CLINICAL STUDY

Thyroid dysfunction in thalassaemic patients: ferritin as a prognostic marker and combined iron chelators as an ideal therapy

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Abstract

Objective: Endocrine complications characterised patients with β thalassaemia (βT). In particular, thyroid dysfunction occurs frequently in βT major, but its long-term natural history is poorly understood.
Design: A total of 72 βT patients were followed for 8 years. The incidence of thyreopathies, defined as the primary study endpoint, was assessed. The aim of this study was to analyse the prognostic role of ferritin for thyreopathies in patients with major and intermedia βT. The power of different iron chelators to treat iron overload and to prevent or reverse thyreopathies was also assessed.
Methods: Patients were treated with chelators with different chelation strategies during the study. Receiver operating characteristics analysis was employed to calculate the area under the curve for serum ferritin to find the best cutoff values capable of identifying thyroid dysfunction in thalassaemic patients. Kaplan–Meier curves were generated to assess incidence of thyreopathy. Adjusted risk estimates for thyreopathy were calculated using univariate followed by multivariate Cox proportional hazard regression analysis.
Results: Patients with thyroid dysfunction were characterised by higher ferritin when compared with patients without thyreopathies (1500 (872–2336) vs 513 (370–698) µg/l; $P < 0.0001$). Patients with ferritin values above 1800 µg/l experienced a significantly faster evolution to endpoint (log-rank (χ^2): 7.7; $P = 0.005$). Ferritin predicted high risk of thyroid dysfunction independently of confounding factors (hazard ratio: 1.20; $P < 0.0001$). The intensification of chelation therapy led to an amelioration of thyroid function.
Conclusions: Ferritin represents a prognostic marker for βT patients and a predictive factor for progression to thyroid dysfunction. Intensive chelation therapy allows the prevention and reversibility of thyroid complications.

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Introduction

Blood transfusions and iron chelation therapy are standard treatment for patients affected by β thalassaemia (βT) major. However, repeated transfusions gradually increase the total body iron load, resulting in secondary hemosiderosis with cardiac, endocrine and hepatic complications (1). Moreover, despite their initial transfusion-independence, βT intermedia patients can still accumulate iron due to increased intestinal absorption (2).

Thyroid dysfunction is known to occur frequently in βT major, but its prevalence and severity varies in different cohorts, and its long-term natural history is poorly understood (3). Previous studies in transfused βT patients showed a variable prevalence of hypothyroidism ranging from 0 to 35%, depending on the age of the study population, duration of blood transfusions, ferritin levels and dose and type of the iron-chelating agent (4, 5).

Although the actual mechanism is not definitive, however, the most likely explanation is related to iron overload and its burden, in addition to lipid peroxidation, oxidative stress and free radicles release (6). However, cardiomyopathy remained the most common and important cause of death, and endocrine morbidities became very common in long-term survivors (7).

The iron status can be assessed by several methods. Serum ferritin has in general been found to correlate with body iron stores. However, as a single value it is not

always reliable because, being an acute phase reactant, it is influenced by other factors such as inflammatory disorders, liver disease and malignancy. Despite this, serial measurements of serum ferritin remain a reliable and the easiest method to evaluate iron overload and efficacy of chelation therapy (8).

Determination of liver iron concentration (LIC) in a liver biopsy specimen shows a high correlation with total body iron accumulation and is considered the gold standard for the evaluation of iron overload. However, liver biopsy is an invasive technique with the possibility (though low) of complications (9).

The three main iron chelators used in current clinical practice are deferoxamine (DFO), deferiprone (DFP) and deferasirox (DFX). Each chelator has different properties influencing the clinical management of iron overload (10).

DFO was the first iron chelation treatment used in thalassaemia. The effectiveness of this parenterally administered chelator is limited partly by poor patient compliance (11). In addition, because cell membranes are relatively impermeable to DFO and DFO–iron complexes, serious cardiac, hepatic and endocrine complications continue to arise, even in compliant patients (12).

DFP, a chelating agent administered orally, has been approved for use as second-line chelation therapy in Europe since 1999. It binds iron in a 3:1 ratio. The majority of the DFP iron complex is excreted in the urine and the remainder in the feces. The most serious side effect associated with its use is agranulocytosis, which occurs in 2% of patients (13).

