Predicting factors for liver iron overload at the first magnetic resonance in children with thalassaemia major

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Background. Transfusion dependency determines iron overload in thalassaemia major, with devastating complications. Significant liver iron overload has been observed from early childhood and we aimed to evaluate factors that could predict liver iron overload at the first magnetic resonance imaging (MRI).

Materials and methods. All transfusion-dependent children who underwent MRI to assess iron overload were retrospectively studied. Age, weight, height, blood requirement, chelation drug and dosage, serum ferritin and liver enzymes were evaluated at three specific steps: start of transfusion regimen, start of chelation therapy, and first MRI.

Results. Among 198 patients, 25 children met inclusion criteria. No differences were detected in all the assessed parameters at start of transfusion regimen and chelation therapy ($p>0.05$) between patients with good iron balance (liver iron concentration [LIC] <7 mg Fe/g dry weight [dw]) and liver iron overload (LIC >7). At the first MRI, patients with iron overload had significantly higher serum ferritin (3,080.3±1,078.5 *vs* 1,672.0±705.3 ng/mL; $p<0.01$) while patients with good iron control maintained a stable ferritin value from the start of chelation therapy but showed significantly lower height Z-score (−1.48±1.02 *vs* -0.36±1.55; $p=0.04$). Serum ferritin >1,770 ng/mL was detected as the best threshold for predicting liver iron overload at the first MRI ($p=0.0003$).

Conclusion. In order to prevent liver iron overload at the first MRI, children should maintain a stable level of serum ferritin below 1,770 from the start of chelation therapy. However, strict monitoring of growth is mandatory.

Keywords: thalassaemia, children, liver iron overload, serum ferritin, magnetic resonance imaging.

Introduction

Iron overload is the leading cause of morbidity and mortality in transfusion-dependent thalassaemia patients. Magnetic resonance imaging (MRI) for the quantitative assessment of iron overload in target organs, such as liver and heart, has produced a dramatic improvement in life expectancy and has lowered the rate of complications in thalassaemia major (TM) patients[1]. Unfortunately, in childhood, the first MRI is generally performed many years after the start of regular transfusions and chelation therapy[2-4] in order to avoid deep sedation or general anaesthesia which increase the clinical risks and the costs of the procedure. Today, the general trend is to defer the first MRI until the patients are able to undergo the scan without anaesthesia, usually after eight years of age, as inferred from MRI studies[2-5]. This time lag means that the start and the management of iron chelation therapy remain quite vague and difficult to define, and this is probably reflected in the high prevalence of moderate to severe liver iron overload observed in approximately 70% of children with TM at the first MRI[2]. The high prevalence of observable liver iron overload at the first MRI has paved the way for a re-assessment of the criteria to start[6-7] and manage chelation therapy during the first years of life. However, robust data are still lacking.

For these reasons, we conducted a retrospective study to evaluate possible predicting factors for iron overload, such as age at start of transfusions or chelation, blood requirement, iron intake, chelation drug and dosage, serum ferritin and liver enzymes in paediatric patients who have undergone their first MRI. The aim of the study was to reduce iron overload and produce reliable scientific data for starting and managing chelation therapy in very young transfusion-dependent children.

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Materials and methods

This was an observational retrospective chart review based study to evaluate factors impacting on iron intake and iron overload before the first MRI scan in children affected by transfusion-dependent thalassaemia followed at the Rare Blood Cell Disease Unit (RBCDU) of "Cardarelli" Hospital in Naples.

The study was conducted in accordance with the Declaration of Helsinki and ICH guidelines for good clinical practice. Inclusion criteria were:

- transfusion-dependent thalassaemia;
- available medical history, including all relevant clinical and laboratory data at the start of transfusion regimen, start of chelation therapy and first MRI assessment;
- at least one MRI scan at age ≤18 years.

All quantitative relevant parameters were collected and recorded at three specific phases of the patients' clinical course: start of transfusion regimen, start of chelation therapy, first MRI scan (Figure 1).

