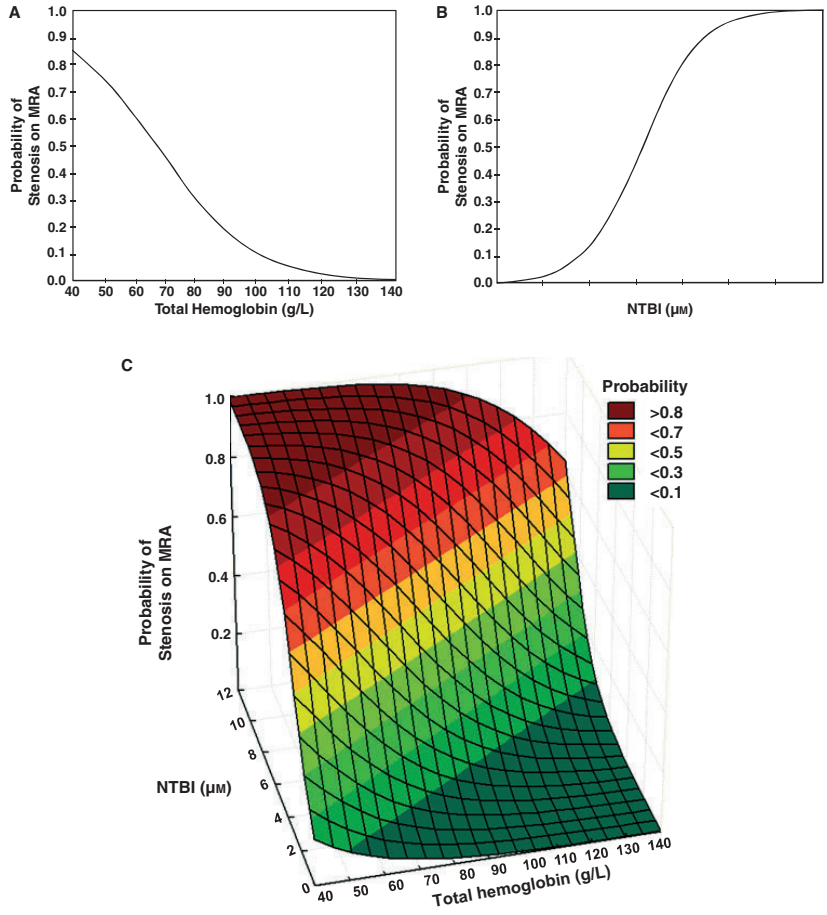

Figure 3 Logistic regression curves showing the probability or stenosis on magnetic resonance angiography as a function of (A) total hemoglobin level, (B) non-transferrin-bound iron (NTBI) level, and (C) both total hemoglobin and NTBI levels.

saturated and is unable to bind excess iron (14). It has been demonstrated that the presence of NTBI in serum can cause oxidative vessel injury (17). Free radicals act directly on the endothelial cells and have a close interaction with lipid peroxidation, causing a modification of low-density lipoprotein and facilitating its deposition, with the consequent formation of atherosclerotic plaques (18). Thus, iron-mediated endothelial dysfunction and a secondary atherosclerotic process may explain cerebral large-vessel disease in patients with TI and echo recent studies supporting the idea that patients with TI exhibit a proatherogenic biochemical phenotype (19, 20). Such large-vessel injury could occur early on with direct iron toxicity (there was no correlation between MRA abnormality and age in our study) and does not require chronic accumulation of iron over time (absence of correlation with LIC or serum ferritin). Whether thrombosis, in this disease with hypercoagulability, is a secondary process or incremental in vessel narrowing warrants investigation. This could be achieved by further study using Doppler/duplex sonography, which can give a better understanding of the morphology (etiology) of stenosis. It should also be noted that five patients (17.2%) had evidence of aneurysms, a prevalence that is higher

than that documented in the healthy adult population (approximately 1–2%) (21) and that is similar to observations in patients with SCD (22, 23). The aforementioned assumptions of vessel wall pathology could be extended to explain the occurrence of these aneurysms.

An association between anemia and cerebrovascular stenosis has never been documented. The association of lower hemoglobin levels with stenosis on MRA in our study may be reflecting an indirect link between phenotype severity and vascular abnormality. Although transfusion status did not correlate with stenosis, most patients in our study were only occasionally transfused in special circumstances, and the role of regular transfusion therapy in ameliorating this large-vessel disease requires further investigation.

Only one of 18 TI patients with silent brain infarction had relevant stenosis on MRA, indicating that large-vessel disease is not a contributing factor in the pathophysiology of these silent white matter lesions. The situation is similar to what have been often described in patients with SCD. It is now established that the majority of symptomatic strokes in patients with SCD are secondary to large-vessel disease. However, the absence of large-artery disease is especially apparent in the context of silent infarcts (24). Whether these infarcts are, thus, secondary to hypercoaulability and smaller arteriolar pathology merits further evaluation.

Silent infarcts observed on brain MRI are associated with a high risk, subsequent overt stroke, and neurocognitive deficits, as evident from several reports on children and adults with SCD (25–31). Furthermore, SCD patients with abnormal MRA findings are at higher risk for stroke (16). Our study demonstrated that the addition of MRA to MRI identifies a greater proportion of TI patients with silent neuroimaging abnormalities. As these abnormalities maybe associated with increased risk of overt stroke or neurocognitive dysfunction, performing both modalities in any diagnostic setting is recommended. Prospective evaluation of a risk assessment model for the development of these abnormalities may help develop a cost-effective screening program of high-risk patients (e.g., patients with severe anemia or iron overload). Such models should also accurately predict which patients are at higher risk of subsequent events and require therapeutic intervention. The role of transfusion and antiplatelet therapy in this regard merit further investigation.

Our study carries several limitations. Using three-dimensional time-of-flight angiography, both high flow and low flow can result in signal void, and high-grade stenoses might, therefore, be misclassified as complete occlusions. A more reliable distinction could be achieved with contrast-enhanced MRA. However, no cases of complete occlusion were detected in this report, making the aforementioned limitation irrelevant. Further, TOF angiography does not provide any information on the etiology (i.e., atherothrombotic vs. embolic vs. dissection, etc.) of the stenosis. Assuming that large-vessel disease in patients with β-thalassemia intermedia might be due to arteriosclerosis as discussed by the authors, it would be desirable to support this hypothesis by imaging data. The MR data should, therefore, be supplemented by Doppler/duplex sonography. Besides precise information on the morphology of stenosis, the suspected degree of stenosis could then be verified. Moreover, our study did not include age-matched healthy individuals as controls. However, in one review of 2000 healthy persons with a mean age of 63.3 yr, the rate of major-vessel stenosis found incidentally on MRI was only 0.5% (95% CI: 0.2–0.8) (32). In another MRA-based study, the rate of mild stenosis ($< 50\%$) on MRA was only 4% (95% CI: 1.4–6.6) in 225 healthy individuals with a mean age of 63 yr (33). Recent large studies in the general population with mean age between 20 and more than 70 yr of age found prevalences of intracranial aneurysms to be 0% (34, 35), 0.1% (95% CI: 0–0.2) (35), 0.2% (95% CI: −0.1 to 0.5) (36), 1.8% (95% CI: 1.2–2.4) (32), and 2% (95% CI: 1.7–2.3) (37). Together, these findings indicate that the vascular abnormalities identified on MRA in our report [large-vessel stenosis 27.6% (95% CI: 11.3–43.9) and intracranial aneurysms 17.2% (95% CI: 3.5–30.9)] are pathological rather than normal variations; especially that the mean age in our cohort (32 yr) is much younger than that reported in the aforementioned references from healthy individuals.

Our study demonstrated that cerebral vasculopathy, evident from large-vessel disease, is common in splenectomized adults with TI. However, it does not explain the occurrence of silent brain infarction. The combined use of MRA and MRI could, thus, better identify splenectomized TI adults with neuroimaging findings that are commonly associated with a high risk for future stroke or functional neurologic deficits.

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Conflict of interests

ATT is a member of Novartis Speakers' Bureau.

Authors' contributions

Study design: KMM, AB, ATT; data collection and assembly: KMM, RH, RR, SK; data analysis and interpretation: KMM, AB, WN, ATT; analysis review and

manuscript preparation: KMM, AB, RH, WN, ATT; final approval for submission: KMM, AB, RH, WN, RR, SK, ATT.

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Brain Positron Emission Tomography In Splenectomized Adults With β-thalassemia Intermedia: Uncovering Yet Another Covert Abnormality

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Brain positron emission tomography in splenectomized adults with β-thalassemia intermedia: uncovering yet another covert abnormality

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Abstract Covert brain infarction is an emerging concern in patients with β-thalassemia intermedia (TI). We have recently observed a high prevalence (60%) of silent brain infarction on brain magnetic resonance imaging (MRI) in 30 splenectomized adults with TI. In this work, we further evaluate cerebral involvement in the same 30 patients using fluorodeoxyglucose positron emission tomography–computed tomography (PET-CT) scanning. The median age was 32 years (range, 18–54 years) with a male to female ratio of 13:17. Nineteen patients (63.3%) had evidence of decreased neuronal function on PET-CT. Involvement was mostly left sided, multiple, and most commonly in the temporal and parietal lobes. Elevated liver iron concentration, beyond 15 mg Fe/g dry weight, characterized patients with decreased neuronal function. The concordance rate between brain MRI and PET-CT for the detection of brain abnormality was only 36.7% (Kappa 0.056, P=0.757), highlighting that both modalities reveal different types of brain pathology. Decreased neuronal function is a common finding in patients with TI and is associated with iron overload. Moreover, the addition of PET-CT to MRI identifies a greater proportion of TI patients with silent neuroimaging abnormalities.

Keywords Thalassemia intermedia · Splenectomy · Brain · PET · Iron overload

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Introduction

It is now apparent that β-thalassemia intermedia (TI) carries more complexity than traditionally recognized [1]. Patients with TI have milder anemia compared to patients with β-thalassemia major (TM), usually present later in childhood, and remain largely transfusion independent [2]. However, the diagnosis of TI is now also associated with several serious morbidities like thromboembolic phenomena [3]. Hypercoagulability in TI results from a combination of several factors including a procoagulant activity of hemolyzed circulating red blood cells, increased platelet activation, coagulation factor defects, depletion of antithrombotic factors, endothelial inflammation, among others

[3]. Clinically, the risk of thromboembolic events increases with age [4–6] and is much higher in splenectomized and never-transfused patients [5–8] in whom hypercoagulability is thought to be much more prominent [7, 9]. Reported thromboembolic events were most commonly venous [5–8]. Strokes, on the other hand, are less frequent in TI compared to TM patients [6] since patients with TM have several other comorbidities that increase stroke risk like diabetes mellitus, cardiac dysfunction, and arrhythmias [10]. Nevertheless, one study showed that 37.5% of patients with TI have asymptomatic brain damage on magnetic resonance imaging (MRI) [11]. In this line, we conducted a brain MRI study on 30 splenectomized adults with TI who were neurologically intact [12]. The rate of silent brain infarcts was as high as 60%. The occurrence and multiplicity of the detected lesions were associated with older age and transfusion naivety [12]. In this current work and for the first time, we evaluate the results of brain fluorodeoxyglucose (^{18}F-FDG) positron emission tomography–computed tomography (PET-CT) scanning in the same 30 patients to further understand cerebral involvement in this patient population.

Materials and methods

Patients

This was a cross-sectional study conducted on all splenectomized TI patients aged 18 years or older (n=43) attending the Chronic Care Center (Lebanon) between June and December 2008. All patients were diagnosed with TI based on described criteria [13]. None of the patients had Hb S, Hb C, Hb E/β, or δβ-thalassemia, coinheritance of α-thalassemia, or coinheritance of determinants associated with increased γ chain production. Exclusion criteria, actively screened for, are summarized in Table 1. After screening patients for exclusion criteria, 30 patients were found eligible and were recruited in the study. The study was approved by the institutional review board of the center and written consents were obtained from all patients.

Patient charts were reviewed for demographics (age and gender) and any history of transfusion therapy. Blood samples were obtained for the assessment of total hemoglobin level, platelet counts, and steady-state serum ferritin levels. Direct determination of liver iron concentration (LIC) was performed by R2 MRI using established methodology [14]. Brain MRI and PET-CT studies were done for all patients on the same day.