The approval of DFX as an orally effective iron-chelating drug in 2005 promised to improve the management of iron overload. It binds to iron in a 2:1 ratio and the complex is excreted almost exclusively in the feces. Due to the relatively long half-life, it may be administered in a once-daily dose, ranging between 10 and 40 mg/kg body weight (14).

Today, the chelation options available are monotherapy with any of these three iron chelators. Combinations of any two of them or even all three provide newer and more effective choices for patients and their physicians. Early intervention with intensification of iron chelation, where appropriate, has in fact allowed the reduction of life-threatening iron overload with reduced mortality from cardiac causes and morbidities related to endocrinopathies (15).

However, there were very few trials for each comparison, and the heterogeneity of the data between the trials together with the different treatment schedules made comparisons and conclusions difficult (16).

The main objective of this prospective study was to establish the incidence and prevalence of thyroid disorders in a cohort of patients with major and intermedia βT, analysing the diagnostic and prognostic role of serum ferritin. We have also evaluated the power of three different iron chelators, administered alone or in combined schemes, to treat the iron overload and to prevent or reverse thyreopathies.

Subjects and methods

Patients and study design

A total of 72 βT patients, who had been referred to the Thalassaemia Unit, Department of Pediatrics, University of Messina, were followed for a period of 8 years. Diagnosis of βT was based on family history, complete transfusion dependence and haemoglobin electrophoresis. A genetic analysis was also performed. In particular, 21 patients suffered from thalassaemia intermedia (TI), whereas 51 patients had thalassaemia major (TM). All TM patients followed a standard treatment protocol and were regularly transfused with packed red cells every 3 weeks, with the aim of maintaining pre-transfusion haemoglobin levels above 9 g/dl. Sixteen TI patients were periodically transfused. At baseline, all patients received chelation monotherapy with DFO (Desferal; Novartis), at a dose of 40 mg/kg per s.c. infusion 5 days per week.

Patients with βT minor and those with acute illness, severe renal and liver disease, heart failure or cardiomyopathy, endocrine complications such as diabetes mellitus, thyroid dysfunction or assuming hormonal therapy were excluded from the study. The study was approved by the local Ethic Committee, and all patients gave written informed consent.

Collection of blood, procedures and definitions

Blood samples were drawn from all patients in fasting state in the morning during their regular visits. Thyroid function was evaluated by measurements of serum-free triiodothyronine (FT_3), serum-free thyroxine (FT_4), thyroid-stimulating hormone (TSH), antithyroglobulin (TGA) and antithyroid peroxidase (TPO). Iron load status was defined by serum ferritin level that was estimated from pre-transfused blood sample and pre-chelation therapy and subsequently every 3 months.

Serum TSH, FT_3, FT_4 and ferritin were measured by chemiluminescent microparticle immunoassay methods on Architect 2000 System (Abbott). TGA and TPO antibody titers were measured by RIA.

Thyroid dysfunction was defined as follows: overt hypothyroidism: low FT_4 and/or FT_3, increased TSH levels; and subclinical hypothyroidism: normal FT_4, FT_3 and increased TSH concentration (> 5 TSH μIU/ml). A central hypothyroidism was defined as an inappropriately low serum TSH concentration in the presence of subnormal serum T_4 and T_3 concentrations.

An ultrasound evaluation of the thyroid gland was done in each patient. In particular, thyroid evaluation by ultrasound was carried out in the supine position with moderate extension of the neck with a machine

equipped with a linear 10 MHz high-resolution transducer and a curved array 7.5 MHz transducer. The thyroid gland was scanned at the long- and short-axis of each lobe. One longitudinal and two transverse diameters were recorded and thyroid volumes were calculated by multiplication of three diameters and the constant value 0.52 (17).

Lipid profile, blood pressure and metabolic parameters were also assessed. In particular insulin resistance was determined by the homoeostatic model assessment insulin resistance (HOMA-IR) and calculated according to the following formula: (Insulin (μU/ml)$\times$serum glucose (mmol/l))/22.5 (18).

Prospective follow-up, end points and randomisation

At baseline, all patients received iron chelation monotherapy with DFO. After baseline assessments, patients were followed prospectively until the end of the first observation period (4 years) or until the primary study endpoint was reached. This latter was defined by the diagnosis of thyreopathy.

