

Ferritin trends do not predict changes in total body iron in patients with transfusional iron overload

Mammen Puliyl,¹ Richard Sposto,^{1,2} Vasilios A. Berdoukas,^{1,3} Thomas C. Hofstra,^{1,3} Anne Nord,¹ Susan Carson,¹ John Wood,^{3,4} and Thomas D. Coates^{1,3,5*}



Ferritin levels and trends are widely used to manage iron overload and assess the efficacy of prescribed iron chelation in patients with transfusional iron loading. A retrospective cohort study was conducted in 134 patients with transfusion-dependent anemia, over a period of up to 9 years. To determine whether the trends in ferritin adequately reflect the changes in total body iron, changes in ferritin between consecutive liver iron measurements by magnetic resonance imaging (MRI) were compared to changes in liver iron concentrations (LIC), a measure of total body iron. The time period between two consecutive LIC measurements was defined as a segment. Trends in ferritin were considered to predict the change in LIC within a segment if the change in one parameter was less than twofold that of the other, and was in the same direction. Using the exclusion criteria detailed in methods, the trends in ferritin were compared to changes in LIC in 358 segments. An agreement between ferritin trends and LIC changes was found in only 38% of the 358 segments examined. Furthermore, the change in ferritin was in opposite direction to that of LIC in 26% of the segments. Trends in ferritin were a worse predictor of changes in LIC in sickle cell disease than in thalassemia ($P < 0.01$). While ferritin is a convenient measure of iron status; ferritin trends were unable to predict changes in LIC in individual patients. Ferritin trends need to be interpreted with caution and confirmed by direct measurement of LIC.

Am. J. Hematol. 89:391–394, 2014. © 2013 Wiley Periodicals, Inc.

■ Introduction

Chronic red cell transfusions are the mainstay of management for many hematological disorders, with secondary iron overload as an important consequence. Prior to effective chelation, iron cardiomyopathy led to premature death, usually during the second decade in thalassemia patients [1]. Modern chelation approaches and iron assessment by magnetic resonance imaging (MRI) have reduced death from iron cardiomyopathy by 70% [2], and increased the projected median survival for thalassemia patients into the seventh decade [3]. Reversal of cardiac and endocrine failure is now possible, provided that total body iron can be normalized [4].

LIC is linearly related to total body iron [5], and is the accepted standard test for assessment of transfusional iron loading. Currently, liver iron concentration (LIC) is estimated non-invasively using MRI methodologies [6,7]. Nevertheless, the serum ferritin assay is relatively well standardized, inexpensive, and widely used for assessment of iron loading and for monitoring chelation therapy [8]. The reliance on ferritin by providers has been reinforced by ferritin-based treatment criteria on packaging information for iron chelators. Serum ferritin levels are often used to monitor patients with severe iron overload in parts of the world where access to non-invasive LIC measurement is not possible.

In the absence of confounding factors such as inflammation, hepatic injury, and vitamin C deficiency, serum ferritin is a reasonable indicator of iron stores, and isolated serum ferritin correlates well with LIC in cross-sectional studies of patient populations with sickle cell disease or thalassemia [9]. However, variability in single ferritin measurements does not allow accurate prediction of iron loading in individual subjects [10,11]. Despite the limitations of isolated serum ferritin measurements, some believe that once the serum ferritin/LIC ratio is known in individual patients, changes in serum ferritin could accurately predict changes in total body iron [9]. This led many providers to assume that changes in ferritin reflect changes in total body iron in individual patients [12]. However, this is not always the case, as seen in Fig. 1, which shows the changes in ferritin and LIC in a representative patient with beta thalassemia major, followed over an 8-year period. Changes in ferritin predicted LIC during the first 4-year period (Segment A), but during the second 4 years (Segment B), ferritin levels suggested rising iron when, in fact, the LIC was unchanged. These data, and the fact that the ability of changes in ferritin to predict changes in LIC in individual patients has not been adequately studied, led us to initiate this study to document this relationship in our population of chronically transfused patients.