Parameters relating to the start of chronic transfusion regimen were: age, weight, height, body mass index (BMI), serum ferritin (ng/mL), aspartate aminotransferase (AST), alanine aminotransferase (ALT). Parameters relating to the start of chelation regimen were: age, weight, height, BMI, total amount of transfused red blood cells (RBCs) (mL), mean haematocrit (Ht) of blood units, iron intake, serum ferritin (ng/mL), AST, ALT, chelation drug, mean dosage. Parameters relating to the first MRI were: age, weight, height, BMI, total amount of transfused RBCs (mL), mean Ht of blood units, iron intake, serum ferritin (ng/mL), AST, ALT, liver T2* (msec), liver iron concentration (LIC) (mg Fe/g dry weight [dw]), heart T2* (msec), chelation drug, mean dosage. Age, weight, height, BMI were considered at the date of start of transfusion, chelation therapy and first MRI ± 8 weeks.

Serum ferritin (ng/mL), AST, ALT were considered as mean value of three different values over a period of 12 weeks, if available. Otherwise, two or single values ± 8 weeks were considered.

Total amount of transfused RBCs, mean Ht of blood units, iron intake, serum ferritin (ng/mL), AST, ALT, chelation drug, and mean dosage were considered between the different consecutive phases.

Iron intake during the study was calculated as: (total amount of RBCs transfused [mL] × 1.16 × Ht of the transfused blood units)[8].

All patients included in the study were scanned with MRI T2* of the liver, and for those patients who gave specific consent, of the heart, using standardised and validated procedures. Liver T2* value was calculated in a large region of interest of standard dimension chosen in a homogeneous area of parenchyma[9].

Magnetic resonance imaging was performed using a 1.5T scanner (GE Excite HD). An 8-element cardiac phased array receiver surface coil and ECG gating was used. For the measurement of myocardial iron overload (MIO), a multislice multiecho T2* approach was adopted[10]. A T2* measurement ≥20 ms was taken as conservative normal value[11]. LIC was calculated from liver T2* images (TE 0.99-16.50 ms) according to the formula: (1/ [T2*/1,000])×0.0254+0.202)[12].

To further analyse data, patients were divided into two groups: LIC <7 mg Fe/g dry weight (dw) (Group 1: good iron control) and LIC >7 mg Fe/g dw (Group 2: iron overload) on the basis of the classification of clinically relevant liver iron overload.

Data analysis

Statistical analyses were performed using MedCalc® (v.10.2.0.0, MedCalc software, bvba, Ostend, Belgium). Results for descriptive statistics were expressed as mean ± standard deviation. Qualitative variables were summarised as counts and percentages; the Kolmogorov-Smirnov test was used to assess the normality of the distributions. Fisher's exact test was used to compare the incidence of different parameters between the two groups of patients. Student's *t*-test was used to compare differences in parametric data. $p<0.05$ was considered statistically significant. The MedCalc statistical

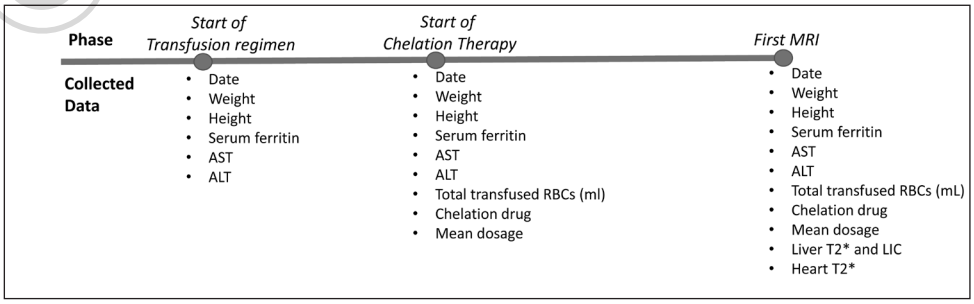


Figure 1 - Study design.
The methodology adopted and data collected at three specific phases (± 8 weeks).
MRI: magnetic resonance imaging; RBCs: red blood cells; AST: aspartate aminotransferase; ALT: alanine aminotransferase.

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software package was used to draw continuous variable receiver operating characteristic (ROC) analysis.

Results

Among 198 transfusion-dependent patients followed at our Institute, 25 patients (15 males and 10 females) were included in the analysis (Table I). The main reasons for patient exclusion were: lack of relevant clinical and laboratory data (especially the total amount of transfused RBCs), and lack of MRI scan during infancy or childhood.