After this first period, two groups were organised: group A was characterised by patients with thyreopathy and group B in which no thyroid alterations were observed. These two groups began a period of follow-up of 2 years. All patients belonging to group A were switched to an intensive combination scheme with DFO and DFP (Ferriprox; Apotex Europe B.V. Leiden, The Netherlands) consisting of both daily oral administration of DFP 75–100 mg/kg per day in three divided doses and DFO injection (20–40 mg/kg, 8–12 h, 2–6 days/week). Individual dosing and frequency of DFO infusions were determined by patients' clinical and laboratory assessments, such as iron overload indices and comorbidities. Group B continued an iron monotherapy chelation with DFO. During this period, the reversibility of thyreopathy and new cases of thyroid dysfunction were evaluated. After the end of these 2 years, a third observational period of 2 years was planned. In particular, patients with a new diagnosis of thyreopathy began a combined chelation therapy, whereas patients with no signs of thyroid dysfunctions continued to be treated with a monotherapy chelation. This latter group was randomised into three arms, based on the type of iron chelation: group C treated

with DFO, group D treated with DFP and group E treated with DFX (Exjade, Novartis).

The patients, physicians, laboratory staff and the epidemiologist who analysed the data were not aware of the intervention for each group. Figure 1 schematises the study design and the different chelation strategies administered during the study.

Statistical analyses

Statistical analyses were performed with NCSS for Windows (version 4.0), the MedCalc (version 11.0; MedCalc Software Acacialaan, Ostend, Belgium) software and the GraphPad Prism (version 5.0; GraphPad Software, Inc., San Diego, CA, USA) package. Differences between groups were established by unpaired *t*-test or by ANOVA followed by Bonferroni's test for normally distributed values and by Kruskal–Wallis analysis followed by Dunn's test for nonparametric values. Dichotomised values were compared using the χ^2-test. Pearson or Spearman correlation coefficients were used as appropriate to test correlations between ferritin and other variables. Receiver operating characteristics (ROC) analysis was employed to calculate the area under the curve (AUC) for serum ferritin to find the best cutoff values capable of identifying thyroid dysfunction in thalassaemic patients.

Kaplan–Meier curves were generated to assess incidence of thyreopathy in subjects with serum ferritin values above and below the optimal ROC-derived cutoff levels. Differences were evaluated using the log-rank test. Adjusted risk estimates for thyreopathy were calculated using univariate followed by multivariate Cox proportional hazard regression analysis. All results were considered significant if *P* was <0.05.

Results

Patients' baseline characteristics

The main auxological and metabolic data of patients, at baseline, are summarised in Table 1. The study group included 38 females and 34 males. TM and TI patients were well matched for sex and age. At baseline, ten patients (48%) with TI were dependent on
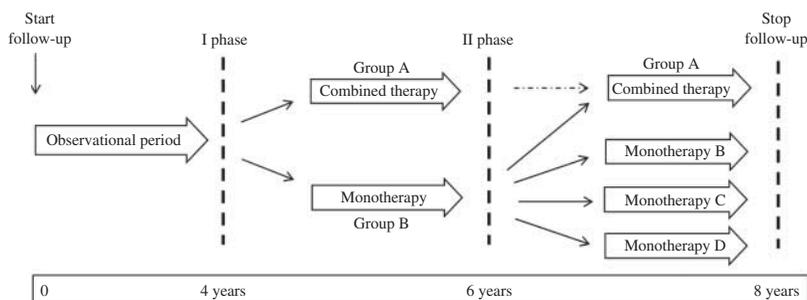

Figure 1 Study design and chelation strategies.

Table 1 Main baseline characteristics of the study cohort and statistical differences between patients with or without thyroid dysfunction progression during the follow-up period.