¹Division of Hematology, Children's Center for Cancer and Blood Diseases, Children's Hospital Los Angeles, Los Angeles, California; ²Department of Preventive Medicine, Keck School of Medicine, University of Southern California, Los Angeles, California; ³Department of Pediatrics, Keck School of Medicine, University of Southern California, Los Angeles, California; ⁴Division of Cardiology, Children's Hospital Los Angeles, Los Angeles, California; ⁵Department of Pathology, Keck School of Medicine, University of Southern California, Los Angeles, California

Conflict of interest: VAB—ApoPharma: Consultancy; SC—ApoPharma: Consultancy; Novartis Inc: Speakers bureau. JW—Novartis: Research funding, speakers bureau; Shire pharmaceuticals: Research funding, Consultancy and speakers bureau; ApoPharma: Consultancy and member of the advisory board; Cooley anemia foundation—member of the advisory board. TDC—Novartis: Speakers bureau; ApoPharma: Consultancy; Shire pharmaceuticals: Consultancy.

***Correspondence to:** Thomas Coates, MD, Division Head for Hematology, Director, Sickle/ Hemoglobinopathy Program, Children's Center for Cancer and Blood Diseases, Children's Hospital Los Angeles, MS # 54, Los Angeles, CA 90027. E-mail: tcoates@chla.usc.edu

Received for publication: 12 December 2013; **Accepted:** 13 December 2013

Am. J. Hematol. 89:391–394, 2014.

Published online: 17 December 2013 in Wiley Online Library (wileyonlinelibrary.com).

DOI: 10.1002/ajh.23650

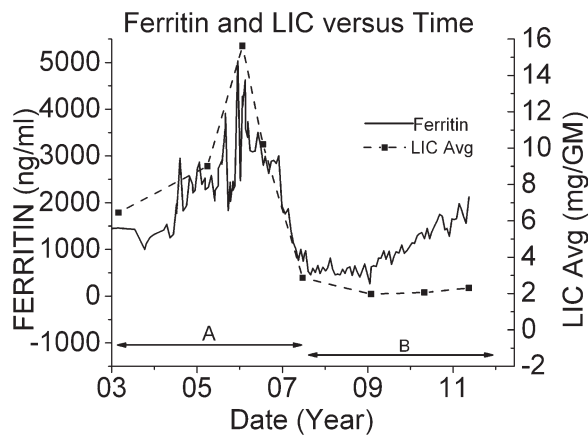


Figure 1. Ferritin levels (solid) and LIC (dashed) are plotted against time for a single patient with transfusion dependent thalassemia major. The changes correlate during time Segment A but not during time Segment B.

Methods

After obtaining institutional ethics board approval, we reviewed the records of all patients followed in the chronic transfusion clinic at Children's Hospital Los Angeles between June 2002 and June 2011. Chelation therapy, including desferrioxamine, deferasirox, and deferiprone or some combination of those, was individualized to promote patient adherence and optimal correction of iron overload. Determination of chelation efficacy, and dose modification was based on changes in LIC. Patients were transfused every 2–4 weeks to maintain pre-transfusion hemoglobin greater than 9.5 g/dL. Ferritin levels were evaluated approximately every 3 weeks after each transfusion by immunometric assay (Vitros ECI: Ortho Clinical Diagnostics). LIC was measured by MRI at approximately annual intervals using the method we described [7]. The LIC values were the average LIC derived from R2 and R2*, expressed in milligram/gram dry weight.