Brain MRI

Brain MRIs were conducted as previously published [12]. In brief, they were performed on a 3.0-T, eight-channel head coil, Achieva Philips Scanner using axial T1-weighted images (repetition time/echo delay time (TR/TE), 450/10), T2-weighted gradient echo images (TR/TE, 731/16), fluid-attenuated inversion recovery (FLAIR) images (TR/TE, 11,000/125), and diffusion-weighted imaging (TR/TE, 2,312/68). Coronal FLAIR images (TR/TE, 11,000/125) as well as coronal and sagittal T2-weighted images (TR/TE, 3,000/80) were also obtained. No contrast material was administered. Two blinded neuroradiologists reviewed the studies, looking for ischemic lesions. Infarction or ischemic lesions were defined as areas of abnormally increased

Table 1 Exclusion criteria

Criterion	Definition and assessment method
Neurological and/or gross cognitive signs or symptoms	Abnormality detected during medical history taking, neurological exam, or MMSE performed by a qualified neurologist (AB)
Use of anticoagulant or antiplatelet therapy	Any current or previous history of anticoagulant or antiplatelet therapy
Diabetes	Use of antidiabetic drugs or a fasting blood sugar ≥126 mg/dl
Hypertension	Use of antihypertensive drugs or a blood pressure ≥140/90 mmHg on two readings 6 weeks apart
Cardiac disease	Any abnormality on electrocardiography or echocardiography including: arrhythmias, valvular disease, dysfunction, presence of atrial or ventricular thrombi, or pulmonary hypertension
Carotid stenosis	Evidence of >50% narrowing of the carotid(s) on color-flow duplex scanning
Thrombophilia	Evidence of factor V Leiden, prothrombin, or MTHFR mutations on genetic studies; or abnormality in protein C, protein S, antithrombin III, lupus anticoagulant, or cardiolipin antibodies levels
Smoking	Any current or previous history of smoking

MMSE mini mental status exam, *MTHFR* methyletetrahydrofolate reductase

signal intensity on the T2- and FLAIR-weighted sequences and were classified by anatomic location.

PET-CT

Brain PET-CT was done on the same day as MRI for all patients. Before undergoing PET-CT, patients were asked to fast for at least 6 h, although oral hydration with glucose-free water was allowed. After ensuring a normal blood glucose level in the peripheral circulation, the patients received an intravenous injection of 370 MBq (10 mCi) ^{18}F-FDG and allowed to rest for 45 min before undergoing scanning. Scans were acquired with a PET scanner combined with a multisection CT scanner (Biograph 6, Siemens). The axes of the two systems were mechanically aligned such that a patient can be moved from the CT to the PET gantry by moving the examination table. CT scanning of the brain was performed according to a standardized protocol, and immediately afterwards, PET scanning was performed with the identical transverse field of view. The acquisition time for PET in static mode was 30 min. The CT data were resized from a 512×512 to a 128×128 matrix to match the PET data so that scans can be fused and CT-based transmission maps generated. PET data sets were reconstructed iteratively using an ordered subset expectation maximization algorithm with segmented attenuation correction. Coregistered scans were then displayed on a workstation with commercially available software (e.soft from Siemens). Visual assessment was determined by two blinded nuclear medicine physicians. Visual (qualitative) interpretation was based on the subjective impression of the degree of ^{18}F-FDG uptake. Decreased uptake was defined as the relative decrease in ^{18}F-FDG uptake in a lobe compared to other lobes, which reflects a decrease of neuronal function (glucose utilization). Review was done independently, and in case of disagreement (two cases), the two experts reviewed the images jointly until a consensus was reached.

Statistical analysis

Descriptive data are presented as medians (range) or percentages. Bivariate correlations between PET-CT abnormality and study parameters were evaluated using the Mann–Whitney *U* test, the Chi-square test, and the Fisher's exact test. A logistic regression analysis was performed to evaluate the probability of a decreased neuronal function on PET-CT, using the variable found to be statistically significant in the bivariate analysis as an independent continuous variable. To determine the optimal variable cutoff for the logistic regression equation that best predicts PET-CT abnormality, receiver operating characteristic (ROC) curve analysis was performed [15]. A concordance Kappa value was calculated for the agreement between brain MRI and PET-CT for the detection of abnormalities. A Kappa value of 1 indicates complete agreement and a value of 0 indicates no agreement at all. All *P* values were two-sided with the level of significance set at <0.05.

Results

Patients' characteristics

A total of 30 patients were included in the analysis. The median age was 32 years (range, 18–54 years) with a male to female ratio of 13:17. Most patients were transfusion independent (n=18, 60%) while 12 patients (40%) were occasionally transfused during infections, surgery, or pregnancy. None of the patients were on iron chelation or hydroxyurea therapy. The median total hemoglobin level was 84 g/l (range, 49–131 g/l) and the median platelet count was 789.5×10^9/l (range, 189–$1{,}602\times10^9$/l). The median serum ferritin level was 1,127.5 μg/l (range, 116–3,158 μg/l) and the median LIC was 10.75 mg Fe/g dry weight (dw) (range, 1–32.1 mg Fe/g dw).

PET-CT

Nineteen patients (63.3%) had evidence of decreased neuronal function on PET-CT. Only 1 patient had bilateral brain involvement while the remaining 18 had left brain involvement. Five (26.3%) out of the 19 patients had single lobe involvement while 14 (73.7%) had multiple lobes involved (11 had two, 3 had three lobes involved). The temporal lobe was most commonly involved (n=18, 94.7%), followed by the parietal (n=14, 73.7%) and frontal lobes (n=3, 15.8%). The occipital lobe was not involved in any patient.

Risk factors for decreased neuronal function

There was no statistically significant correlation between the evidence of decreased neuronal function on PET-CT and any of age, gender, transfusion history, total hemoglobin level, or platelet count. The median serum ferritin level was higher in patients with PET-CT abnormality than those without (1,215 vs. 861.5 μg/l), although the association did not reach statistical significance (P=0.053). Moreover, the median LIC was significantly higher in patients with evidence of decreased neuronal activity than those without (16.3 vs. 3.4 mg Fe/g dw, P=0.003). A logistic regression model was also performed to estimate the probability of abnormality on PET-CT using LIC as an independent variable (Fig. 1). The model was significant (P<0.001) and had a predictive value of 76.7%. On ROC curve

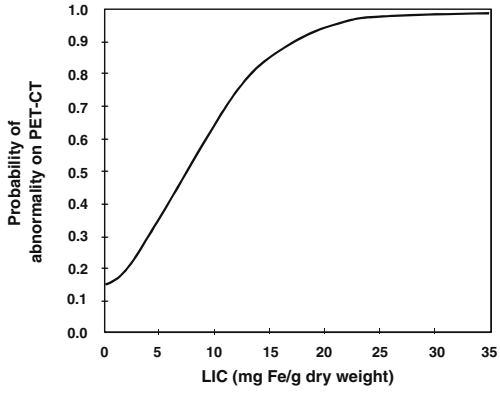

Fig. 1 Logistic regression curve showing the probability or PET-CT abnormality as a function of LIC

analysis, a LIC cutoff of 15 mg Fe/g dw was the best predictor of decreased neuronal function on PET-CT with an area under the curve of 0.828±0.075 (95% confidence interval 0.681–0.975, P=0.003), a sensitivity of 52.6%, and a specificity of 100%.

PET-CT vs. brain MRI

Among the group of 30 patients, 18 (60%) had evidence of silent infarcts on brain MRI, all in the white matter [12]. A total of 11 patients (36.7%) had evidence of brain abnormality on both MRI and PET-CT while 26 patients (86.7%) had evidence of brain abnormality on either MRI or PET-CT (Table 2). The concordance rates between brain MRI and PET-CT were 36.7% for the detection of abnormality (Kappa 0.056, P=0.757); 23.3% for the detection of multiple abnormalities (Kappa 0.062, P= 0.732); 3.3% for the detection of bilateral brain abnormality (Kappa 0.086, P=0.245); and 6.7% (Kappa 0.036, P= 0.713), 10% (Kappa 0.164, P=0.338), 3.3% (Kappa 0.045, P=0.406), and 0% (Kappa N/A, P = N/A) for the detection of frontal, parietal, temporal, or occipital abnormalities, respectively (Fig. 2).

Discussion

Unlike MRI, PET-CT imaging does not seem helpful in detecting silent white matter infarcts in patients with TI. Nevertheless, PET-CT imaging revealed that decreased neuronal function is a common finding in this patient population, which is associated with iron overload. Thus, the addition of PET-CT to MRI identifies a greater proportion of TI patients with silent neuroimaging abnormalities and provides additional information on the neurophysiologic status of these patients.

The association of iron overload, evident from the elevated LIC, with decreased neuronal function on PET-CT in our study is the first report of its kind for patients with hemoglobinopathies. Transfusion-independent patients with TI still develop iron overload due to increased intestinal absorption and show considerably high levels of LIC [16, 17], which may explain why both transfused and nontransfused patients had similar rates of PET-CT abnormality in our study. Although cardiac siderosis and disease do not seem to be a consequence of iron overload in this patient population [18–20], an association with other clinical complications in several organ systems has been observed [5]. Although a causal relationship may be hard to confirm, our study adds brain involvement to the growing list of iron overload-related morbidity in TI patients. Iron is an essential element for the multiple functions of the brain. The abnormal distribution of brain iron has been implicated in neuronal injury and death in several neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease, and amyotrophic lateral sclerosis. Multiple iron chelators have been shown to possess neuroprotective and neuro-

Table 2 Brain abnormalities as detected by MRI or PET-CT in the 30 patients

Parameter	MRI [12]	PET-CT	Both MRI and PET-CT	Either MRI or PET-CT
Abnormal finding	18 (60)	19 (63.3)	11 (36.7)	26 (86.7)
Number of abnormalities				
Single	4 (13.3)	5 (16.7)	1 (0)	8 (26.7)
Multiple	14 (46.7)	14 (46.7)	7 (23.3)	21 (70)
Bilateral abnormality	13 (43.3)	1 (3.3)	1 (3.3)	13 (43.3)
Location of abnormality				
Frontal	17 (56.7)	3 (10)	2 (6.7)	18 (60)
Parietal	9 (30)	14 (46.7)	3 (10)	20 (66.7)
Temporal	1 (3.3)	18 (60)	1 (3.3)	18 (60)
Occipital	3 (10)	0 (0)	0 (0)	3 (10)

MRI magnetic resonance imaging, *PET* positron emission tomography, *CT* computed tomography

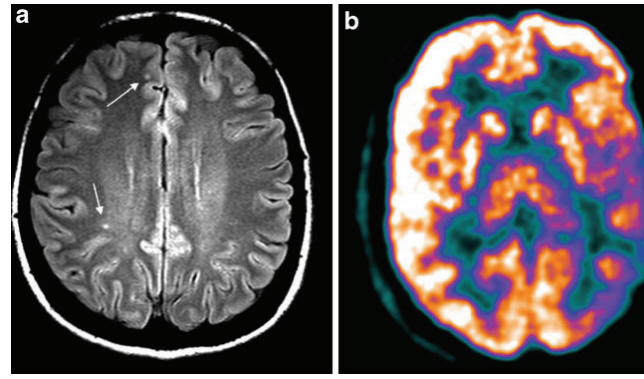

Fig. 2 Example of poor concordance between brain MRI and PET-CT. A 30-year-old female with **a** two ischemic lesions (<0.5 cm, *arrows*) in the right frontal and parietal lobes on MRI and **b** hypometabolism in the left parietal and temporal lobes on PET-CT

restorative properties in these diseases, suggesting that iron chelation might be promising therapeutics [21]. Whether the same applies for patients with TI merits further evaluation. The risk factors for silent brain infarcts detected on MRI remain different. There is no evidence that iron overload can be associated with cerebral small vessel disease or silent infarcts [12]. In fact, transfusion therapy seems to be protective against the development of these silent white matter lesions [12], probably due to the beneficial role of transfusions in improving hypercoagulability and vascular disease in TI by decreasing the concentrations of damaged red blood cells with thrombogenic potential, among other factors [3]. Although the occurrence and multiplicity of silent brain infarcts increase with age in TI patients [12], such observation was not noted for the decrease in neuronal function detected on PET-CT. In line with our findings, it could be hypothesized that neuronal damage could occur early on with direct iron toxicity, unlike vascular damage where the accumulation of risk factors over time may be necessary.

Most acquired knowledge on cerebral involvement in hemoglobinopathies comes from studies on patients with sickle cell disease (SCD) [22]. The application of PET in SCD subjects was first published in 1988 in a preliminary study on six adults who had no history of neurological events but were found to have significant glucose hypometabolism in the frontal areas of the brain [23]. Our finding that MRI and PET-CT reveal different types of silent brain pathology (thus the low concordance rate) is in agreement with a similar study on patients with SCD [24]. Among 30 patients with no evidence of neurological dysfunction, 13 (43%) patients were found to have silent brain infarcts on MRI. PET identified 12 additional subjects, with normal MRI, to have silent brain abnormality (total=83%). Two valid questions are (1) why would PET-CT fail to reveal silent brain infarcts detected on MRI and (2) can patients with decreased neuronal function on PET-CT have normal MRI? First, in the aforementioned study on patients with SCD [24], the concordance rate between abnormal PET and MRI scans was 80% for MRI-identified gray matter lesions, dropping to ~50% for white matter lesions [24]. These observations may be partly attributed to the naturally low glucose utilization in the white matter and could explain the low concordance for silent stroke detection between both imaging modalities, especially in our study where all silent MRI lesions were detected in the white matter. Thus, PET imaging cannot replace MRI to identify white matter lesions in the watershed areas [25–28]. Second, the areas of functional abnormality are usually greater than the structural neuronal loss defined by MRI or CT; thus, PET-CT abnormality can be detected with a normal MRI [29]. Moreover, there was a low concordance rate for the location of abnormality between both imaging techniques. Silent infarcts on MRI were bilateral and most commonly involved the frontal and parietal lobes, whereas neuronal dysfunction evident on PET-CT was mainly left sided, involving the parietal and temporal lobes. Although this may be attributed to the aforementioned difference in the type of brain pathology revealed by the two imaging modalities, it still warrants further discussion. The diffuse nature of silent infarcts on brain MRI in our study and their high prevalence in the frontal and parietal white matter is in total agreement with studies on SCD patients [30, 31]. In SCD patients, it was shown that the geographic distribution of the involved small penetrating arteries in the brain is derived from the carotid rather than the vertebrobasilar circulation, as a result of several anatomic and hemodynamic factors [32]. However, PET-CT abnormalities in this study were mostly detected in the temporal and/or parietal lobes. Temporoparietal hypometabolism on ^{18}F-FDG PET is indeed the classic metabolic abnormality associated with Alzheimer's neuronal dysfunction [33], which has been associated with selective iron accumulation and oxidative damage [34, 35]. Whether a similar mechanism applies in

patients with TI warrants further pathological investigation. However, the finding that PET-CT abnormalities in patients with TI are mainly confined to the dominant, left hemisphere (all patients in this report were right-handed) is difficult to interpret using the available evidence. Nevertheless, it may still be attributed to chance, considering the small sample size in this study.