	Baseline data		Follow-up (4 years)		
	TM patients ($n=51$)	TI patients ($n=21$)	TIR ($n=26$)	NO-TIR ($n=46$)	**P**
Age (years)	34.4 ± 9.7	38.5 ± 19.4	43.6 ± 11	31.2 ± 12.6	0.0001
BMI (kg/m^2)	21.2 ± 2.6	21.3 ± 3.2	19.05 ± 1.8	22.6 ± 2.4	<0.0001
SBP (mmHg)	100.1 ± 13	104.4 ± 12.2	133.2 ± 10	101.5 ± 12	<0.0001
DBP (mmHg)	62.8 ± 7.7	64.3 ± 7.4	64.3 ± 8.4	62.9 ± 7.7	0.49
Total cholesterol (mg/dl)	119.3 ± 23.2	132 ± 41.9	130.6 ± 32.6	143.6 ± 28.4	0.16
TG (mg/dl)	105.9 ± 44.6	96 ± 40.4	132.6 ± 40.3	121.8 ± 33.7	0.11
Glucose	98.7 ± 18.8	78.7 ± 11.1	121.5 ± 14.3	98.5 ± 19	<0.0001
HOMA-IR	1.1 ± 0.4	0.9 ± 0.3	2.9 ± 1.2	1.1 ± 0.3	<0.0001
Hb (g/l)	9.3 ± 0.4	9.4 ± 1	8.4 ± 0.6	9.9 ± 0.6	<0.0001
Ferritin (µg/l)	872 (541–1921)	670 (480–1345)	1500 (872–2336)	513 (370–698)	<0.0001
Creatinine	0.5 ± 0.2	0.5 ± 0.4	0.7 ± 0.3	0.6 ± 0.5	0.34
AST (IU/l)	43.6 ± 38.4	30.3 ± 14.8	45.7 ± 24.6	41.5 ± 21.3	0.22
ALT (IU/l)	66.6 ± 14.8	35.6 ± 30.2	52.5 ± 30.2	49.7 ± 28.3	0.16
TSH (µIU/ml)	3.2 ± 2.1	2.9 ± 1.7	5.3 ± 2.4	2.7 ± 1	<0.0001
FT$_4$ (pmol/l)	14.8 ± 1.9	15.1 ± 2.3	14.7 ± 5.1	13.8 ± 3.4	0.86
Splenectomy (%)	29 (56%)	8 (38%)	20 (76%)	17 (37%)	0.006

TIR, group with thyroid dysfunction; NO-TIR, group without thyroid dysfunction; Hb, haemoglobin; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglycerides; HOMA-IR, homoeostatic model assessment insulin resistance; TSH, thyroid-stimulating hormone; FT$_4$, free thyroxine; AST, aspartate transaminase; ALT, alanine transaminase.

hemotransfusion therapy, six patients (28%) were occasionally transfused, whereas five patients (24%) did not receive blood transfusions. Haemoglobin levels in TM patients were 9.3 ± 0.4 g/dl before blood transfusions.

Furthermore, splenectomy was applied to 29 TM and eight TI patients before the enrollment. Fasting glucose levels (FG) were within normal range in all patients, as well as insulin sensitivity HOMA-IR. All patients had systolic and diastolic blood pressure values within the normal range. A normal lipid profile was also assessed in all enrolled patients, at baseline.

Serum ferritin level in TM patients was significantly higher than in TI patients (872 (541–1921) vs 670 (480–1345) µg/l, $P<0.0001$). At baseline no patients had thyroid dysfunctions, with normal thyroid hormones levels and ultrasound parameters in the normal range.

First observational period and progression to end-point

Patients were followed over a period of 4 years, evaluating the incidence of thyreopathy. During this period, 26 patients (36%) had a thyroid dysfunction, whereas 46 patients had no alterations on thyroid hormones and echographic parameters. In particular, 16 patients had a subclinical hypothyroidism, whereas ten patients were affected by an overt hypothyroidism. Autoimmune thyroiditis was diagnosed in only two patients. No case of central hypothyroidism was observed. The ultrasound exam demonstrated a reduced antero–posterior diameter of the thyroid in eight patients (11.1%) and features of dishomogeneity of the glandular tissue were revealed in 26 patients (36%). Thyroid nodules had been observed in 21 patients (29%) and the mean values of thyroid volumes were 12.3 ± 3.9.

The mean level of serum FT$_4$ concentration of the group with thyreopathy (TIR group) was 14.7 ± 5.1 pmol/l, without statistical differences when compared with non progressor patients (NO-TIR group) (13.8 ± 3.4 pmol/l; $P=0.37$). Moreover the mean serum level of TSH in the NO-TIR group was lower when compared with TIR group (2.7 ± 1.08 vs 5.3 ± 2.4 µIU/ml).