In order to determine whether the trends in ferritin predicted the change in LIC, we calculated the percent change in ferritin between consecutive LIC measurements. Only LIC measurement intervals between six months and two years were included in order to allow enough time for the liver iron to change, but yet not so long that meaningful patterns in iron change might be overlooked. Each measurement interval, referred herein as a segment, is the unit of comparison. Because of the variability in individual ferritin measurements we used linear regression to fit all of the ferritin values in a segment, and then used this fit to determine the ferritin values at the start (Fer_1) and end (Fer_2) of the segment (see Fig. 2). Regression analysis was performed on the log ferritin scale to stabilize variance. Linear regression on this scale uniformly provides a good fit to the observed ferritin values. In order to obtain an accurate estimation of the slope, segments that did not include at least five ferritin measurements or that did not show even distribution of the data were excluded from the analysis. Ferritin measurements were considered to be unevenly distributed if the standard deviation (SD) for the time of ferritin measurements within the segment was less than 0.5 times that of the maximum theoretical SD for that segment [13]. The derived start and end ferritin values were used to calculate the percent changes in ferritin $((Fer_2 - Fer_1)/Fer_1 \times 100)$. To further determine whether trends in ferritin predicted changes in LIC, we compared the percent change in derived ferritin to the percent change in LIC $((LIC_2 - LIC_1)/LIC_1 \times 100)$. We considered that the trends in ferritin predicted the change in LIC within a segment if the change in one parameter was less than twofold that of the other, and was in the same direction. All other segments were considered discordant. Discordant segments included those where the ferritin change was in the opposite direction to the change in LIC and those where the change in one parameter was more than twofold that of the other.

The difference in the relationship of change in ferritin to change in LIC in thalassemia and sickle cell disease was determined by regression analysis of log-transformed data. The effect of diagnosis on the ability of trends in ferritin to predict changes in LIC was tested using the Pearson's chi squared test. The effect of age, gender, and initial LIC (LIC_1 in Fig. 2) was determined by univariate and multivariate regression analyses.

Results

Population relationship between LIC and Ferritin values

A total of 646 MRI-LIC measurements in 134 patients were screened. One hundred seventeen patients had ferritin measurements within 1

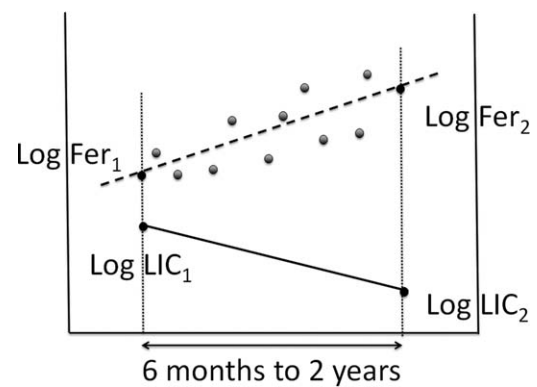


Figure 2. Schematic showing the method of estimating the ferritin values on the day of the LIC measurements by using regression analysis, assumed linearity on the log scales. We used these derived values to calculate the percent change in the ferritin $((Fer_2 - Fer_1)/Fer_1 \times 100)$ and compared this to the percent change in the LIC $((LIC_2 - LIC_1)/LIC_1 \times 100)$

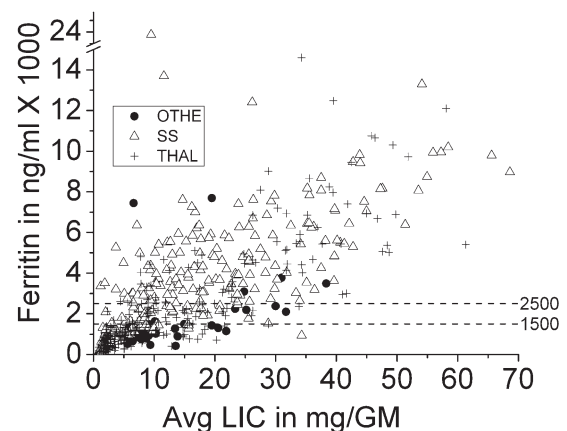


Figure 3. Relationship between repeated measurements of LIC and serum ferritin concentration of 117 patients (61 with sickle cell disease, 46 with thalassemia major, and 10 other subjects). There were 527 observations of LIC and corresponding raw ferritins values.