The main limitation of our study is the lack of neurocognitive testing. Despite the terminology, "silent infarcts" observed on brain MRI are clinically significant given their association with subsequent overt stroke and neurocognitive deficits, as evident from studies in children and adults with SCD [30, 36–41]. Decreased neuronal function on PET scanning in patients with SCD is also associated with an intelligence quotient lower than the normal mean [24, 28] and a broader region of cerebral dysfunction that may be a prelude to clinical stroke [42, 43]. Whether such correlations exist in patients with TI merits evaluation.

In conclusion, our study demonstrated that decreased neuronal function evident on PET-CT is common in patients with TI, especially those characterized by elevated LIC. Moreover, it seems that the combined use of PET-CT and MRI could better identify splenectomized TI adults at high risk for stroke or functional neurologic deficits by highlighting the extent of physiologic dysfunction alongside the anatomic loss of neuronal tissue. However, larger studies are needed to confirm these findings before recommendations for screening can be made and to avoid unnecessary radiation exposure. More importantly, the exact mechanisms behind these abnormalities should be understood and their correlation with neurocognitive and long-term sequelae should be prospectively examined, thus allowing for optimal risk classification and timely preventive intervention.

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Conflicts of interest ATT is a member of Novartis Speakers' Bureau.

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Chapter 5

Health-Related Quality of Life

Health-related Quality Of Life In Adults With Transfusion-independent Thalassaemia Intermedia Compared To Regularly Transfused Thalassaemia Major: New Insights

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Health-related quality of life in adults with transfusion-independent thalassaemia intermedia compared to regularly transfused thalassaemia major: new insights

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Abstract

Background: In patients with β thalassaemia intermedia (TI), the milder anaemia and transfusion independence imply better health-related quality of life (HR-QoL). However, the unbalanced pathophysiology of the disease allows for several serious clinical complications to manifest, which may have a negative impact on HR-QoL. *Methods:* This was a cross-sectional study on adult patients with transfusion- and iron chelation-independent TI and β thalassaemia major (TM) attending the Chronic Care Center, Hazmieh, Lebanon. A total of 80 patients agreed to participate in the study [32 TI (median age 24 yr) and 48 TM (median age 23 yr)]. The RAND SF-36 survey was used to assess HR-QoL. Data on patient demographics, clinical complications and socioeconomic status were collected. *Results:* Patients with TI and TM were comparable with age and gender, but patients with TM had a significantly longer median duration with a known thalassaemia diagnosis. Patients with TI had a higher proportion of multiple complications. Socioeconomic parameters were comparable, except for patients with TI being more commonly married. The mean Total, Physical Health and Mental Health Scores were significantly lower in patients with TI compared to TM, indicating poorer HR-QoL. There was a statistically significant positive correlation between the duration with a known thalassaemia diagnosis and a higher Mental Health Score (r_s = 0.73, P = 0.020). The mean Physical Health Score was significantly lower in patients with multiple clinical complications compared to patients with single or no complications (P = 0.012). Associations remained independently significant at multivariate analysis. *Conclusion:* Patients with transfusion-independent TI have lower HR-QoL compared to TM patients. At a comparable age, the shorter duration since diagnosis and the multiplicity of complications may explain these findings.

Key words thalassaemia intermedia; thalassaemia major; chronic disease; quality of life; clinical complications; health-related quality of life

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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) that refers to those patients whose clinical course is characterised by profound anaemia, who are presented to medical attention very early in life, and who subsequently require regular blood transfusions and iron chelation therapy for survival. The term β thalassaemia intermedia (TI) was first suggested to describe patients who have a milder anaemia compared to patients with TM, and who usually present to medical

attention later in childhood and remain largely transfusion independent (1).

Global public health efforts alongside advances in medical management, especially the introduction of safe blood transfusion practices and iron chelation therapy, surely translated into prolonged survival, lower morbidity and enhanced health-related quality of life (HR-QoL) in patients with TM (2–4). In patients with TI, however, the situation is far from ideal. Knowledge of the molecular and pathophysiological mechanisms underlying the disease process in TI has only recently started to evolve (5). It is now apparent that the diagnosis of TI carries higher morbidity than previously recognised, especially in the transfusion-independent patient where the mechanism of disease remains largely unbalanced (6–8). However, significant efforts are still needed before evidence-based management guidelines become available for this thalassaemia phenotype (9). Hence, both patient with the TI and the caring physician may be faced with challenges towards understanding the true burden of the disease and its optimal management. With these observations in mind, we evaluated reported HR-QoL of adults with transfusion-independent TI as compared to regularly transfused TM using a standardised instrument and further explored determinants of the observed differences.

Patients and methods

This was a cross-sectional study of adult (≥18 yr) patients with TI and TM attending to the Chronic Care Center, Hazmieh, Lebanon, the national thalassaemia centre in the country. Over a period of 1 yr, all adult patients presenting to the centre on two specified days of every week (total of 52 TI and 85 TM) were approached for inclusion in the study, and 80 consecutive patients (32 TI and 48 TM) agreed to participate. The remaining patients refused to participate for being unable or unwilling to spend more time at the centre for completion of the questionnaire. The study was approved by the Institutional Review Board at the centre, and written informed consent was obtained from each patient. All patients with TI were diagnosed based on established criteria (10) and were transfusion and iron chelation independent, while all patients with TM were regularly transfused (every 2–3 wk) and iron chelated with desferrioxamine (started before the age of 7 yr, in a daily dose of 30–50 mg/kg, given 5–6 times weekly). Retrieved data included demographics (age and gender), age at diagnosis, splenectomy status, history of clinical complications (Table 1) (11–16), education level (illiterate, elementary school, middle school, high school, university), marital status, presence of dependents, employment status, personal monthly income, household monthly income, monthly expenditure on disease and any history of previous psychosocial support.

For each recruited patient, the RAND 36 item Short Form Health Survey (SF-36) was administered to assess the HR-QoL, by self-administration or face-to-face interviews (for illiterate persons or those with other difficulties). This instrument was previously translated to Arabic, adapted and validated on the Lebanese population using the International Quality of Life Assessment methodology (17). The SF-36 is suitable for self-administration, computerised administration or administration

Table 1 Definitions of evaluated clinical complications

Complication	Definition
EMH	Radiologic evidence of extramedullary haematopoietic foci with or without symptoms
Leg ulcers	An ischaemic or necrotic skin lesion on the lower extremity by general visual inspection
PHT	A systolic pulmonary artery pressure >35 mm Hg, which corresponds to a tricuspid regurgitant velocity on Doppler echocardiography of >2.8 m/s (11) + Exertional dyspnoea without evidence of left heart disease
Thrombosis	Compression ultrasonography, contrast venography or angiography evidence of thrombus
HF	Modified Framingham criteria (12)
Abnormal liver function	ALT >50 U/L
DM	A fasting blood sugar ≥126 mg/dL, or 2-h postprandial blood sugar ≥200 mg/dL, or Symptoms of hyperglycaemia and a casual (random) plasma glucose ≥200 mg/dL (13)
Hypothyroidism	TSH > 4.7 μU/L and a free T4 < 0.8 ng/dL (14)
Osteoporosis	Bone densitometry *T*-score – 2.5 SD (15)
Hypogonadism	Females: >13 yr, not yet Tanner B2 (i.e. prepubertal breast development) *or* >14 yr requiring oestrogen replacement therapy *or* >15 yr with primary amenorrhoea Males: >14 yr, not yet Tanner G2 (i.e. prepubertal genital development) *or* on androgen replacement therapy *or* >17 yr, not yet Tanner G4 (i.e. midpubertal genital development) (16)

EMH, extramedullary haematopoiesis; PHT, pulmonary hypertension; HF, heart failure; ALT, alanine transaminase; DM, diabetes mellitus; TSH, thyroid-stimulating hormone.

by a trained interviewer in person or by telephone to persons aged 14 and older. It is a generic questionnaire, widely used in various clinical conditions and populations (18). It consists of 36 questions that are clustered to yield eight health status scales: Physical Functioning, Role-Physical, Bodily Pain, General Health, Vitality, Social Functioning, Role-Emotional and Mental Health. The health concepts described by the SF-36 range in score from 0 to 100, with higher scores indicating higher levels of function and/or better health. The subjects' responses are presented as a profile of scores calculated for each scale. Two summary measures aggregate these status scales, namely the Physical and Mental Health Scores (17).

Statistical analysis

Descriptive statistics are reported as medians (range), means ± standard deviation (SD) or percentages where appropriate. To evaluate for differences between patients with TI and TM, bivariate correlations were made using the independent samples Mann–Whitney *U* test or *t*-test for continuous variables and the chi-square or Fisher's exact tests for categorical variables. Spearman's correlation coefficient (r_s) was used to examine the relationship between SF-36 scores and any continuous variables. Multivariate linear regression analysis was carried out to evaluate for independent associations when needed. All *P*-values are two sided with the level of significance set at < 0.05.

Results

Patient characteristics

A total of 80 patients (32 TI and 48 TM) were included in this analysis. Table 2 summarises characteristics of patients. Patients with TI and TM were comparable in median age at study and gender distribution. However, patients with TM had a significantly younger median age at diagnosis and subsequently a longer median duration with a known thalassaemia diagnosis compared to patients with TI (median of 22.3 vs. 16 yr, $P < 0.001$). There was no statistically significant difference in the proportion of patients who had undergone splenectomy between the two groups. Similarly, the proportions of patients who had any history of clinical complications, as defined in this study, were similar in both groups. However, patients with TI had a higher proportion of patients with multiple complications compared to TM (50.3% vs. 30.4% among those who had complications in TI and TM, respectively). Patients with TI were more commonly married, but a comparable proportion of patients had received an education or were employed in the two groups. There were no significant differences in financial income or expenditure on disease between both groups.

Table 2 Characteristics of patients

Parameter	Thalassaemia intermedia (n = 32)	Thalassaemia major (n = 48)	*P*-value
Median age at study (range), yr	24 (18–46)	23 (18–42)	0.734
Median age at diagnosis (range), yr	5 (0.1–31)	0.6 (0.2–5.5)	<0.001
Median duration with diagnosis (range), yr	16 (1–39)	22.3 (16.5–41.5)	<0.001
Male, %	37.5	45.8	0.460
Splenectomised, %	64.5	64.6	0.995
History of clinical complications, %	46.9	47.9	0.927
Single, %	21.9	33.3	
Multiple, %	25	14.6	
Educated, %	100	97.9	0.411
Elementary school, %	9.4	16.7	
Middle school, %	28.1	22.9	
High school, %	18.8	25	
University, %	43.8	33.3	
Married, %	29	10.4	0.034
With dependents, %	29	12.5	0.067
Employed, %	45.2	40.9	0.714
Median personal monthly income (range), USD	50 (0–3300)	75 (0–3000)	0.417
Median household monthly income (range), USD	1750 (450–3300)	1500 (350–3000)	0.176
Median monthly expenditure on disease (range), USD	47.5 (0–500)	150 (0–500)	0.238
Previous psychosocial support, %	0	6.2	0.149

Health-related quality of life

The mean Total SF-36 Score was significantly lower in patients with TI compared to TM (66.5 ± 16.1 vs. 75.8 ± 18.8, $P = 0.021$), indicating poorer HR-QoL. Data for the eight scales of the SF-36 were also analysed. Looking at summaries, both the mean Physical Health Score (TI: 66.2 ± 16.8 vs. TM: 77.1 ± 18.1, $P = 0.008$) and the Mental Health Score (TI: 62.8 ± 17.3 vs. TM: 71.7 ± 21.2, $P = 0.042$) were significantly lower in patients with TI compared to TM. The most notable differences were in the Physical Functioning, General Health and Vitality scales (Fig. 1).

For the whole study group, there was a statistically significant positive correlation between the duration with a known thalassaemia diagnosis and the Mental Health Score ($r_s = 0.73$, $P = 0.020$) but not the Physical Health Score ($r_s = 0.34$, $P = 0.256$). There were no statistically significant differences in the mean Physical ($P = 0.188$) or Mental Health Scores ($P = 0.260$) between married and single patients. However, the mean Physical but not the Mental Health Score was significantly lower in patients with multiple clinical complications compared to patients with single or no complications ($P = 0.012$) (Fig. 2).