These data are referred to all thalassaemic patients. Distinguishing among TM and TI patients, we have noted that incidence of thyreopathy was higher in TM than those of TI patients (out of 26 thyreopatic patients, nine had TI). Furthermore, thyreopatic TM patients had mean FT$_4$ levels lower than thyreopatic TI patients (12.6 ± 5.7 vs 16.6 ± 1.4 pmol/l; $P=0.03$). TM patients with thyroid dysfunction also had higher THS levels than thyreopatic TI patients (4.2 ± 1.4 vs 2.7 ± 1.4 µIU/ml: $P=0.01$). Furthermore, the decision to start a T$_4$ treatment was individualised and based on patients' clinical history and comorbidities. According to ferritin levels, the TIR group was characterised by higher ferritin levels when compared with the NO-TIR group (1500 (872–2336) vs 513 (370–698) µg/l; $P<0.0001$).

Univariate correlations and multiple regression analysis

Serum ferritin was strictly correlated with markers of thyreopathy. In particular, we demonstrated that ferritin was directly correlated with TSH ($r=0.71$; $P<0.0001$), thyroid nodular lesions ($r=0.52$; $P=0.005$) and age ($r=0.72$; $P=0.0004$), whereas an inverse correlation was found with FT$_4$ levels ($r=-0.51$; $P=0.007$) and thyroid volume ($r=-0.75$; $P<0.0001$) (Fig. 2).
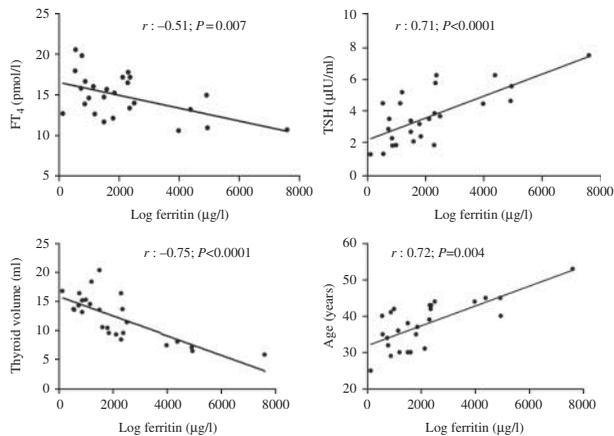
Figure 2 Univariate and multiple regression analyses of ferritin at baseline in thalassaemic patients.

Using ferritin as the dependent variable in a multiple regression model including all previously reported univariate correlates, only the associations with TSH ($\beta = 0.71$, $P = 0.004$) and thyroid volume ($\beta = -0.75$, $P = 0.0004$) remained significant.

Ferritin as a diagnostic marker of thyreopathy

ROC analyses were performed in order to define the diagnostic profile of serum ferritin in identifying thyreopathy among thalassaemic patients. The AUC for ferritin was 0.955. When the cutoff values of ferritin were set at 1800 µg/l, sensitivity and specificity of the marker used for the diagnosis were 84.6 and 97.7% (Fig. 3).

Patients with serum ferritin values above 1800 µg/l experienced a significantly faster evolution to endpoint (log-rank (χ^2): 7.7; $P = 0.005$; hazard ratio (HR): 0.3 (95% CI, 0.1–0.7)), with a mean follow-up time of 14 months to progression compared with >40 months for serum ferritin below the cut-off (Fig. 4).

Univariate/multiple cox regression analysis and incidence of thyroid dysfunction

To identify putative risk factors associated with incidence of thyroid dysfunction, we performed a Cox regression analysis, inserting in the model all variables that were different at baseline in patients who reached the end-point during the whole follow-up period.

Univariate analysis showed that only age, BMI, systolic blood pressure, serum glucose, HOMA-IR, haemoglobin levels, splenectomy, TSH and serum ferritin were significantly associated with endpoint. Table 2 summarises the unadjusted HRs for the study outcome associated with various parameters taken into account. A multiple Cox regression was constructed, simultaneously inserting into the model all of the

variables found to be significantly associated with the endpoint at univariate analysis. Results from this analysis indicated that serum ferritin, as well as haemoglobin levels, splenectomy and TSH predicted higher risk of thyroid dysfunction independent of BMI and age. In detail, an increase of serum ferritin was associated with a 20% increased risk of progression (HR: 1.20; 95% CI, 1.10–1.26; $P < 0.0001$), such as a decrease in haemoglobin levels increased this risk by 10% (HR: 1.10; 95% CI, 1.07–1.16; $P = 0.0005$). The risk of progression associated with an increment in TSH levels was 15%, whereas a patient with splenectomy had an increased risk of 8%. Table 2 summarises data from multivariate Cox analysis.