month of the LIC measurement. The overall Spearman correlation between LIC and measured ferritin values was 0.8 ($P < 0.001$), essentially identical to the relationship between LIC and corresponding ferritin previously determined by other methods [10,11]. An overall correlation of 0.8 describes the population data but can be very misleading. This corresponds to an r^2 of 0.6, suggesting that 60% of the variance is accounted for by the regression. Figure 3 shows these data with arbitrarily placed horizontal lines at ferritin = 1,000 and 2,500 ng/mL to emphasize this variability and the broad range of LIC values associated with these single ferritin levels. There is considerable scatter making it impossible to use these values to predict total body iron from an individual ferritin measurement. The correlation coefficient for thalassemia ($r = 0.8$; $P < 0.001$) was greater ($P < 0.001$) than that for sickle cell disease ($r = 0.7$; $P < 0.001$).

Patient and segment characteristics

To determine whether the change in ferritin predicts the change in LIC, we analyzed the calculated change in ferritin and LIC in each individual time segment between LIC measures. After applying the indicated restrictions (see Methods) on data points per segment, segment length, and ferritin point clustering, a total of 358 segments between LIC measurements were available for analysis in 86 patients. Ferritin values on the day of LIC measurement were derived from regression analysis of all of the ferritin measurements within each segment to reduce the day to day fluctuation in ferritin.

TABLE I. The Number of Patients and Segments by Diagnosis

Diagnosis	N ^a (%)	Segments ^b (%)	Follow-up ^c (Range)	Median ^d (Range)	Mean age ^e (Range)	Sex ^f (%)
Thalassemia syndromes	38 (44)	184 (51)	262 (57–414)	5 (1–9)	16 (3–33)	17 (45)
Sickle syndromes	41 (48)	143 (40)	201 (47–444)	3 (1–7)	16 (4–34)	24 (59)
Other anemias ^g	7 (8)	31 (9)	238 (59–382)	6 (1–6)	11 (2–29)	1 (14)
Total	86 (100)	358 (100)	238 (47–444)	4 (1–9)	16 (2–34)	42 (49)

^a Number of patients.
^b Number of segments.
^c Follow-up in weeks.
^d Median number of segments per patient.
^e Mean age per segment in years.
^f Number of female.
^g Diamond blackfan (3), Congenital Dyserythropoetic anemia (3), Sideroblastic anemia (1).

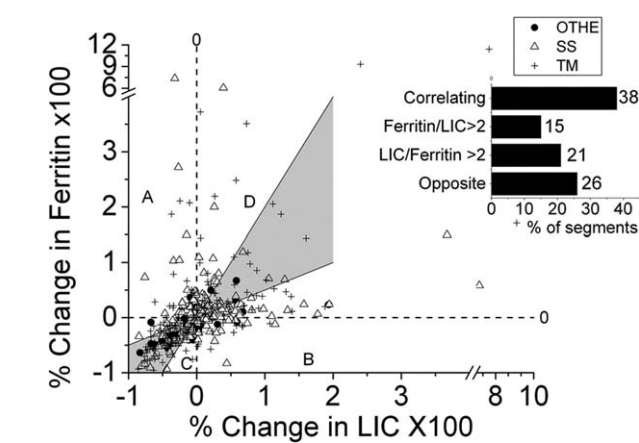


Figure 4. Plot of the percent change in ferritin versus percent change in LIC. The shaded area represents the region where the change in LIC and ferritin is less than a twofold difference. Inset Histogram showing the distribution of segments in each category.

Table I shows the characteristics of the patients analyzed for the segments. The median duration of follow-up was 238 weeks with a minimum of 47 weeks and a maximum of 444 weeks. No patients were seropositive for hepatitis C.