A stepwise multivariate linear regression model was built with Physical or Mental Health Scores as the dependent variables and: thalassaemia diagnosis (TM or TI), marital status (married or single), duration with a known thalassaemia diagnosis and multiplicity of clinical complications (yes or no) as the independent variables. A longer duration with a known thalassaemia diagnosis was the only independent variable correlating with higher Mental Heath Scores ($P = 0.039$) while multiplicity of clinical complications was the only independent variable correlating with lower Physical Heath Scores ($P = 0.032$).

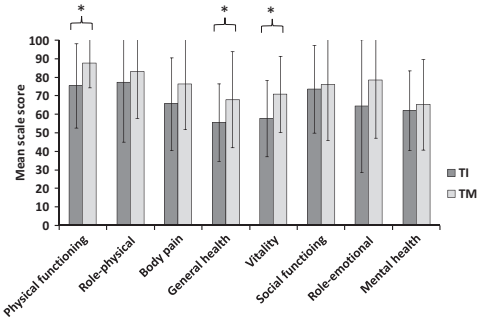

Figure 1 Bar chart showing the mean (bars) and standard deviation (whiskers) of the eight SF-36 health status scales in patients with thalassaemia intermedia (TI) and thalassaemia major (TM). *Statistically significant difference ($P < 0.05$) by the independent samples *t*-test.

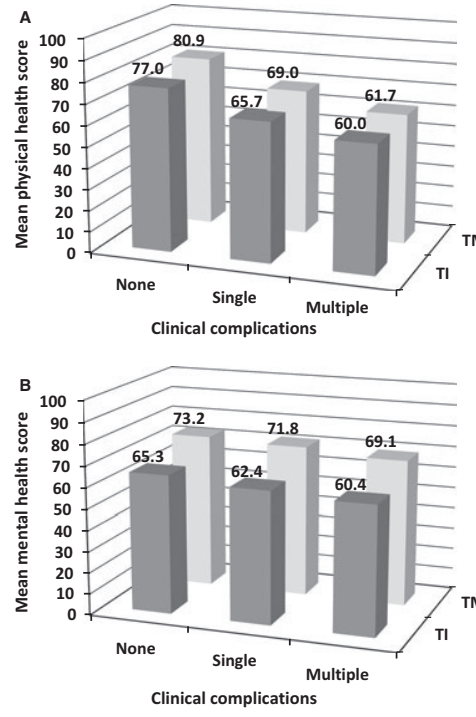

Figure 2 Bar charts showing mean (A) Physical Health Score and (B) Mental Health Score, for patients with thalassaemia intermedia (TI) and thalassaemia major (TM) according to history of clinical complications.

Discussion

Our study demonstrates that patients with transfusion-independent TI have impaired HR-QoL compared to patients with regularly transfused TM of similar age and gender. This finding is contrary to what one would normally assume and echoes recent evidence that highlights the true burden of TI (9).

Several studies evaluated HR-QoL in children (19–23) and adults (24–28) with TM. Reported HR-QoL in patients with TM has markedly improved over the years, especially with the introduction of safe blood transfusions and iron chelation therapy (2, 4). However, several challenges continue to exist, especially for the thalassaemic child transitioning into adulthood (29), allowing the HR-QoL in patients with TM to remain lower than that reported for normal individuals (19–28). Data on

patients with TI are scarce (30). Our study is the first to evaluate reported HR-QoL, using a standardised instrument, for adult patients with TI. Although normative data for the SF-36 using a Lebanese population are not available for comparison, a clinically meaningful gap (~10 scale points) was noted between patients with TI and TM for both the mean Physical and Mental Health Scores. The difference becomes even more meaningful knowing that patients with TM evaluated in this report were receiving the subcutaneous iron chelator desferrioxamine, which may be associated with lower HR-QoL as compared to the newer oral chelators deferiprone and deferasirox (31–34). Only one previous report evaluated HR-QoL in patients with TI. In a cohort of children and adolescents, Pakbaz *et al*. (30) compared 19 TI to 29 TM patients using the Dartmouth Care Cooperative Chart System questionnaire. Similar to our findings in adults, a higher proportion of TI children reported impaired HR-QoL compared to their TM colleagues.

In the study by Pakbaz *et al*. (30) the authors could not evaluate the association between clinical complications and HR-QoL. However, in our study, the report of poor physical health status in patients with TI seems to be attributed to a higher proportion of patients having multiple clinical complications. Severity of the disease and multiplicity of clinical complications have been associated with compromised HR-QoL in patients with TM (19, 24, 28) and sickle cell anaemia (35). Recent evidence continues to highlight that a substantial proportion of patients with TI suffer from serious cardiovascular (namely venous thromboembolism or pulmonary hypertension with secondary right heart failure), endocrinological, hepatic or skeletal complications (7). These complications were mostly evident in patients who never received transfusion or iron chelation therapy (7). In TI, the prevailing approach has been avoidance of early blood transfusions and the concomitant requirement for chelation therapy. However, in a recent study on 584 patients with TI, subjects who were placed on transfusion regimens suffered fewer complications relevant to chronic anaemia, ineffective erythropoiesis and haemolysis (7). Other observational studies have also confirmed that patients with transfused TI suffer fewer thromboembolic events, pulmonary hypertension and silent brain infarcts as compared to transfusion-independent patients (36–39). We herein provide further evidence that multiplicity of complications in the non-transfused patient is also associated with compromised HR-QoL. As such, the introduction of transfusion therapy aimed at preventing the consequences of chronic haemolytic anaemia may benefit patients with TI and improve HR-QoL. Rather than enforcing the regular transfusion regimens implemented in TM, blood transfusion in TI should be individually tailored to meet patient needs. 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 (7, 40).

There was a negative correlation between mental health status and duration with a known thalassaemia diagnosis, which may explain the lower Mental Health Score observed in patients with TI compared to TM. When comparing a TI to a TM patient of similar age, the patient with TI would have lived a shorter duration of time carrying a diagnosis of thalassaemia, because patients with TI usually present to medical attention and get diagnosed later in childhood (1). Thus, it may be worthwhile proposing that the patient with TM could have had a longer duration of time and greater opportunity to adapt to the disease, its complications and treatment (41). Moreover, as many patients get diagnosed in late childhood or adolescence, it may be harder for them to adjust to the disease because adolescence is a challenging period by itself even without the illness, and this will make them feel even more different than their peers while all they want is to be able to fit in (29, 42, 43). Although patients with TI and TM are usually treated at the same comprehensive care centre, the more frequent presence of TM patients at the centre, to receive blood transfusions, allows them to establish stronger bonds with the health care staff and to get more involved in educational activities aimed at expanding their knowledge about the disease. The health care system has also traditionally been biased towards displaying TM as the more serious condition requiring considerable attention, although this notion is gradually starting to change as the severity of TI is starting to unfold (9). Because broad knowledge about TI and its management has only recently started to become available (9), during initial stages following diagnosis, the patient with TI may be a victim of clinical misinterpretations or inappropriate management, allowing for confusion about the taught and observed nature of the disease to develop. In other words, the patient may personally start to realise that the disease carries more complications than he or she had anticipated or been taught. All the aforementioned factors may have negatively impacted the HR-QoL in patients with TI, especially the reported mental health status.

The study was limited by the small sample of patients recruited for each thalassaemia group. The lack of SF-36 measurement for a sample of healthy Lebanese controls did not allow comparison of HR-QoL for both thalassaemia groups to normal values. Moreover, our findings may not be generalizable to thalassaemia patients in other countries, because HR-QoL may be influenced by standards of health care and other environmental factors.

Contrary to previous belief, patients with TI report substantially impaired HR-QoL. We recommend that all patients with TI undergo frequent HR-QoL assessment, preferably by a standardised instrument like the SF-36. This should also be supported with proper utilisation of psychological interventions and relevant patient education in line with current understanding of the disease. Such efforts should be introduced directly after diagnosis and be maintained throughout patient follow-up. Prospective clinical trials are also urgently needed to evaluate the role of transfusion and other interventions in preventing clinical complications in TI, which are associated with compromised HR-QoL.

Disclosures

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Chapter 6

Management

Age-related Complications In Treatment-naïve Patients With Thalassaemia Intermedia

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Age-related complications in treatment-naïve patients with thalassaemia intermedia

Numerous efforts have been made to understand the molecular and pathophysiological basis of the intermediate forms of thalassaemia (Taher *et al*, 2006). Although the term thalassaemia intermedia (TI) lacks specific molecular correlates, several studies highlight established differences in the underlying pathophysiology and associated clinical sequelae as compared to patients with thalassaemia major (Taher *et al*, 2006). However, much of these differences rely on the treatment approaches -splenectomy, transfusion, iron chelation, and foetal haemoglobin induction- undertaken after initial diagnosis. Moreover, the shortage of clinical trials that investigate treatment strategies for TI resulted in a wide variety of management practices (Taher *et al*, 2009a). Consequently, the natural history of the disease remains poorly understood.

We revaluated data collected for the Thalassaemia Intermedia Registry, a database of 584 TI patients currently registered at six comprehensive care centres in Lebanon, Italy, Iran, Egypt, United Arab Emirates, and Oman. Institutional review boards (IRBs) at each centre approved the study protocol. All patients were diagnosed with TI based on previously described criteria (Camaschella & Cappellini, 1995). Patients who had never received any treatment intervention (splenectomy, transfusion therapy, iron chelation therapy, foetal haemoglobin inducing agents) were identified and included in this study ($n = 120$). Retrieved data included demographics (age and gender); identified mutation; mean haemoglobin (Hb) and steady-state serum ferritin (SF) levels of three consecutive measurements within the year corresponding to patient age; viral hepatitis status; and presence of complications [extramedullary haematopoiesis (EMH), leg ulcers, thrombosis, pulmonary hypertension (PHT), heart failure (HF), cholelithiasis, abnormal liver function (ALF), diabetes mellitus (DM), hypothyroidism, osteoporosis, hypogonadism] according to criteria described elsewhere (Taher *et al*, 2009a). Patients were divided into four quartiles ($n = 30$ each) according to their age: ≤10, 11–20, 21–32, and >32 years, representing 37·5%, 21·3%, 17·3%, and 15·8% of the whole cohort's ($n = 584$) corresponding age intervals, respectively. Bivariate correlations between age and SF or Hb levels were evaluated using Spearman's (r_s) correlation coefficients. To estimate the incidence density ratios (rate ratios) in those who had *versus* those who did not have the complication, odds ratios were calculated, with 95% confidence intervals. We tested for linear trend with advancing age by examining the significance of the coefficients with a Chi-squared test (*p*-trend). All *P*-values are two sided with the level of significance set at <0·05.

The mean age of the patients was 21·4 ± 13·4 years (range: 2–56 years). The male to female ratio was 61:59. Homozygosity for IVS-I-6 (T→C) was the most common mutation (87·5%), followed by IVS-I-5 (G→C) (8·3%), IVS-II-1 (G→A) (2·5%) and Codon 39 (C→T) (1·7%). There was no statistically significant difference between age quartiles in the proportion of patients with co-inheritance of α thalassaemia [α^+ (-$\alpha^{3.7}$ and -$\alpha^{4.2}$) or α^0 ($-^{Med}$ and $-^{SEA}$)] or determinants associated with increased γ-chain production (Xmn-I +/+ genotype at position −158 of *HBG2*). Moreover, none of the patients had evidence of hepatitis B or C infection. The mean Hb and SF levels of the whole study group were 77 ± 16 g/l (range: 41–110 g/l) and 610·7 ± 515·1 μg/l (range: 16·7–2520 μg/l) respectively. There was a statistically significant negative correlation between age and Hb level ($r_s = -0{\cdot}679$, $P < 0{\cdot}001$; Fig 1A) and a statistically significant positive correlation between age and SF ($r_s = 0{\cdot}653$, $P < 0{\cdot}001$; Fig 1B). With advancing age, there was a statistically significant trend towards a higher rate of EMH ($P = 0{\cdot}001$), leg ulcers ($P = 0{\cdot}004$), thrombosis ($P = 0{\cdot}030$), PHT ($P = 0{\cdot}010$), hypothyroidism ($P = 0{\cdot}039$), and osteoporosis ($P = 0{\cdot}018$) (Fig 1C and Table I).

This study demonstrated a significant role for advancing age (even among paediatric and adult patients) in acquiring complications in TI. Three main factors are responsible for the clinical sequelae of TI: ineffective erythropoiesis, chronic haemolytic anaemia, and iron overload (Taher *et al*, 2006).