First randomisation: two chelation schemes in comparison

The intensification of chelation with combined therapy (DFO+DFP) and the reduction in serum ferritin levels, led to an amelioration of thyroid function with a significant increase in FT_4 and reduction in TSH levels both in euthyroid as well as in hypothyroid thalassaemic patients (Table 3).

In particular, 8/12 (67%) discontinued T_4 therapy and 4/12 (33%) reduced their T_4 dose. These patients were older, had the highest ferritin levels, as well as the worst blood pressure and glycemic control, when compared with patients who suspend the treatment. In addition, new cases of hypothyroidism were not noted after combined chelation. In the combined treatment group, there was a significant reduction in ferritin, from a median value of 1500 µg/l at baseline to 569 µg/l ($P < 0.0001$) at 24 months. A moderate reduction in
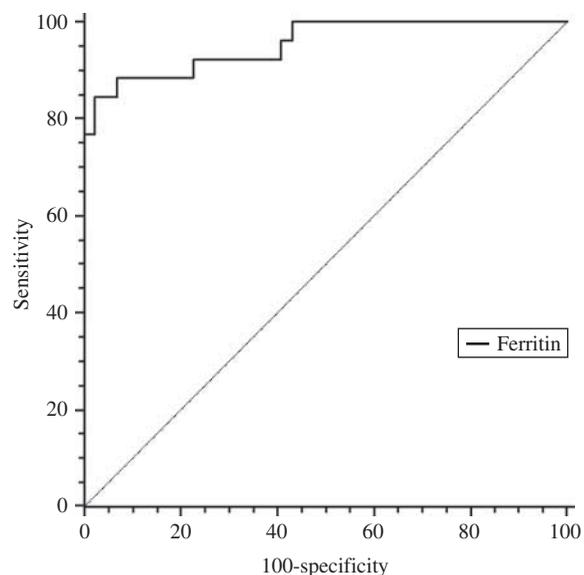

Figure 3 Receiver operating characteristics curves of serum ferritin considering onset of thyroid dysfunction as status variable.
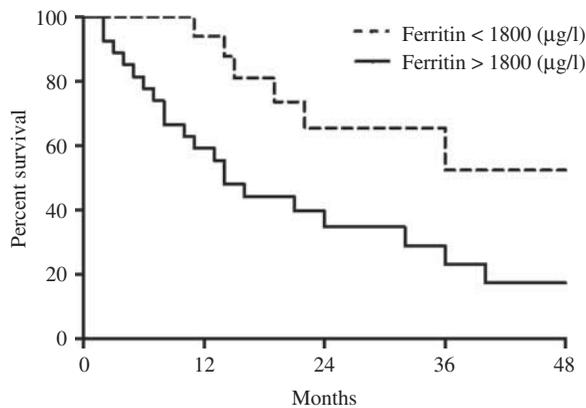
Figure 4 Kaplan–Meier survival curves of end-point (thyroid dysfunction incidence during a follow-up period) in patients with serum ferritin levels above and below the optimal receiver operating characteristics cut-off level.

ferritin levels was also assessed in the group treated with DFO alone, but nine new cases of thyreopathy were observed.

Preventive therapy: three arms compared and ferritin levels

After 6 years of follow-up, 37/72 patients (51%) treated with DFO alone had no signs of thyreopathy. This condition occurred in 32.4% (12/37) of patients after randomisation and during a follow-up of 2 years. In particular, 25% (3/12) of patients were in the DFO group, 42% (5/12) were in the DFP group and 33% (4/12) were in the DFX group; the difference was not statistically significant ($P = 0.56$). At baseline, there were no significant differences of risk factors for thyroid dysfunction in the three groups. Furthermore, we did not find statistical differences between serum ferritin changes in all groups after therapy with the three different iron chelators (Table 4).