Relationship between LIC and ferritin trends in individual patients

We compared the percent change in derived ferritin to the percent change in LIC to determine whether this parameter could also be a better predictor of LIC (see Methods). When the percent change in derived ferritin was plotted against the percent change in LIC, 38% of the segments were considered to be concordant (shaded region of Fig. 4). Importantly, the direction of change in ferritin was opposite to that of LIC in 26% of the segments (Fig. 4: regions A and B). In 36% of the segments, the change in ferritin differed by more than twofold, but was in the same direction as the change in LIC (Fig. 4: regions C and D), suggesting that change in ferritin cannot predict change in LIC.

Based on the analysis of log-transformed derived ferritin fold change (Fe_2/Fe_1) and LIC fold change (LIC_2/LIC_1), the correlation coefficient for ferritin prediction of LIC change in thalassemia ($r = 0.7$; $P < 0.001$) was greater ($P < 0.001$) than that for sickle cell disease ($r = 0.5$; $P < 0.001$). The slopes of the lines were different as well ($P = 0.01$; Fig. 5), indicating a small but significant difference in the relation between change in ferritin and change in LIC in these two disorders.

The Pearson's chi-squared test was used to determine whether the diagnosis impacted the ability of ferritin trends to predict the change in LIC. In thalassemia, 46% of the segments were concordant. The direction of change in ferritin was opposite to that of the LIC in 21%

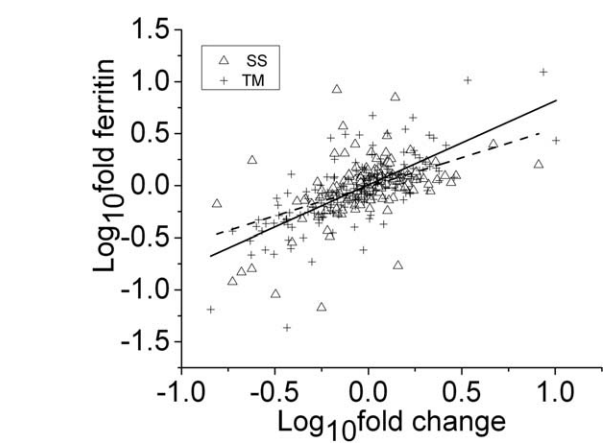


Figure 5. Log fold change ferritin versus Log fold change LIC in sickle cell disease (dashed line; $r = 0.5$; $P < 0.001$) and thalassemia (solid line; $r = 0.7$; $P < 0.001$).

of the segments. Thirty-three percent of the segments were in the same direction, but differed by more than twofold. In sickle cell disease, only 29% of the segments were concordant, and the direction of change in ferritin was opposite to that of the LIC in 32% of the segments. Thirty-nine percent of the segments were in the same direction, but differed by more than twofold ($P < 0.01$). Thus, although it is still not acceptable, the concordance between change in ferritin and change in LIC is better in thalassemia than in sickle cell disease.

There was no significant effect of age ($P = 0.70$) or gender ($P = 0.40$) on the number of segments where changes in ferritin and LIC were concordant. Lower initial LIC was associated with better concordance ($P < 0.05$).

Discussion

Serum ferritin is widely used as a marker of total body iron burden in many disease states. In particular, it is used to monitor chelation therapy in multiple transfused patients with iron overload. While the inadequacy of ferritin as a measure of total iron at any time point is recognized [10,11], trends in ferritin continue to be used to estimate changes in LIC and to adjust chelation management in chronically transfused patients [9,12]. The data presented here directly address whether trends in ferritin predict changes in LIC in a heterogeneous group of patients with transfusion-dependent anemia, consisting primarily of sickle cell disease and thalassemia.

The methodology used allowed us to compare the change in ferritin to the change in LIC during multiple time segments in individual patients. A conservative criterion was employed where less than a

10098652, 2014, 4, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/ajh.23650, Wiley Online Library on [28/04/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

twofold difference between the change in ferritin and change in LIC represents concordance. The data revealed concordance in only 38% of the segments. Notably, 26% of the segments showed changes between ferritin and LIC that were in opposite directions (Fig. 4). The failure of ferritin trends to predict changes in LIC was clearly evident, including when the variability in ferritin was reduced by regression methods (Fig. 2).