The degree of ineffective erythropoiesis is the primary determinant for the development of anaemia, while peripheral haemolysis of mature red blood cells remains secondary (Rund & Rachmilewitz, 2005). Although the first is mainly associated with skeletal complications attributed to compensatory EMH (Taher *et al*, 2006), the latter has been linked to more severe complications, such as PHT (Aessopos *et al*, 2007), with secondary HF, and thromboembolic phenomena (Taher *et al*, 2008). This study demonstrated a decreasing trend in Hb level with advancing age. Although our study did not specifically measure relevant markers, this may reflect progressive worsening of ineffective erythropoiesis, haemolysis, hypersplenism, or all of these, and explain the associated increasing rate of EMH, leg ulcers, PHT, and thrombosis**.** Age-related changes in adaptation to anaemia have been observed in patients with Hb E β-thalassaemia. O'Donnell *et al* (2007) suggested that advancing age had an independent and direct effect on the background level of erythropoietin production in response to anaemia. In another study, there appeared to be a difference in the overall pattern of erythropoietin response to anaemia between children and adults, although this did not reach

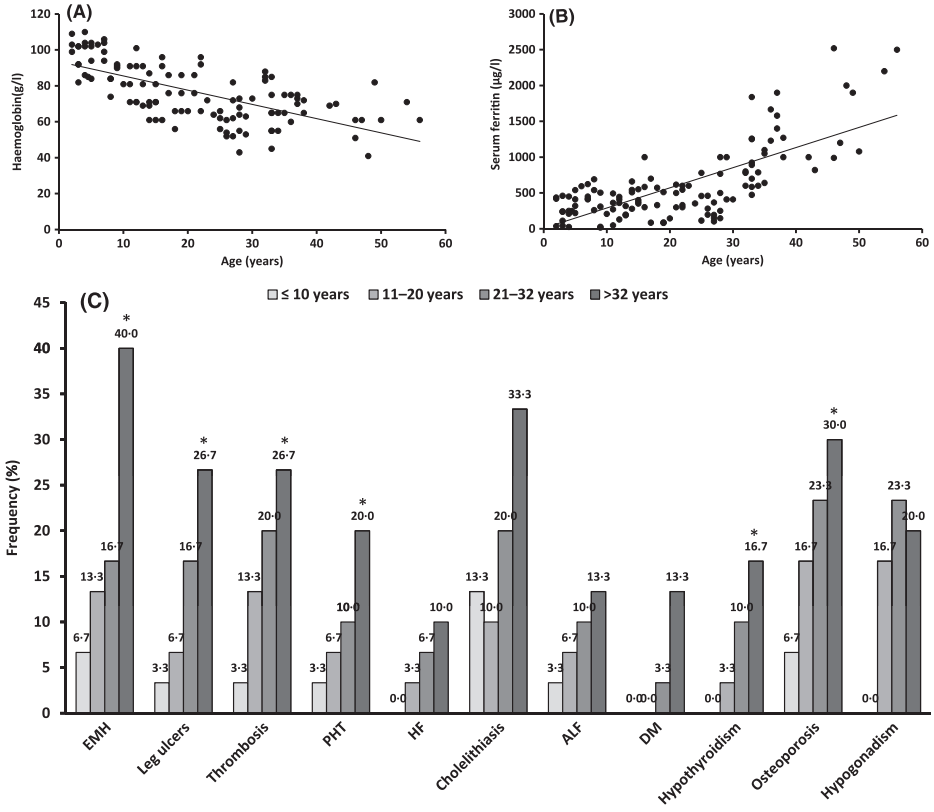

Fig 1. (A) Scatter plot of age and haemoglobin level. (B) Scatter plot of age and steady-state serum ferritin level. (C) Bar chart showing the frequency of complications across the different age quartiles. (*, statistically significant trend, EMH, extramedullary haematopoiesis; PHT, pulmonary hypertension; HF, heart failure; ALF, abnormal liver function; DM, diabetes mellitus).

statistical significance (Sukpanichnant *et al*, 1997). Moreover, in a study of erythropoietin response in patients with sickle cell anaemia, 10 adults seemed to show a lower response at approximately the same Hb level as those of children at the same level (Sherwood *et al*, 1986).

Our study also confirmed a positive correlation between advancing age and SF levels. Although no liver iron concentration measurements were done in this study, this association most probably reflects iron accumulation over time in patients with TI, which in turn explains the higher occurrence of iron-overload related endocrinopathy in older patients. The combination of ineffective erythropoiesis and chronic anaemia/hypoxia in TI results in hepcidin suppression, increased intestinal iron absorption, and increased release of recycled iron from the reticuloendothelial system. This results in depletion of macrophage iron, relatively low levels of SF, and preferential portal and hepatocyte iron loading (Taher *et al*, 2009b). Hence, transfusion-independent TI patients may still be at risk of iron-overload related morbidity, especially as older adults where iron accumulation may surpass acceptable levels.

Our study has an important clinical implication. It calls for close clinical follow up of TI patients as they get older. 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. Prospective clinical studies are thus urgently invited to assess the optimal type and timing of treatment initiation that needs to be offered to this group of patients to avoid disease-related morbidity, because an increasing percentage of patients will require treatment as they advance in age.

Authorship

ATT, KMM, MDC were responsible for conception and design, data analysis and interpretation, and manuscript writing; KMM performed statistical analysis; AE, MK, SD, KB, MSS

Table I. Incidence rate ratios for complications according to age.

	EMH		Leg ulcers		Thrombosis		PHT	
Age quartile	RR	95% CI	RR	95% CI	RR	95% CI	RR	95% CI
Q1	1·00	Referent	1·00	Referent	1·00	Referent	1·00	Referent
Q2	2·15	(0·36–12·76)	2·07	(0·18–24·15)	4·46	(0·47–42·51)	2·07	(0·18–24·15)
Q3	2·80	(0·50–15·73)	5·80	(0·64–53·01)	7·25	(0·82–64·46)	3·22	(0·32–32·89)
Q4	9·33	(1·87–46·7)	10·55	(1·23–90·66)	10·55	(1·23–90·66)	7·25	(0·82–64·46)
p-trend	0·001		0·004		0·010		0·030	
	HF		Cholelithiasis		ALF		DM	
Age quartile	RR	95% CI	RR	95% CI	RR	95% CI	RR	95% CI
Q1	1·00	Referent	1·00	Referent	1·00	Referent	1·00	Referent
Q2	1·00	(0·06–16·76)	1·23	(0·94–1·61)	2·07	(0·18–24·15)	1·00	(0·06–16·76)
Q3	2·07	(0·18–24·15)	1·07	(0·78–1·47)	3·22	(0·32–32·89)	1·00	(0·06–16·76)
Q4	3·22	(0·32–32·89)	1·25	(0·94–1·65)	4·46	(0·47–42·51)	4·46	(0·47–42·51)
p-trend	0·225		0·300		0·141		0·118	
	Hypothyroidism		Osteoporosis		Hypogonadism			
Age quartile	RR	95% CI	RR	95% CI	RR	95% CI		
Q1	1·00	Referent	1·00	Referent	1·00	Referent		
Q2	1·00	(0·06–16·76)	2·80	(0·50–15·73)	5·80	(0·64–24·15)		
Q3	3·22	(0·32–32·89)	4·26	(0·81–22·73)	8·85	(1·01–76·92)		
Q4	5·80	(1·23–90·66)	5·99	(1·17–30·30)	7·25	(0·82–64·46)		
p-trend	0·039		0·018		0·058			

Q1 = ≤10 years; Q2 = 11–20 years; Q3 = 21–32 years; Q4 = >32 years; EMH, extramedullary haematopoiesis; PHT, pulmonary hypertension; HF, heart failure; ALF, abnormal liver function; DM, diabetes mellitus.

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Overview On Practices In Thalassemia Intermedia Management Aiming For Lowering Complication Rates Across A Region Of Endemicity: The OPTIMAL CARE Study

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Overview on practices in thalassemia intermedia management aiming for lowering complication rates across a region of endemicity: the OPTIMAL CARE study

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Despite recent advances in understanding the pathophysiologic mechanisms behind the thalassemia intermedia (TI) phenotype, data on the effects of treatment are deficient. To provide such data, we evaluated 584 TI patients for the associations between patient and disease characteristics, treatment received, and the rate of complications. The most common disease-related complications were osteoporosis, extramedullary hematopoeisis (EMH), hypogonadism, and cholelithiasis, followed by thrombosis, pulmonary hypertension (PHT), abnormal liver function, and leg ulcers. Hypothyroidism, heart failure, and diabetes mellitus were less frequently observed. On multivariate analysis, older age and splenectomy were independently associated with an increased risk of most disease-related complications. Transfusion therapy was protective for thrombosis, EMH, PHT, heart failure, cholelithiasis, and leg ulcers. However, transfusion therapy was associated with an increased risk of endocrinopathy. Iron chelation therapy was in turn protective for endocrinopathy and PHT. Hydroxyurea treatment was associated with an increased risk of hypogonadism yet was protective for EMH, PHT, leg ulcers, hypothyroidism, and osteoporosis. Attention should be paid to the impact of age on complications in TI, and the beneficial role of splenectomy deserves revisiting. This study provides evidence that calls for prospective evaluation of the roles of transfusion, iron chelation, and hydroxyurea therapy in TI patients. (Blood. 2010; 115:1886-1892)

Introduction

Knowledge of the molecular basis of thalassemia intermedia (TI) has progressed significantly in the last decade, including an increased understanding of the genetic mutations that lead to the associated phenotypes.[1,2] It is now established that such clinical phenotypes lie in severity between those of thalassemia minor (clinically silent, mildly hypochromic, and microcytic anemia) and transfusion-dependent thalassemia major (TM), although there is substantial clinical overlap between the 3 conditions.[3] Three main factors are responsible for the clinical sequelae of TI: ineffective erythropoiesis, chronic anemia, and iron overload.[3] The degree of ineffective erythropoiesis is the primary determinant of the development of anemia, whereas peripheral hemolysis of mature red blood cells (RBCs) remains secondary.[4] Although the first is mainly associated with skeletal complications attributed to compensatory extramedullary hematopoiesis (EMH),[5] the latter has been linked to more severe complications, such as pulmonary hypertension (PHT),[6,7] with secondary heart failure (HF), and thromboembolic phenomena.[8] Moreover, chronic anemia leads to an increase in gastrointestinal iron absorption,[9] resulting in iron overload, which in turn can cause several serious complications, including HF and endocrine abnormalities, such as diabetes mellitus, hypothyroidism, osteoporosis, and hypogonadism.[3,10,11] There are several options that may be available for managing patients with TI, including splenectomy, transfusion therapy, iron chelation therapy, and modulation of fetal hemoglobin (HbF) production.[3,5,12-15] However, despite the availability of several treatment options, these modalities have rarely been evaluated in TI patients, and the lack of clear guidelines still presents a significant clinical challenge.[5]

We herein present the largest overview to date on the current status of TI patients, in 6 comprehensive care centers, by assessing the rate of disease-associated complications in relation to currently practiced treatment options.

Methods

This was a retrospective review of the medical charts of all TI patients currently registered at 6 comprehensive care centers in Lebanon, Italy, Iran, Egypt, United Arab Emirates, and Oman. Institutional review boards at each center approved the study protocol. All patients were diagnosed with TI based on criteria previously described.[16,17] Data included demographics (age and sex), splenectomy status, mean hemoglobin (Hb), alanine transaminase, and steady-state serum ferritin levels of all available laboratory records (mean, 18.6 ± 8.2, 8.1 ± 6.2, and 13.5 ± 6.6 readings, over 10.6 ± 4.2, 5.1 ± 3.2, and 8.5 ± 3.6 years, respectively), and type of treatment received (RBC transfusion, iron chelation, and hydroxyurea). The main indications for splenectomy were growth retardation or poor health; leukopenia; thrombocytopenia; increased transfusion demand; or symptomatic splenomegaly. For transfusion status, data were categorized as follows:

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Table 1. Clinical definitions required to confirm identified complications

Complication	Definition
EMH	Radiologic evidence of extramedullary hematopoietic foci with or without symptoms
PHT	Systolic pulmonary artery pressure > 35 mm Hg, which corresponds to a tricuspid regurgitant velocity on Doppler echocardiography of > 2.8 m/s[18] plus exertional dyspnea without evidence of left heart disease
HF	Modified Framingham criteria[19]
Thrombosis	Compression ultrasonography, contrast venography, or angiography evidence of thrombus
Cholelithiasis	Ultrasonographic evidence of gallbladder stones
Abnormal liver function	ALT > 50 U/L
Leg ulcers	Ischemic or necrotic skin lesion on the lower extremity by general visual inspection
DM	Fasting blood sugar ≥ 126 mg/dL, or 2-hour postprandial blood sugar ≥ 200 mg/dL, or symptoms of hyperglycemia and a casual (random) plasma glucose ≥ 200 mg/dL[20]
Hypothyroidism	TSH > 4.7 μU/L and a free T_4 < 0.8 ng/dL[21]
Osteoporosis	Bone densitometry T score: 2.5 SD[22]
Hypogonadism	Females: > 13 y, not yet Tanner B2 (ie, prepubertal breast development) or > 14 y requiring estrogen replacement therapy or > 15 y with primary amenorrhea; males: > 14 y, not yet Tanner G2 (ie, prepubertal genital development) or on androgen replacement therapy or > 17 y, not yet Tanner G4 (ie, midpubertal genital development)[23]

ALT indicates alanine transaminase; DM, diabetes mellitus; TSH, thyroid stimulating hormone; and T_4, thyroxine.

regular transfusion (patients on regular-interval transfusion protocols [once every 1-3 months for a pretransfusion Hb of ≥ 90 g/L] initiated mainly for failure to thrive in childhood; bone deformities; progressive splenic enlargement; persistent worsening anemia; or development of complications during the course of the disease); occasional transfusion (patients who required incidental transfusions for transient severe anemia secondary to infections, surgery, or pregnancy); and never transfused. Iron chelation therapy had to be administered for at least one year or else the patient was considered nonchelated. Complications were defined according to Table 1.[18-23] The prevalence of other elements (family history of cardiovascular or endocrine disease, acquired or inherited thrombophilia, anticoagulant or antiplatelet use for reasons other than overt thrombosis, malignancy, orthopedic surgery, hepatitis C or B virus infection) in the patients' medical history that could modify the rate of complications was low; and hence, these parameters were not included in further analysis.