Data on the overall population (All patients) are reported in Table I. Mean Ht of RBC units was 65%, as reported by the Transfusion Medicine Department. Patients were divided into two groups: LIC <7 mg Fe/g dw (Group 1: good iron control) and LIC >7 mg Fe/g dw (Group 2: iron overload) on the basis of the classification of clinically relevant liver iron overload.

Table I - Total collected data in the overall population (All patients) and in the two groups*.

	All patients (n=25)	**Group 1 LIC <7 (n=14)**	**Group 2 LIC >7 (n=11)**	**p 1 *vs* 2**
Sex	15 M (60%) / 10 F (40%)	8 M (57%) / 6 F (43%)	7 M (64%) / 4 F (36%)	p=1.00
Data at start of transfusion regimen				
Age (months)	17 (2-39)	17 (4-39)	17 (2-39)	p=0.59
Weight Z-score	−0.47±1.30	−0.6±1.4	−0.3±1.2	p=0.65
Height Z-score	0.31±1.81	−0.17±1.6	0.93±1.9	p=0.13
BMI	16.0±2.4	16.3±2.0	15.6±2.8	p=0.45
Serum ferritin (ng/mL)	339.9±283.0	434.8±357.3	245.0±146.1	p=0.14
ALT	25.3±15.7	25.6±15.7	25.0±16.5	p=0.93
AST	36.0±13.7	35.5±17.2	36.6±8.1	p=0.85
Data at start of chelation therapy (from start of transfusion regimen)				
Age (months)	36 (23-59)	36 (23-59)	34 (23-59)	p=0.60
Weight Z-score	0.57±1.40	0.2±1.5	1.1±1.1	p=0.12
Height Z-score	0.02±1.15	−0.34±1.2	0.48 ±0.9	p=0.08
BMI	17.2±2.0	16.9±1.8	17.6±2.1	p=0.35
Serum ferritin (ng/mL)	1,820.2±664.1	1,710±759.1	1,960.6±520.1	p=0.36
ALT	63.2±51.3	77±54.5	46.2±43.4	p=0.14
AST	44.5±19.4	50±21.2	37.4±14.8	p=0.10
Total RBCs (mL)	3,831.9±1,807.2	3,747.2±2019.3	3,939.8±1,585.7	p=0.85
Blood consumption (mL/kg/year)	184.2±50.0	183.4±54.8	185.2±45.7	p=0.93
Total iron intake (mg)	3,430.7±1,555	3,320.6±1,670.2	3,570.9±1,464.0	p=0.72
Data at first MRI (from start of chelation therapy)				
Age (years)	8 (4-17)	8 (7-17)	8 (4-17)	p=0.97
Weight Z-score	−0.21±1.08	−0.48±1.11	0.1±1.0	p=0.17
Height Z-score	**−0.99±1.37**	**−1.48±1.02**	**−0.36±1.55**	**p=0.04**
BMI	18.8±4.0	18.7±4.2	18.9±3.9	p=0.87
Serum ferritin (ng/mL)	**2,291.6±1,123.9**	**1,672.0±705.3**	**3,080.3±1,078.5**	**p<0.01**
ALT	42.5±53.4	31.1±14.9	58.4±80.6	p=0.22
AST	32.7±27.0	26.2±9.1	41.0±38.9	p=0.18
Total RBCs (mL)	39,164.9±31,284.6	37,535.5±29,632.8	41,238.6±34,626.1	p=0.78
Blood consumption (mL/kg/year)	188.2±44.4	193.8±52.3	181.0±32.5	p=0.48
Total iron intake (mg)	35,562.9±27,824.9	33,851.1±25,485.1	37,741.6±31,693.5	p=0.71
T2* liver	**4.9±3.9**	**7.1±4.0**	**2.2±0.9**	**p<0.01**
LIC	**8.8±7.3**	**4.6±1.6**	**14.1±8.3**	**p<0.01**
T2* heart	31.6±9.7	30.9±10.7	32.5±8.9	p=0.73

*p-values are referred to differences between the two groups at different phases. Chelation drugs and mean dosage, and Ht of RBCs were not significant different (p>0.05, data in the text). In bold statistically significant data.
LIC: liver iron concentration; BMI: body mass index; ALT: alanine aminotransferase; AST: aspartate aminotransferase; RBC: red blood cell; MRI: magnetic resonance imaging.