We found that concordance between ferritin trends and change in LIC in individual patients was better in thalassemia than in sickle cell disease. Several mechanisms have been offered to explain why ferritin is a worse predictor of LIC in sickle cell disease. Hepatic injury and inflammation, which are more common in sickle cell disease [14,15], affects iron transport and ferritin levels. Additionally, in sickle cell disease, despite ongoing transfusions, there is ongoing hemolysis and increased effective erythropoiesis, unlike in thalassemia major where there is ineffective erythropoiesis. Serum ferritin underestimates LIC in non-transfusion-dependent thalassemia, which is associated with significant ineffective erythropoiesis [16,17]. These differences in erythropoiesis among disease states may affect iron distribution and contribute to the variability in ferritin levels. Though ferritin trends predict LIC change better in thalassemia, these trends are still unable to predict changes in LIC more than half the time.

We do not know why the changes in ferritin closely predict changes in LIC during some time periods and not during others. It is possible that factors affecting single ferritin measurements, such as vitamin C level, inflammation, and hepatic injury, may vary over time, thereby impacting ferritin trends, and impairing the predictive ability of ferritin trends, independently of total body iron. Changes in chelation between time periods may also affect the distribution of iron stores differently, in turn altering how ferritin trends relate to changes in LIC.

We empirically chose a factor of two as the threshold for concordance between changes in ferritin and LIC, but a different value might be used. The exact percentage of segments that are predictive is not as important as the fact that the ferritin trends inaccurately represent the change in LIC in a significant number of segments, and can even be in the opposite direction. These discrepancies may lead to inappropriate management decisions. Perhaps more importantly, as ferritin trends are often used as a surrogate for adherence to therapy, changes in ferritin such as those depicted in segment B of Fig. 1 could lead to inaccurate

assumptions about patient adherence. Lack of adherence is the most common cause of treatment failure in iron overload. Given the lifelong requirement for chelation in these chronic disorders, we fear that inaccurate conclusions regarding patient adherence based on ferritin trends could have a negative effect on patient morale, and in fact, reduce adherence.

High ferritin levels are associated with poor prognosis [18–20], and serum ferritin remains an inexpensive and easily available tool for assessment of iron overload. However, direct measurement of liver iron is important before critical changes are made in the chelation plan. Our data show that the change in ferritin is in the same direction as the change in LIC in 74% of the time. Therefore, ferritin trends may be used to optimize the timing of liver iron measurements. Direct measurement of liver iron should be performed if ferritin trends are not consistent with the clinical scenario. For example, when rising ferritin levels were observed (Segment B) in our patient (Fig. 1), who was known to be adherent, an MRI was immediately scheduled to determine the actual iron status. At our center, we perform MRI for LIC assessment yearly in chronically transfused patients and will strongly consider performing LIC assessments sooner if there are unexpected changes in ferritin over a period of 3 months.

The overwhelming conclusion from these analyses is that neither absolute LIC nor change in LIC can be predicted accurately enough from ferritin measurements to support critical clinical decisions. These data clearly demonstrate that in spite of methods to decrease the variability in ferritin, trends in ferritin can be dramatically different from changes in LIC as assessed by MRI.

■ Author Contributions

TDC was responsible for the conception of the idea and study design and writing the manuscript. MP contributed to the study design, literature search, data analysis, data collection and writing of the manuscript. RS performed the statistical analysis. TDC, RS, VB, TCH, AN, JW were actively involved in data interpretation and writing of the manuscript.

■ Acknowledgment

Authors thank Martine Torres, PhD for editorial assistance.