Statistical analysis

Descriptive statistics are expressed as percentages or means. For each complication, a univariate analysis was done to determine the effect of study parameters (age, sex, serum ferritin level, Hb level, splenectomy, transfusion, hydroxyurea, and iron chelation therapy) using χ^2 and Fisher exact test. Multivariate logistic regression analysis was done for each complication as a dependent variable to determine the independent effect of study parameters; where all variables with a P value less than .1 (on univariate analysis) were entered into the model. In the multivariate model age was divided into 2 groups (≤ 35 and > 35 years) and transfusion status was defined as transfused versus never transfused to preserve sample size. Differences in the mean number of complications between different treatment modalities were evaluated using the independent samples t test. All P values are 2-sided with the level of significance set at less than .05.

Table 2. Patient and disease characteristics of the study population

Parameter	Frequency, no. (%)
Age, y	
Less than 18	172 (29.5)
18-35	288 (49.3)
More than 35	124 (21.2)
Male:female	291 (49.8):293 (50.2)
Splenectomized	325 (55.7)
Serum ferritin, μg/L	
Less than 1000	376 (64.4)
1000-2500	179 (30.6)
More than 2500	29 (5)
Treatment	
Hydroxyurea	202 (34.6)
Occasional transfusion	143 (24.5)
Regular transfusion	302 (51.7)
Iron chelation	336 (47.5)
Complications	
Osteoporosis	134 (22.9)
EMH	124 (21.2)
Hypogonadism	101 (17.3)
Cholelithiasis	100 (17.1)
Thrombosis	82 (14)
PHT	64 (11)
Abnormal liver function	57 (9.8)
Leg ulcers	46 (7.9)
Hypothyroidisim	33 (5.7)
HF	25 (4.3)
Diabetes mellitus	10 (1.7)

Results

Patient, disease, and treatment characteristics

A total of 584 TI patients were identified in the 5 participating centers. The mean age was 25.44 plus or minus 13.86 years (range, 2-76 years) with a male-to-female ratio of 291:293. A total of 325 (55.7%) patients were splenectomized. The mean Hb, alanine transaminase, and steady-state serum ferritin levels of the whole study group were 89 plus or minus 14.9 g/L (range, 49-140 g/L), 31.68 plus or minus 32.42 IU/L (range, 6-148 IU/L), and 967.5 plus or minus 853.9 μg/L (17-10 793 μg/L), respectively. The most common disease-related complications were osteoporosis, EMH, hypogonadism, and cholelithiasis, followed by thrombosis, PHT, abnormal liver function, and leg ulcers. Hypothyroidism, HF, and diabetes mellitus were less frequently observed (Table 2). The types of treatment received are summarized in Table 2.

Determinants of complication rate

Results of univariate analysis are summarized in Table 3. On multivariate analysis (Table 4), older age and splenectomy were independently associated with an increased risk of most disease-related complications; splenectomy was protective only against the development of EMH. The probability of having 0, 1 to 3, and more than 3 complications at different age intervals is summarized in Figure 1. Female sex was associated with an increased risk of osteoporosis, hypogonadism, and cholelithiasis. Although a mean Hb more than or equal to 90 g/L was only associated with a reduced risk of thrombotic events, transfusion therapy was protective for thrombosis, EMH, PHT, HF, cholelithiasis, and leg ulcers. However, transfusion therapy was associated with an increased risk of endocrinopathy (hypothyroidism, osteoporosis, and hypogonadism). Whereas a mean serum ferritin level of more than 1000 μg/L

Table 3. Univariate analysis for determinants of complication rate

	EMH	PHT	HF	Thrombosis	Cholelithiasis	Abnormal liver function	Leg ulcers	DM	Hypothyroidism	Osteoporosis	Hypogonadism
Age, y											
Less than 18 (n = 172)	36	2.3	1.7	4.1	2.3	6.4	2.9	0	0.6	2.3	14
18-35 (n = 288)	12.8	11.5	5.2	13.9	17	11.5	6.2	1.4	6.2	20.5	21.5
More than 35 (n = 124)	20.2	21.8	5.6	28.2	37.9	10.5	18.5	4.8	11.3	57.3	12.1
P	< .001*	< .001*	.145	< .001*	< .001*	.199	< .001*	0.005*	< .001*	< .001*	.026*
Sex											
Male (n = 291)	21	9.6	5.2	11	12.4	10	7.2	1	5.8	17.2	10.7
Female (n = 293)	21.5	12.3	3.4	17.1	21.8	9.6	8.5	2.4	5.5	28.7	23.9
P	.873	.303	.299	.035*	.002*	.868	.555	.206	.842	.001*	< .001*
Ferritin, μg/L											
Less than 1000 (n = 376)	21.8	9.8	4.5	10.1	17	8	6.1	2.1	5.1	18.1	13.3
1000 or more (n = 208)	20.2	13	3.8	21.2	17.3	13	11.1	1	6.7	31.7	24.5
P	.059	.245	.700	< .001*	.930	.051	.034*	.298	.400	< .001*	< .001*
Hemoglobin, g/L											
Less than 90 (n = 282)	21.6	11	4.6	19.5	16.7	11.7	9.6	1.8	4.6	24.1	18.1
90 or more (n = 302)	20.9	10.9	4	8.9	17.5	7.9	6.3	1.7	6.6	21.9	16.6
P	.820	.980	.704	< .001*	.777	.127	.141	.913	.293	.516	.625
Splenectomy											
No (n = 259)	26.3	3.9	1.9	3.5	5.0	10	2.7	0.4	1.5	9.3	13.9
Yes (n = 325)	17.2	16.6	6.2	22.5	26.8	9.5	12	2.8	8.9	33.8	20
P	.008*	< .001*	.012*	< .001*	< .001*	.840	< .001*	.027*	< .001*	< .001*	.053
Transfusion											
None (n = 139)	60.4	20.1	14.4	26.6	27.3	7.2	13.7	0.7	0	15.1	2.2
Occasional (n = 143)	19.6	14.3	0	18.2	19.6	14.7	13.3	1.4	9.8	44.1	18.2
Regular (n = 302)	4	5.3	1.7	6.3	11.3	8.6	2.6	2.3	6.3	16.6	23.8
P	< .001*	< .001*	< .001*	< .001*	< .001*	.066	< .001*	.459	0.001*	< .001*	< .001*
Hydroxyurea											
No (n = 382)	23.8	13.9	3.1	17	20.9	9.4	11.5	2.6	8.4	34.6	11.5
Yes (n= 202)	16.3	5.4	6.4	8.4	9.9	10.4	1	0	0.5	1	28.2
P	.035*	.002*	.061	.004*	.001*	.707	< .001*	.020*	< .001*	< .001*	< .001*
Iron chelation											
No (n = 248)	19.4	16.1	6.5	17.7	28.2	10.5	10.9	3.2	8.1	32.7	12.9
Yes (n = 336)	22.6	7.1	2.7	11.3	8.9	9.2	5.7	0.6	3.9	15.8	20.5
P	.340	.001*	.026*	.027*	< .001*	.613	.020*	.015*	.030*	< .001*	.016*

All data except *P* values are presented as percentages. DM indicates diabetes mellitus.
*Statistically significant.

Table 4. Multivariate analysis for determinants of complication rate

Complication/parameter	RR	95% CI	*P*
EMH			
Age > 35 y	0.85	0.46-1.58	.610
Ferritin ≥ 1000 μg/L	0.85	0.51-1.44	.548
Splenectomy	0.44	0.26-0.73	.001*
Transfusion	0.06	0.03-0.09	< .001*
Hydroxyurea	0.52	0.30-0.91	.022*
PHT			
Age > 35 y	2.59	1.08-6.19	.032*
Splenectomy	4.11	1.99-8.47	< .001*
Transfusion	0.33	0.18-0.58	< .001*
Hydroxyurea	0.42	0.20-0.90	.025*
Iron chelation	0.53	0.29-0.95	.032*
HF			
Splenectomy	2.88	0.99-8.32	.051
Transfusion	0.06	0.02-0.17	< .001*
Hydroxyurea	1.84	0.98-3.47	.057
Iron chelation	0.45	0.18-1.12	.086
Thrombosis			
Age > 35 y	2.60	1.39-4.87	.003*
Female	1.27	0.74-2.19	.387
Hb ≥ 90 g/L	0.41	0.23-0.71	.001*
Ferritin ≥ 1000 μg/L	1.86	1.09-3.16	.023*
Splenectomy	6.59	3.09-14.05	< .001*
Transfusion	0.28	0.16-0.48	< .001*
Hydroxyurea	0.56	0.28-1.10	.090
Iron chelation	0.97	0.56-1.68	.912
Cholelithiasis			
Age > 35 y	2.76	1.56-4.87	< .001*
Female	1.96	1.18-3.25	.010*
Splenectomy	5.19	2.72-9.90	< .001*
Transfusion	0.36	0.21-0.62	< .001*
Hydroxyurea	0.55	0.29-1.02	.058
Iron chelation	0.30	0.18-0.51	< .001*
Abnormal liver function			
Ferritin ≥ 1000 μg/L	1.74	1.00-3.02	.049*
Transfusion	1.56	0.76-3.17	.224
Leg ulcers			
Age > 35 y	2.09	1.05-4.16	.036*
Ferritin ≥ 1000 μg/L	1.29	0.67-2.47	.449
Splenectomy	3.98	1.68-9.39	.002*
Transfusion	0.39	0.20-0.76	.006*
Hydroxyurea	0.10	0.02-0.43	.002*
Iron chelation	0.68	0.35-1.34	.269
DM			
Age > 35 y	2.00	0.53-7.62	.309
Splenectomy	5.79	0.71-47.21	.101
Hydroxyurea	0.24	0.03-2.20	.208
Iron chelation	0.40	0.10-1.62	.197
Hypothyroidism			
Age > 35 y	1.01	0.46-2.23	.984
Splenectomy	6.04	2.03-17.92	.001*
Transfusion	13.3	1.78-100.00	.012
Hydroxyurea	0.05	0.01-0.45	.003*
Iron chelation	0.49	0.22-1.07	.073
Osteoporosis			
Age > 35 y	3.51	2.06-5.99	< .001*
Female	1.97	1.19-3.27	.009*
Ferritin ≥ 1000 μg/L	1.60	0.96-2.68	.072
Splenectomy	4.73	2.72-8.24	< .001*
Transfusion	3.10	1.64-5.85	< .001*
Hydroxyurea	0.02	0.01-0.09	< .001*
Iron chelation	0.40	0.24-0.68	.001*
Hypogonadism			
Age > 35 y	1.05	0.51-2.15	.900
Female	2.98	1.79-4.96	< .001*
Ferritin ≥ 1000 μg/L	2.63	1.59-4.36	< .001*
Splenectomy	1.65	0.97-2.77	.056
Transfusion	16.13	4.85-52.63	< .001*
Hydroxyurea	4.32	2.49-7.49	< .001*
Iron chelation	2.51	1.48-4.26	.001*

RR indicates adjusted relative risk; CI, confidence interval; and DM, diabetes mellitus.

*Statistically significant.

was only independently associated with an increased risk of hypogonadism, iron chelathion therapy was protective for a multitude of other complications (hypogonadism, PHT, cholelithiasis, and osteoporosis). Hydroxyurea treatment was associated with an increased risk of hypogonadism yet was protective for EMH, PHT, leg ulcers, hypothyroidism, and osteoporosis. The mean number of complications for different management schemes are summarized in Figure 2. The lowest mean number of complications (0.827) reflected patients who received the 3 treatment modalities, whereas the highest mean number of complications (2.43) reflected patients who received no treatment; the difference between both means was statistically significant (*t* test; $P < .001$). The difference remained statistically significant after controlling for the effects of age and splenectomy ($P = .03$).