Discussion

Findings from this study clearly indicate that ferritin represents a risk factor for βT patients and a predictive marker for progression of thyroid dysfunctions. Progressor subjects presented higher ferritin levels at baseline, compared with non progressor, and were characterised by a faster evolution to thyreopathy. Furthermore, if predictive values of baseline haemoglobin levels confirm the general suggestion that a worse anaemic status associated with a condition of splenectomy and TSH values is an important factor for the subsequent progression to endocrine and thyroid alterations, remarkably ferritin levels showed a most impressive predictive power in such a contest even after adjustment for the confounding factors.

In fact, splenectomised patients are characterised by a decreased extrahepatic iron-buffering capacity, with an accelerated iron deposition in several tissues, including thyroid gland [19].

This suggests that ferritin would not be a simple surrogate index of low haemoglobin levels, requiring more blood transfusions and then iron overload, but a marker on its own, predicting thyreopathy progression beyond the information provided by haemoglobin levels or TSH values.

Moreover, we have demonstrated that ferritin was strictly related to TSH levels and echographic parameters such as the thyroid volume. Although ultrasonography is one of the techniques most frequently used to evaluate the volume and structure of thyroid gland, autoimmune thyroiditis are not ubiquitous in βT patients and most echogenicity patterns are not specific [20]. However, several studies have revealed that a diffuse spotty echogenicity or a dishomogeneity of the parenchyma is indicative of thyroid dysfunction [21, 22].

Furthermore, although endocrine and metabolic abnormalities are quite common in patients with βT, there are few studies analysing these complications among patients with TI. It was demonstrated that LIC

Table 2 Univariate and multivariate Cox proportional hazards regression model for incidence of thyroid dysfunction among thalassaemic patients.

	Univariate analysis			**Multivariate analysis**		
	HR	95% CI	*P* value	HR	95% CI	*P* value
Age	1.06	1.02–1.10	0.03	1.02	0.98–1.03	0.12
BMI	0.95	0.93–0.98	0.04	0.96	0.95–1.01	0.31
SBP	1.00	0.97–1.04	0.65			
Serum glucose	1.00	0.96–1.03	0.55			
HOMA-IR	1.01	0.95–1.07	0.22			
Haemoglobin	0.86	0.80–0.90	<0.0001	0.90	0.83–0.96	0.0005
Splenectomy	1.15	1.19–1.29	0.0004	1.08	1.06–1.10	0.004
TSH	1.16	1.09–1.20	0.0006	1.15	1.12–1.21	<0.0001
Serum ferritin	1.36	1.22–1.59	<0.0001	1.20	1.10–1.26	<0.0001

SBP, systolic blood pressure; HOMA-IR, homoeostatic model assessment insulin resistance; TSH, thyroid-stimulating hormone.

Table 3 Effects of combined iron chelation therapy on thyroid dysfunction in thalassaemic patients.

	Baseline therapy DFO	Group A DFO+DFP	P
Total thyreopathies, n (%)	26 (36%)	8 (11%)	0.0004
Thyroid nodules, n (%)	21 (80%)	8 (30%)	0.0001
Autoimmune thyroiditis	2 (7%)	2 (7%)	1.0
Overt hypothyroidism (%)	10 (38%)	4 (15%)	0.06
Subclinical hypothyroidism	16 (61%)	3 (11%)	0.0001
Thyroid dishomogeneity	26 (100%)	10 (38%)	<0.0001
Thyroid volume (ml)	12.3±3.9	14.7±3.4	0.04
L-thyroxine therapy, n (%)	12 (46%)	4 (15%)	0.002
Laboratoristic parameters			
TSH	5.3±2.4	3.01±2.4	<0.0001
FT$_4$	14.7±5.1	16.1±3.4	0.003
Serum ferritin	1500 (872–2336)	569 (372–936)	<0.0001
SBP	133.2±10	125.5±8	0.02
DBP	64.3±8.4	60.6±5.4	0.15
HOMA-IR	2.9±1.2	1.8±0.6	0.002

DFO, desferrioxamine; DFP, deferiprone; SBP, systolic blood pressure; DBP, diastolic blood pressure; HOMA-IR, homoeostatic model assessment insulin resistance; TSH, thyroid-stimulating hormone; FT$_4$, free thyroxine.

was associated with an increased rate of morbidity in patients with βT intermedia, with an increased incidence of vascular morbidity and an earlier appearance of endocrine and bone disease (23).

Moreover, the need for iron chelation therapy in these patients who have never been transfused or have received only occasional transfusions has just recently started to emerge after documenting substantially high LIC and non-transferrin-bound iron values in such patients (24, 25).