■ References

- Borgna-Pignatti C, Cappellini MD, De SP, et al. Survival and complications in thalassemia. *Ann NY Acad Sci* 2005;1054:40–47.
- Modell B, Khan M, Darlison M, et al. Improved survival of thalassaemia major in the UK and relation to T2* cardiovascular magnetic resonance. *J Cardiovasc Magn Reson* 2008;10:42.
- Delea TE, Edelsberg J, Sofrygin O, et al. Consequences and costs of noncompliance with iron chelation therapy in patients with transfusion-dependent thalassemia: A literature review. *Transfusion* 2007;47:1919–1929.
- Farmaki K, Tzoumari I, Pappa C, et al. Normalisation of total body iron load with very intensive combined chelation reverses cardiac and endocrine complications of thalassaemia major. *Br J Haematol* 2010;148:466–475.
- Angelucci E, Brittenham GM, McLaren CE, et al. Hepatic iron concentration and total body iron stores in thalassemia major. *N Engl J Med* 2000;343:327–331.
- StPierre TG, Clark PR, Chua-anusorn W, et al. Noninvasive measurement and imaging of liver iron concentrations using proton magnetic resonance. *Blood* 2005;105:855–861.
- Wood JC, Enriquez C, Ghugre N, et al. MRI R2 and R2* mapping accurately estimates hepatic iron concentration in transfusion-dependent thalassemia and sickle cell disease patients. *Blood* 2005;106:1460–1465.
- Brittenham GM. Iron-chelating therapy for transfusional iron overload. *N Engl J Med* 2011;364:146–156.
- Fischer R, Harmatz PR. Non-invasive assessment of tissue iron overload. *Hematol Am Soc Hematol Educ Program* 2009;2009:215–221.
- Fischer R, Tiemann CD, Engelhardt R, et al. Assessment of iron stores in children with transfusion siderosis by biomagnetic liver susceptibility. *Am J Hematol* 1999;60:289–299.
- Brittenham GM, Cohen AR, McLaren CE, et al. Hepatic iron stores and plasma ferritin concentration in patients with sickle cell anemia and thalassemia major. *Am J Hematol* 1993;42:81–85.
- Cappellini MD, Porter J, El-Beshlawy A, et al. Tailoring iron chelation by iron intake and serum ferritin: The prospective EPIC study of deferasirox in 1744 patients with transfusion-dependent anemias. *Haematologica* 2010;95:557–566.
- Steel RGD, Torrie JH. *Principles and Procedures of Statistics with Special Reference to the Biological Sciences*. McGraw Hill; London and New York, 1960.
- Harmatz P, Butensky E, Quirolo K, et al. Severity of iron overload in patients with sickle cell disease receiving chronic red blood cell transfusion therapy. *Blood* 2000;96:76–79.
- Adamkiewicz TV, Abboud MR, Paley C, et al. Serum ferritin level changes in children with sickle cell disease on chronic blood transfusion are non-linear, and are associated with iron load and liver injury. *Blood* 2009;114:4632–4638.
- Pakbaz Z, Fischer R, Fung E, et al. Serum ferritin underestimates liver iron concentration in transfusion independent thalassemia patients as compared to regularly transfused thalassemia and sickle cell patients. *Pediatr Blood Cancer* 2007;49:329–332.
- Taher A, El RF, Isma'eel H, et al. Correlation of liver iron concentration determined by R2 MRI with serum ferritin in patients with thalassemia intermedia. *Haematologica* 2008;93:1584–1586.
- Gabutti V, Piga A. Results of long-term iron-chelating therapy. *Acta Haematol* 1996;95:26–36.
- Olivieri NF, Nathan DG, MacMillan JH, et al. Survival in medically treated patients with homozygous beta-thalassemia. *N Engl J Med* 1994;331:574–578.
- Telfer PT, Prestcott E, Holden S, et al. Hepatic iron concentration combined with long-term monitoring of serum ferritin to predict complications of iron overload in thalassaemia major. *Br J Haematol* 2000;110:971–977.