Discussion

Our study confirms previously reported complication rates in TI and further highlights the high prevalence of those specific complications that are thought to be more frequent in TI compared with TM: thrombosis, PHT, EMH, leg ulcers, and cholelithiasis.[3,10,24]

In our group, splenectomized patients had significantly higher rates than nonsplenectomized patients for almost all complications. Few clinical observations have suggested that splenectomy in TI can contribute to an increased susceptibility to thrombosis.[24,25] The development of these complications has been ascribed to the presence of high platelet counts and aggregation after splenectomy[26,27] and/or to increased number of RBCs with negatively charged membranes that carry thrombogenic potential.[28] In splenectomized TI patients, thrombin generation is significantly higher than in control subjects and patients who had not undergone splenectomy.[25] A study by Atichartakarn et al also noted that splenectomized thalassemia patients have a high frequency of PHT, mostly attributed to chronic thromboembolic disease.[29] Our study confirms these findings and recognizes additional complications that have never been associated with splenectomy. The higher incidence of iron overload-related complications in splenectomized patients suggests 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.[30] As per expert opinion and this report, the current indications for splenectomy in TI include growth retardation or poor health, leukopenia, thrombocytopenia, increased transfusion demand, or symptomatic splenomegaly.[5] However, our data call for a review of splenectomy as a procedure of choice, especially with its potential role in increasing

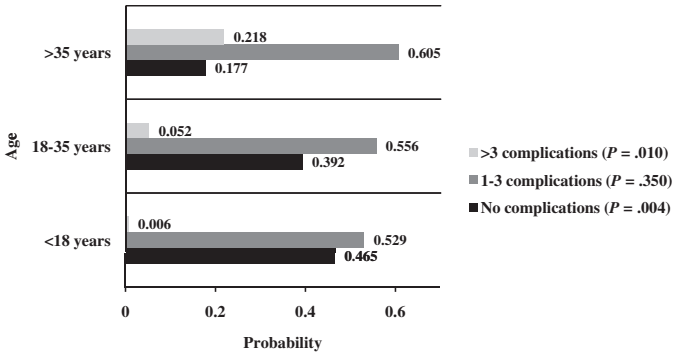

Figure 1. Probability of acquiring disease-related complications at different age intervals.

TI-related complications and the inherent risk of infection associated with the procedure, even for persons without hematologic disorders.[31]

In patients with TM, a remarkable improvement in life expectancy and prevention of morbidity have been achieved in recent decades.[32] This may be attributed to several key factors, including improved methods of blood transfusion, better understanding of iron toxicity, and a continuous improvement in iron chelation therapy.[32] TI has, however, been largely regarded as a mild to moderate disease with limited complications, and the prevailing approach has been avoidance of early blood transfusions and the concomitant requirement for chelation therapy. Consequently, unlike TM, evaluation of the role of transfusion and iron chelation therapy in the management of TI and prevention of its complications has been limited. In this study, it seems that patients who were placed on transfusion regimens had fewer complications relevant to chronic anemia, ineffective erythropoiesis, and hemolysis (mainly EMH, PHT, and thrombosis) while having a higher rate of iron overload-related endocrinopathy. Unlike TM, in which cardiac siderosis is the predominant cause of morbidity and mortality,[33] in TI increased thrombosis and PHT dominate the clinical picture.[24,34] These dismal complications observed with increasing frequency in untreated TI patients suggest that earlier intervention may benefit TI patients.[35] 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. This was also supported in our study in which patients who receive iron chelation therapy had fewer iron overload complications.

Thus, although current recommendations suggest initiating transfusion therapy after complications have manifested, it may be worthwhile considering earlier initiation as a preventive approach that will also help alleviate the increased risk of alloimmunization with delayed initiation of transfusion.[36] The initiation of chelation therapy in TI patients depends primarily on the extent of iron overload and rate of endogenous iron accumulation; but, as with other aspects of the management of TI, clear guidelines are not available.[5] In light of our recommendation of early initiation of transfusion therapy, iron chelation therapy would follow the same recommendations as with patients with TM.[5]

Increasing the synthesis of HbF can help alleviate anemia and ineffective erythropoeisis and therefore improve the clinical status of patients with TI.[37] Production of HbF is reactivated during recovery from marrow suppression after treatment with cytotoxic drugs; therefore, it is postulated that these agents may alter the pattern of erythropoiesis and increase the expression of γ-chain genes. Several cytotoxic agents with this effect have been identified, including cytosine arabinoside and hydroxyurea.[38-40] Our data support the encouraging results of a single report, evaluating 6 years of hydroxyurea therapy in transfusion-dependent patients with TI.[12]

Few studies outlined the effect of age in TI patients on the risk of iron overload and thrombosis.[34,41] Our study further expands on these findings and attributes a high rate of most disease-related complications to age. This brings further attention to our aging TI patient population. It directly calls for earlier intervention to prevent serious long-term sequelae and fortifies the notion that complications substantially increase as thalassemia patients enter adulthood.[42]

Our study carries the limitation of being retrospective in nature without a clear identification of the onset and chronology of complications with respect to treatment options received. Another limitation of our study is the use of serum ferritin instead of liver R2 magnetic resonance imaging for the assessment of iron

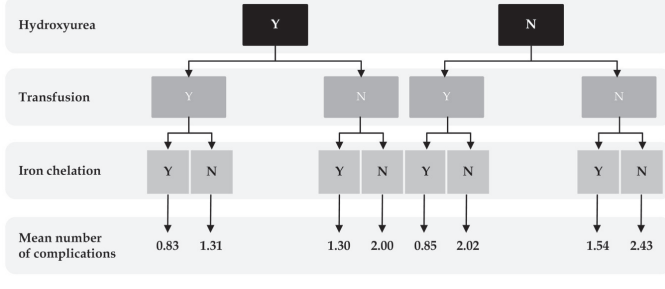

Figure 2. The mean number of complications for different management schemes.

overload, as studies have shown that serum ferritin may underestimate iron burden in TI,[41] and the use of echocardiography instead of cardiac catheterization for the diagnosis of PHT, which may increase the rate of false-positive findings. However, our patients were mainly screened for PHT after presenting with exertional dyspnea with no evidence of left heart disease. Moreover, echocardiography is still the modality of choice used in many studies on thalassemia and sickle cell anemia for financial/practical reasons and relying on reports of good relationship between Doppler estimates and invasive measurements of pulmonary arterial pressure at baseline and after treatment, despite the variable echocardiographic cutoff values used to label patients with PHT.[7,10,34,43]

Despite several 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. Our study confirms some of these recommendations yet challenges others through novel findings from a large cohort of patients. This should hopefully bridge the gap between evidence and practice by calling for prospective clinical trials that evaluate the efficacy, safety, and cost-effectiveness of these therapies. Such studies are expected to evaluate the optimal timing; dose and duration of transfusion, iron chelation, or hyrdoxyurea theapy; and the added advantage of multimodal therapy. Moreover, our study turns the attention toward an increasing complication rate in the elderly TI population and questions the role of splenectomy in this patient population. Until solid evidence-based guidelines are available, a system-centered risk stratification model that individualizes patient treatment should be entertained.

Authorship

Contribution: A.T.T., K.M.M., and M.D.C. were responsible for conception and design, data analysis and interpretation, and manuscript writing; K.M.M. performed statistical analysis; M.K., A.E.-B., K.B., S.D., and M.-S.E.S. gave administrative support and helped in provision of study material or patients; A.-H.E.-C. and M.R.F. helped in collection and assembly of data; and all authors gave final approval of the manuscript for submission.

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Optimal Management Of β Thalassaemia Intermedia

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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

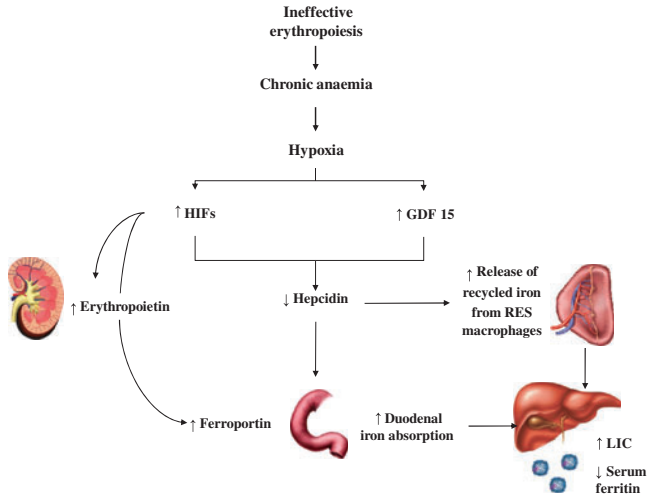

Fig 1. Iron metabolism in transfusion-independent patients with thalassaemia intermedia. The combination of ineffective erythropoiesis [leading to increased growth and differentiation factor 15 (GDF 15)] and chronic anaemia/hypoxia [altering the expression of hypoxia-inducible transcription factors (HIFs)] 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.

Disclosures

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Summary / Samenvatting

SUMMARY

The research presented in this thesis provides several novel insights regarding the β-thalassemia intermedia phenotype. Earlier studies observed that patients with β-thalassemia intermedia experience a clinical complications profile that is different from that in patients with β-thalassemia major; which was primarily attributed to their transfusion-independence. In this work, a variety of clinical morbidities were explored and their associations with the underlying disease pathophysiology and risk factors were examined. The morbidities evaluated throughout the studies involved several organs and organ systems including the vasculature (venous thrombosis, pulmonary artery hypertension, cerebrovascular disease, and leg ulcers), heart, liver, kidney, endocrine glands (diabetes mellitus, hypothyroidism, and hypogonadism), bone (osteoporosis), and the hematopoietic system (extramedullary hematopoietic tumors).

In **Chapter 2**, we first identified that growth differentiation factor-15 (GDF-15) -a newly identified marker that allows measurement of the severity of ineffective erythropoiesis- is elevated and associated with the multiplicity of morbidity. This was the first study to examine GDF-15 levels in β-thalassemia intermedia, providing a formal confirmation of the substantial role of ineffective erythropoiesis in the pathophysiology and clinical severity of β-thalassemia intermedia. Several new attempts are now being made

to identify targets for modulation of ineffective erythropoiesis in β-thalassemia intermedia; our study suggests that such efforts if successful, will help lessen the morbidity in this disease. In the second study presented in this chapter, a similar approach was undertaken to evaluate the association between fetal hemoglobin concentration and clinical morbidity in β-thalassemia intermedia. Increases in fetal hemoglobin concentration were shown to ameliorate disease severity in sickle cell disease, which led to the design of several pivotal trials on fetal hemoglobin modulators. These agents later became standard of care in patients with sickle cell disease after showing remarkable efficacy in reducing the rate of complications. The role of fetal hemoglobin modulation in patients with thalassemia, however, was not yet thoroughly investigated. Nonetheless, recent genome-wide association studies have identified several genetic variants that modulate fetal hemoglobin levels in patients with sickle cell disease and thalassemia and these approaches are being carried forward to identify novel targets for therapy. We realized that before any such attempts are applied to the β-thalassemia intermedia patient, evidence on the benefit of increasing fetal hemoglobin concentration is warranted. Our study not only confirmed that elevated levels are associated with less morbidity, but also provided details of the threshold needed to ensure absence of morbidity.

In **Chapter 3**, we revisit iron overload in patients with β-thalassemia intermedia. Although patients with β-thalassemia intermedia remain largely nontransfused, they can still accumulate iron secondary to intestinal iron absorption signaled by a high, although ineffective, erythropoietic demand. The translation of these findings into the clinical setting was never extensively explored. In the first study, we provided laboratory evidence that patients with β-thalassemia intermedia have high levels of non-transferrin-bound iron, the toxic species responsible for target-organ damage. The question that remained was whether iron accumulation can reach high-enough levels to cause clinical complications in β-thalassemia intermedia. The second study in **Chapter 3** provided a clear answer to this question. By measurement of liver iron concentration –a surrogate marker of total body iron levels- we were able to identify a high proportion of patients with increased hepatic iron levels as well as an association between elevated liver iron concentration and vascular, endocrine, and bone morbidity. Modifiers of this association were determined and thresholds of liver iron concentration that discriminate patients who develop complications were established. These findings suggest the need for iron chelation therapy in patients with β-thalassemia intermedia, and should help the design of future clinical trials. The two subsequent studies in this chapter used cardiac magnetic resonance imaging technology to provide evidence that despite elevated liver iron concentration, patients with β-thalassemia intermedia do not develop cardiac

siderosis. This finding has important implications for a tailored management of iron overload in patients with β-thalassemia intermedia. Unlike the case with β-thalassemia major, a reduction in mortality rate from cardiac iron overload will not be a target outcome in β-thalassemia intermedia. A study of renal function in β-thalassemia intermedia is then presented in this chapter. The study of renal manifestations in patients with β-thalassemia has primarily revolved around the potential nephrotoxic effects of iron overload and chelators used to treat transfusional iron overload in β-thalassemia major patients. However, data on the role of primary, nontransfusional iron overload was not available. Our study concluded that iron overload in transfusion-independent patients with β-thalassemia intermedia is associated with overt proteinuria, possibly attributed to damaging effects on tubular cells.