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Fourteen patients were included in the first group (good iron control) and 11 patients in the second group (iron overload). No differences between the two groups was detected in weight, height, BMI, serum ferritin, liver enzyme value, age at start of transfusion regimen and chelation regimen and first MRI, total amount of transfused RBCs, iron intake, chelation drug and dosage at the start of transfusion regimen and between the start of chelation therapy and the first MRI ($p>0.05$ for all quantitative parameters) (Table I). At the start of chelation therapy all the patients were on deferoxamine at mean dosage of 28.07±6.03 mg/kg/day 7 days per week *vs* 25.5±5.00 mg/kg/day, 7 days per week in group 1 and group 2, respectively ($p>0.05$). At the first MRI, chelation therapy was deferoxamine at the mean dosage of 33.33±5.01 mg/kg/day *vs* 34.17±8.01 mg/kg/day 7 days per week in group 1 and 2, respectively ($p>0.05$). At the first MRI scan, there was no difference in the following parameters between the two patient groups: age, weight, height, BMI, total amount of transfused RBCs, iron intake, liver enzymes and cardiac T2* ($p>0.05$), but patients with LIC >7 mg Fe/g dw had significantly higher serum ferritin (3,080.3±1,078.5 ng/mL *vs*. 1,672.0±705.3 ng/mL; $p<0.01$) (Figure 2). Patients with LIC <7 mg Fe/g dw, instead, showed significantly lower height Z- score (−1.48±1.02 *vs* −0.36±1.55; $p=0.04$) (Figure 3). The area under the ROC curve (AUCROC) was used to determine whether any level of serum ferritin could be used to differentiate between patients with and without liver iron overload. The error in the estimate of cut-off point to create the ROC curve was not negligible due to the small sample size. The highest value of the AUCROC in predicting a LIC >7 mg Fe/g dw was found for serum ferritin concentrations with a threshold value of 1,773 ng/mL (AUCROC=0.83 [95% CI: 0.62-0.95; $p=0.0003$]). The sensitivity and specificity for this variable were 100% and 66.67%, respectively (Figure 4).

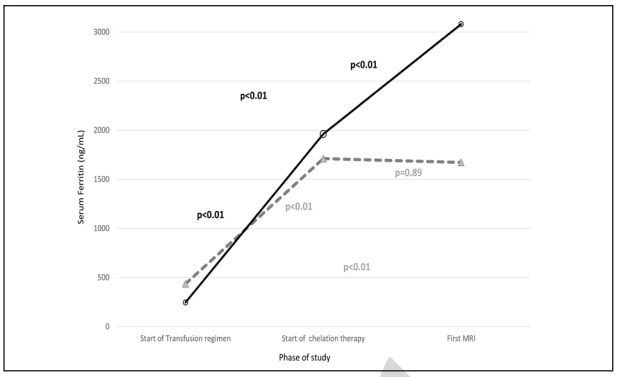


Figure 2 - Serum ferritin trend in the two groups.
Liver iron concentration (LIC) <7 mg Fe/g dw: grey dotted line; LIC > 7 mg Fe/g dw: black continuous line. p-values are referred to differences in the same group at three different phases.

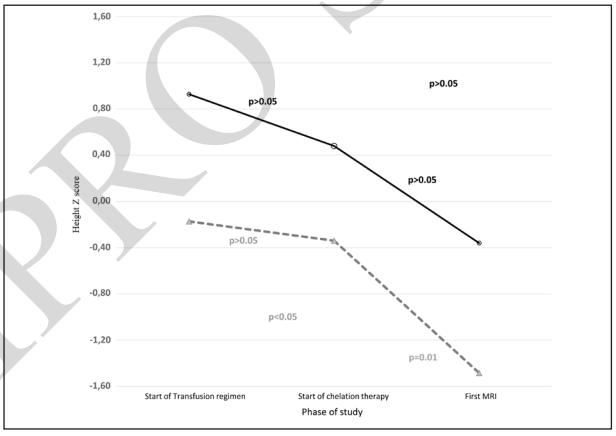


Figure 3 - Height Z-score trend in the two groups.
Liver iron concentration (LIC) <7 mg Fe/g dw: grey dotted line; LIC >7 mg Fe/g dw: black continuous line. p-values are referred to differences in the same group at three different phases.