Our results confirm these observations, demonstrating that patients affected by βT intermedia could develop a thyreopathy, presenting an iron overload, irrespective of their transfusion status, attributed mainly to increased intestinal iron absorption due to ineffective erythropoiesis (26).

However, as for other aspects of the management of βT intermedia, clear guidelines on initiation of chelation therapy are not available.

Our patients were also affected by other endocrine complications, such as an altered glucose homeostasis associated with insulin resistance. This condition is common in multi-transfused thalassaemic patients and could be attributed to early impaired β-cell function (27).

The prevalence of diabetes mellitus in thalassaemia has been shown to correlate with serum ferritin concentration and pancreatic iron deposition. Therefore, the incidence of disturbed glucose homeostasis

depends on adherence to chelation treatment, the adequacy of the dosage and the chemical properties of the chelating agent (8).

It was in fact demonstrated that poor chelation, and subsequently hemosiderosis of the pancreas and other organs, appears to be a major cause for glycaemic abnormalities in thalassaemic patients (28).

The availability of three iron chelators allows physicians to personalise the chelation regimens to the needs of different patients. Therefore, it is important to establish whether these drugs have various effects on different organs. Our study demonstrated the efficacy of combined chelation therapy with DFO and DFP in reducing total body iron load and endocrine complications such as thyreopathy. Our data clearly supported that iron-induced tissue damage is reversible, suggesting that iron reductions may prevent or reverse thyroid dysfunction. However, the risk of progression to overt hypothyroidism would remain if the patients were not on intensive chelation therapy. In fact, the oldest thalassaemic patients with a late onset of chelation therapy and the highest ferritin levels were characterised by a permanent impairment of thyroid function, suggesting that iron-induced toxicity is also time dependent. However, it is imperative to start an early intensive chelation treatment to avoid the onset of irreversible mechanisms.

We have also compared the three iron chelators in monotherapy with no differences both on ferritin reduction and prevention of thyreopathies. The newer challenges of chelation therapy include the prevention and reversal of iron-related morbidities by reducing and maintaining iron and free iron to very low levels.

Our study has some limitations that should be mentioned. It was a single-centre study, and the cohort of patients, especially after randomisation, was relatively small. A larger study population could make the results more reliable. Nevertheless, the primary endpoint was

Table 4 Comparison of three iron chelators effects on reduction in ferritin levels and prevention of thyroid dysfunction.

	DFO (n=13)	DFP (n=12)	DFX (n=12)
Age (years)	30.2±7.3	28.8±8.9	31.4±7.4
Splenectomy, n (%)	7 (53%)	5 (42%)	5 (42%)
Thyroid disease, n (%)	5 (38%)	3 (25%)	4 (33%)
Hb levels before transfusion (g/dl)	9.5±0.9	9.4±0.5	9.8±0.3
T-0 serum ferritin (μg/l)	573 (470–648)	628 (490–708)	592 (499–681)
T-end serum ferritin (μg/l)	97 (64–119)	105 (86–118)	94 (83–123)

DFO, deferoxamine; DFP, deferiprone; DFX, deferasirox; Hb, haemoglobin; T-0, at baseline, before randomisation; T-end, at the end of the follow-up period of 2 years.

reached by one-third of the participants, and the statistical model was powerful enough to establish independent relationships between ferritin and incidence of thyreopathy. Furthermore, although high ferritin level is an indicator of iron overload, however, being a positive acute phase protein, it is increased in the presence of associated acute and chronic disorders particularly inflammatory and hepatic conditions, such as chronic hepatitis. This may affect and limit the validity and effectiveness of ferritin in reflecting iron status in βT.

In conclusion, serum ferritin represents a prognostic marker for βT patients and a predictive factor for progression to thyroid dysfunction. Moreover, intensive chelation therapy using combined DFO and DFP achieves a negative iron balance, allowing the reduction of total body iron load and the prevention and/or reversibility of thyroid complications. These findings reinforce the importance of the regular follow-up of patients with TM and TI for early detection and management of thyroid complications. However, well designed, prospective and randomised comparative clinical trials are clearly necessary in order to establish whether other intensified regimens, such as combined treatment with the two oral chelators DFP and DFX, will offer comparable benefits.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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