Chapter 4 shifts to the study of vascular disease in patients with β-thalassemia intermedia. The hypercoagulability in β-thalassemia intermedia and the common practice of splenectomy were shown to be associated with the occurrence of venous thrombosis. Our first study in this chapter further identified the characteristics of splenectomized β-thalassemia intermedia patients who develop venous thrombosis which shed more light on the underlying mechanisms, provided valuable information for any risk-assessment strategies to be applied for preventive measures, and suggested a beneficial role for some already available interventions. The second

study used a similar approach to evaluate β-thalassemia intermedia patients with pulmonary artery hypertension. The subsequent three studies investigated cerebrovascular disease in this patient population. Remarkable advances have been made in understanding central nervous system involvement in sickle cell disease. However, data in β-thalassemia intermedia patients are lacking despite several similarities between both hemoglobinopathies. It was already established that the frequency of stroke in β-thalassemia intermedia patients is high but lower than β-thalassemia major patients who have several stroke-related risk factors like cardiac failure and arrhythmias. However, an earlier study in the 1990s highlighted that β-thalassemia intermedia patients have a high rate of silent cerebral infarction. We performed three imaging studies on a carefully selected group of splenectomized adults with β-thalassemia intermedia. We confirmed a high frequency of silent ischemic lesions on brain magnetic resonance imaging especially in patients who had never received transfusion therapy. For the first time, we also showed that large-vessel disease on magnetic resonance angiography is common in β-thalassemia intermedia and is associated with levels of toxic iron species. Moreover, using positron emission tomography, we also uncovered a novel finding in β-thalassemia intermedia patients, neuronal dysfunction that is proportional to the degree of iron overload. These new insights on brain involvement in β-thalassemia intermedia will stimulate further

research of the long-term sequelae of such covert findings and their optimal prevention strategies.

Chapter 5 determined that the multiplicity of complications affects quality of life in β-thalassemia intermedia patients, enforcing the need for appropriate patient management. **Chapter 6** explores the role of currently available treatment interventions for β-thalassemia intermedia patients. The first study in this chapter clearly established that without intervention patients with β-thalassemia intermedia will suffer greater morbidity as they advance in age. The following study remains one of the largest conducted on β-thalassemia intermedia patients and provided several valuable clues. It demonstrated the frequency of different complications in a group of patients attending specialized care centers. More importantly, it observed that management in β-thalassemia intermedia does not follow any clear guidelines as different measures were implemented for different patients, primarily relying on the caring physician's knowledge and expertise. Data from this study showed that splenectomy is not only associated with an increased risk of venous thrombosis but also a variety of other morbidities. This indicated the need for a careful, as opposed to the previously liberal, approach to splenectomy reserving the procedure to patients who have extreme indications. Moreover, the beneficial roles of transfusion, iron chelation, fetal hemoglobin induction, and combinations of these therapies were established. Results were joined to those retrieved from our other work presented

in this thesis to compile a management guideline (third section of **Chapter 6**) that shall remain useful until more evidence-based findings from trials are available.

In conclusion, the work presented in this thesis has added to our knowledge of the various clinical morbidities that β-thalassemia intermedia patients endure and will hopefully invite further work on this topic. Findings confirm that β-thalassemia intermedia should no longer be regarded as a mild form of thalassemia as patients experience serious manifestations involving almost every organ system. Moreover, with better understanding of the mechanisms and risk factors of disease, as well as the roles of available treatments, patient care should start transforming into optimal standards.

SAMENVATTING

Het in dit proefschrift beschreven onderzoek heeft tot enkele nieuwe inzichten geleid over de aandoening β-thalassemie intermedia. In eerder onderzoek was al aangetoond dat patiënten met β-thalassemie intermedia andere complicaties ervaren dan patiënten met β-thalassemie major, hetgeen vooral werd toegeschreven aan de mindere behoefte aan bloedtransfusies. In dit proefschrift worden in een aantal hoofdstukken de klinische manifestaties nagegaan en in verband gebracht met onderliggende aandoeningen en risicofactoren. De onderzochte ziektebeelden betreffen verscheidene orgaansystemen, zoals het vaatsysteem (veneuze trombose, pulmonaalhypertensie, cerebrovasculaire accidenten, ulcera van het been), het hart, de lever, de nieren, de hormoonhuishouding (suikerziekte, schildklier, geslachtshormonen), bot (osteoporose) en het bloedcelproducerende systeem.

In hoofdstuk 2 toonden we aan dat ‘groei- en differentiefactor-15’ (GDF-15), een recent ontdekt eiwit dat een merker is voor de ernst van een gestoorde aanmaak van rode bloedcellen, verhoogd is in thalassemie intermedia en samenhangt met de ernst van de aandoening. Dit onderzoek toonde het belang van een gestoorde bloedaanmaak in thalassemie intermedia aan, en ontwikkelingen om deze te proberen te beïnvloeden kunnen daarom van therapeutisch belang zijn. In hoofdstuk 2 keken we ook naar de rol van foetaal

hemoglobine, waarvan eerder is aangetoond dat het in sikkelcelanemie de ernst van de aandoening bepaalt. In analogie van de bevindingen bij sikkelcelanemie vonden wij dat verhoogde niveaus in het bloed van foetaal hemoglobine bij thalassemie intermedia samenhangen met een verminderde morbiditeit.

In hoofdstuk 3 onderzochten wij de effecten van ijzerstapeling bij β-thalassemie intermedia. Hoewel deze patiënten zelden bloedtransfusies nodig hebben, kunnen zij toch ijzer stapelen door een verhoogde absorptie in de darm, gestimuleerd door de, zij het inefficiënte, verhoogde aanmaak van rode bloedcellen. In het eerste onderzoek lieten wij zien dat patiënten met β-thalassemie intermedia hoge bloedspiegels hebben van aan transferrine gebonden ijzer, waarvan bekend is dat het orgaanschade kan veroorzaken. De vraag of de verhoogde spiegels inderdaad zo hoog zijn dat zij schadelijk kunnen zijn, beantwoorden wij door te kijken naar de ijzerconcentratie in de lever, hetgeen een reflectie is van de totale ijzervoorraad in het lichaam. Niet alleen vonden wij een groot aantal patiënten met verhoogde waarden, maar ook zagen wij een relatie met afwijkingen in de vaten, de hormoonhuishouding en de botten. Deze bevindingen suggereren dat ook bij patiënten met β-thalassemie intermedia vermindering van de ijzervoorraad (ijzerchelatietherapie) nut zou kunnen hebben. In ditzelfde hoofdstuk werd met ‘magnetic resonance imaging (MRI)’ aangetoond dat de verhoogde ijzerconcentratie in de lever bij β-

thalassemie intermedia niet gepaard lijkt te gaan met een ophoping van ijzer in het hart. Dit impliceert dat de behandeling van ijzerstapeling bij β-thalassemie intermedia toegespitst zal moeten zijn op zorgvuldig geselecteerde patiënten en niet, in tegenstelling tot bij β-thalassemie major, als primaire doel zal hebben de complicaties van ijzerstapeling in het hart tegen te gaan. Daarom werd in dit hoofdstuk ook gekeken naar de effecten van ijzerstapeling op de nieren en wij vonden een samenhang met eiwitverlies via de nieren.

Hoofdstuk 4 behandelt het optreden van vaatziekten bij patiënten met β-thalassemie intermedia. De verhoogde stolbaarheid van het bloed en het frequent voorkomen van verwijdering van de milt verhoogden de kans op veneuze trombose. In het eerste onderzoek van dit hoofdstuk keken wij naar de karakteristieken van zulke patiënten. Vervolgens keken wij naar het optreden van pulmonaalhypertensie en cerebrovasculaire aandoeningen bij β-thalassemie intermedia. Eerder was reeds aannemelijk gemaakt dat er een verhoogde frequentie van cerebrovasculaire accidenten (CVA) is bij patiënten met β-thalassemie intermedia, maar dat dit minder sterk verhoogd is dan bij β-thalassemie major. Met MRI van de hersenen toonden wij aan dat ‘stille’ herseninfarcten, d.w.z. die niet tot duidelijke symptomen en een diagnose hadden geleid, bij β-thalassemie intermedia patiënten bij wie de milt verwijderd was, frequent voorkwamen.

Uit het onderzoek beschreven in hoofdstuk 5 bleek dat de veelheid aan complicaties de kwaliteit van leven van patiënten met β-thalassemie intermedia negatief beïnvloedt. In hoofdstuk 6 beschreven wij de behandelingen die momenteel voorhanden zijn voor patiënten met β-thalassemie intermedia. Allereerst bleek dat zonder behandeling er met het voortschrijden van de jaren een toenemende ziektelast is. Ook bleek dat het beleid bij β-thalassemie intermedia geen duidelijke consensus of richtlijnen volgt, en dus gebaseerd is op de ervaring en kennis van de individuele arts. Verwijdering van de milt bleek duidelijk geassocieerd met een verhoogde kans op veneuze trombose, waaruit wij concluderen dat deze behandeling alleen in uitzonderingen dient te worden overwogen. Daarentegen bleken gunstige effecten van transfusies, chelatietherapie, verhoging van het foetaal hemoglobine en combinaties van deze behandelwijzen. Een en ander heeft geleid tot de behandelingsrichtlijnen in hoofdstuk 6.

Het in dit proefschrift beschreven onderzoek heeft onze kennis van β-thalassemie intermedia vergroot en vormt hopelijk de opmaat voor meer onderzoek op dit onderwerp. Het moge duidelijk zijn dat β-thalassemie intermedia niet slechts als een milde vorm van thalassemie gezien dient te worden, aangezien deze patiënten ernstige complicaties kunnen ontwikkelen, in vrijwel ieder orgaansysteem. Een beter begrip van de mechanismen die hierbij

een rol spelen, en het effect van de beschikbare behandelingsvormen, moeten uiteindelijk leiden tot een optimalisering van de zorg voor patiënten met β-thalassemie intermedia.

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Curriculum Vitae

CURRICULUM VITAE

Khaled M. Musallam, M.D.

Khaled Musallam (1982, Amman, Jordan) graduated from the National Orthodox School in Amman, Jordan with high distinction (1999). He then continued his education at the American University of Beirut in Lebanon where he received his Bachelor of Science (B.Sc.) in Biology degree with distinction (2004) and Doctor of Medicine (M.D.) degree (2009). He then completed two years of postdoctoral Clinical Research Fellowship at the Division of Hematology and Oncology, Department of Internal Medicine, American University of Beirut Medical Center (2009-2011) and is currently a Clinical Research Fellow at the Angelo Bianchi Bonomi Haemophila and Thrombosis Center, Department of Medicine and Medical Specialties, IRCCS Ca' Granda Foundation Maggiore Policlinico Hospital in Milan, Italy; working under the supervision of Dr. Flora Peyvandi (2011-2012). He conducted the work described in this thesis during his three years of postdoctoral Clinical Research Fellowship.

Dr. Musallam has devoted his career to clinical research development ever since he was a medical student. For three years now, he has published four book chapters and over 110 articles in international peer-reviewed journals, many in leading general

medicine and hematology journals. His main research interests are the congenital anemias including β-thalassemia, especially thalassemia intermedia, and sickle cell disease; acquired anemias; as well as thrombosis, hemostasis, and vascular disease. Dr. Musallam has collaborated with investigators and research groups from the Middle East, Europe, USA, and Canada and follows the mentorship of leaders in the field of hemoglobinopathy research including Sir Professor David Weatherall and Professor David Nathan. He himself also mentored over ten physicians-in-training and fortified their interest in academic medicine.

Dr. Musallam is also a peer-reviewer for twenty medical journals, an Associate Editor for the European Journal of Internal Medicine, and an Associate Faculty Member at Faculty of 1000 Medicine.

Ali T. Taher, M.D., F.R.C.P.

Ali Taher (1960, Tyre, Lebanon) graduated from the Rawda High School in Beirut, Lebanon with distinction (1979). He then continued his education at the American University of Beirut in Lebanon where he received his Bachelor of Science (B.Sc.) in Biology degree (1982) and Doctor of Medicine (M.D.) degree (1986). He then completed a Residency in Internal Medicine at the American University of Beirut (1986-1989) and a Fellowship in Hematology and Oncology both from the American University of Beirut (1989-1991) and the Royal Free Hospital in London, UK (1991-1992). He was appointed as an Assistant Professor of Medicine at the Division of Hematology-Oncology, Department of Internal Medicine, American University of Beirut Medical Center in 1993, and was promoted to the rank of Professor in 2005. He also serves as Associate Chair for Research at the Department of Internal Medicine since 2011. In addition, he is a Consultant Hematologist at the Thalassemia Department of the Chronic Care Center in Hazmieh, Lebanon. He was recently appointed a Fellow of the Royal College of Physicians (2011).

Dr. Taher's research focuses on hemoglobinopathies, notably thalassemia and sickle cell disease, as well as thrombosis & hemostasis. Within thalassemia, his research interest relies in the detection of iron overload and the efficacy and safety of novel oral

iron chelators. Moreover, he investigates the pathophysiology and clinical implications of thalassemia intermedia. In thrombosis & hemostasis, he investigates inherited thrombophilia and bleeding disorders, as well the incidence and prophylaxis of venous thromboembolic events across medical and surgical settings.

Dr. Taher has been nationally and internationally active in the fields of thalassemia and hemoglobinopathies for more than 18 years. He has shown leadership in creating local and regional scientific interest groups and associations that promote partnerships in science and dissemination of knowledge both to physicians and the community.

Dr. Taher has published around 300 peer-reviewed articles in leading international hematology journals. He is also an author of three textbook chapters, an editor of the Thalassaemia International Federation Treatment Guidelines for Thalassemia, a reviewer for the top ten hematology journals, and an Associate Editor for the journal Hemoglobin. He is a regular chair and invited speaker at national and international meetings including the European Hematology Association, and among the faculty of the European School of Hematology.

Dr. Taher was the principal investigator on several clinical trials studying the oral iron chelator deferasirox including the ESCALATOR and THALASSA trials.