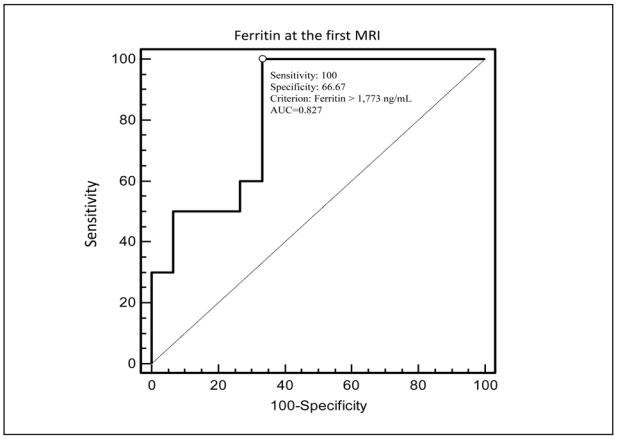


Figure 4 - Receiver-operating characteristic (ROC) curves for serum ferritin levels at first magnetic resonance imaging (MRI).
AUC: area under the curve.

Discussion

The dangers of delayed or bad management of iron overload in paediatric thalassaemia are well known. In fact, delayed initiation of chelation therapy has been reported to be correlated with a significantly higher risk of delayed puberty[13] or multiple endocrinopathies later in life[14]. Furthermore, children with short stature and delayed puberty maintain higher levels of serum ferritin during infancy compared to children without complications[15]. However, to date, no studies have evaluated the appropriateness of criteria for initiating iron chelation treatment (after 10-20 transfusions or when serum ferritin is above 1,000 ng/mL)[8], nor have these ever been correlated with MRI parameters. LIC is a reliable indicator of total body iron and is correlated to development of hepatic and extrahepatic complications[15].

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In paediatric TM patients, pathological iron levels occur at first and mainly in the liver and there is a pressing need for preventative measures, as moderate to severe liver iron overload has been reported in 60% of paediatric patients and cardiac overload in 21%[2]. This proportion may even increase in the near future, as recent studies have demonstrated that children with TM born in the last decade (2006-2016) start regular transfusions earlier and with higher pre-transfusional haemoglobin (Hb) values[7], thus suggesting a higher iron intake during early childhood.

In this study we evaluated all the quantifiable parameters in the transfusion and chelation history of children who underwent MRI, in order to determine risk factors for liver or cardiac iron overload at the first MRI. In fact, paediatric TM patients undergo their first MRI many years after starting transfusion and iron chelation regimes, and clinicians do not have reliable data on how to manage chelation therapy to avoid iron overload at the first MRI. Our data show that there is no difference among children with and without iron overload in age at start of transfusions, total amount of blood transfused, age at start of chelation, type of drug and dosage, serum ferritin level at start of chelation. The only significant parameter was serum ferritin at the first MRI, which was doubled in patients with iron overload, while in patients without iron overload it was stable compared to start of chelation. These results confirm the observation that patients who maintained higher levels of serum ferritin or had poor compliance to chelation therapy during adolescence, developed more and worst clinical complications[13,14,16]. Furthermore, even recently, a high rate of endocrine complications[17] and multiple endocrinopathies[14] have been observed in a cohort of TM adolescents who had initiated chelation at an average five years of age and had mean serum ferritin values above 6,000 ng/mL[17]. So, higher level of serum ferritin had previously been associated to the development of clinical complications later in life. Our study, for the first time, establishes a cut-off level of serum ferritin that could discriminate the presence of liver iron overload at the first MRI in children. In order to prevent liver iron overload, children have to maintain a stable serum ferritin level from the start of chelation therapy of around 1,700 ng/mL. Our results may provide significant help in the management of iron overload in paediatric TM patients living in low income countries with limited access to MRI, and also provide a quantifiable therapeutic target to be reached while waiting until the child is able to perform MRI. However, in adult patients, the value of serum ferritin predictive for liver iron overload was 882 ng/mL[18], which is lower than the cut-off observed in our cohort. There are probably two reasons for this: firstly serum ferritin value can increase by multiple infections and inflammatory conditions, very frequent in children. Secondly, in the adult cohort the threshold chosen to identify liver iron overload was more conservative (hepatic R2* >158 Hz, LIC >4 mg Fe/g dw). The higher target of LIC chosen in our study reflects the high prevalence of liver iron overload in a paediatric population since, in a large cohort of TM children assessed with MRI, moderate to severe liver iron overload (LIC >7 mg Fe/g dw) was detected in 50% of patients and around 80% of children presented a LIC value >3 mg Fe/g dw[2]. In a multicentre study considering a LIC, threshold of 7 mg Fe/g dw, as in our analysis, serum ferritin was predictive at 2,000 ng/mL[19], similar to our results. Probably, by optimising chelation therapy and using a more conservative threshold for LIC the cut-off of serum ferritin may be lower also in children. Unfortunately, at our centre, pancreas MRI was not routinely performed in paediatric TM patients, so no data on pancreas iron overload were collected.

Since all parameters were comparable among groups, we can hypothesise that the only explanation for the difference in serum ferritin and LIC at the first MRI is compliance to chelation treatment. Unfortunately, to date there is no effective and reliable way to measure compliance, particularly in a retrospective study. But we believe that this observation is quite interesting from a clinical point of view, especially taking into account recent studies that suggest modification of criteria for beginning and managing chelation therapy in childhood[6,7].

In our patient population, criteria for starting and managing chelation therapy were followed for all patients, since the age at start of transfusions and chelation, serum ferritin, total iron intake, growth parameters, type of drug and dosage were all similar. Considering the criteria currently applied for management of transfusions and chelation in paediatric TM patients, the only factor predicting iron overload is the increase in serum ferritin at the first MRI. In our cohort, this factor is probably associated to compliance to treatment, as there are no significant differences between other quantifiable parameters. One element that confirms our hypothesis is the significant reduction in height Z-score in patients with good control of iron overload at the first MRI, which could be secondary to known toxic effects of deferoxamine on growth[20].

Many children currently start iron chelation with oral drugs and we believe it is important to acquire further data on growth during the first years of life, taking into account possible toxic effects of treatment.

It has been proven that deferasirox does not influence growth in children treated during the first six years of life[21] and there are data on early use of deferiprone in childhood[22]. Unfortunately, we could not analyse children on oral chelating drugs because of the relatively

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recent use of those medications in very young patients, and the fact that, during the first decade of life, these patients are generally unable to undergo MRI.

It is, therefore, essential to balance the iron chelation effect of drugs and their toxicity during infancy. Recent guidelines for management of TM indicate: "If chelation therapy begins before 3 years of age, particularly careful monitoring of growth and bone development is advised, along with reduced dosage"[8].

The major limitations of our study concern the retrospective nature of the observations and the limited number of patients. The most important difficulty encountered was establishing the exact amount of blood transfused prior to MRI. This underlines the importance of improving data storage and transfusional management history in order to conduct a more precise analysis on a larger group of patients.

Conclusions

Criteria currently applied to start chelation therapy in children seem reliable in our cohort. The only factor correlated to liver iron overload at the first MRI is the increase of serum ferritin from the start of chelation regimen. In order to prevent liver iron overload in childhood, it is important to maintain serum ferritin levels stable below 1,700 ng/mL from the beginning of chelation therapy. However, evaluation of growth is important to reduce the toxic effects of chelation drugs, thus striking a balance between chelation and toxicity.

Authorship contributions

MC and AF are responsible for study concept design. Data collection and assembly were carried out by MC, MM, MA, AS, SC, PC, PR, and AF. Data analysis and interpretation were performed by MC, AF, and MA. The manuscript was drafted by MC, AF, and MM. All Authors are responsible for critical revision of the manuscript for intellectual content. All Authors approved the manuscript before submission.

The Authors declare no conflicts of interest.

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