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ORIGINAL ARTICLE

Iron overload in non-transfusion-dependent thalassemia: association with genotype and clinical risk factors

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Abstract In the present study, we sought to determine the prevalence of iron overload in patients with non-transfusion-dependent thalassemia (NTDT) and its association with genotype and other clinical risk factors, and to evaluate the correlation between serum ferritin (SF) and liver iron concentration (LIC). Myocardial and liver iron concentration was measured by MRI using a T2* gradient multi-echo sequence in NTDT patients, aged 10–50 years. Of 91 patients, 54 (59 %) had hepatic iron overload. None had cardiac iron overload. The clinical risk factors for hepatic iron overload were age >20 years (adjusted OR 30.2, 95 % CI 4.5–203, $p < 0.001$), hemoglobin level <7 g/dL (adjusted OR 6.3, 95 % CI 1.01–39.5, $p = 0.049$), and cumulative RBC transfusion >10 units (adjusted OR 53.6, 95 % CI 3.2–884, $p = 0.005$). Beta-thalassemia genotype was associated with higher risk of iron overload by univariate analysis, but the association was not significant when adjusted for other clinical factors. The correlation coefficient between SF and LIC was 0.60 ($p < 0.001$). In conclusion, the prevalence of hepatic iron overload is high in NTDT. Older age, lower hemoglobin level, and higher cumulative RBC transfusion are significant risk factors. SF and LIC show a significant positive correlation.

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Introduction

Non-transfusion-dependent thalassemia (NTDT) is a spectrum of thalassemia that does not require regular lifelong transfusion for survival. This group of thalassemia may require occasional transfusions in special circumstances such as infection, pregnancy or growth retardation and may require more regular transfusions later in life due to complications or splenomegaly [1, 2]. The NTDT group largely includes beta-thalassemia intermedia, hemoglobin (Hb) E/beta-thalassemia, and Hb H and Hb H/Constant Spring (Hb H/CS) disease.

Although perceived as a milder form of thalassemia, NTDT has a different pathological mechanism which relates mainly to chronic hemolysis and hypoxia, and is associated with several complications that differ from those seen in transfusion-dependent thalassemia (TDT). These complications are extramedullary hematopoiesis, pulmonary hypertension and thrombosis [3].

Iron overload is also one of the major complications in NTDT [4, 5]. The condition is mainly a result of increased gastrointestinal absorption from ineffective erythropoiesis which leads to iron deposit in the liver [6, 7]. Origa et al. showed that in thalassemia intermedia, iron accumulation was seen predominantly in hepatocytes and the serum ferritin level was low when comparing to thalassemia major. This was associated with the low level of the iron-regulating hormone hepcidin in thalassemia intermedia [8]. Iron overload is associated with other complications therefore early detection and prompt treatment is needed [3].

In Thailand, the prevalence of all types of thalassemia is high. Especially unique in northern Thailand, the prevalence of Hb H and Hb H/CS disease is as high as 1:65 of general population [9]. Currently available data of complications and iron overload are mostly derived from beta-thalassemia. Recently, beta-thalassemia genotype was reported to be an independent risk factor for pulmonary hypertension in NTDT patients [10]. The proposed explanation was the more severe phenotype of beta-thalassemia. The knowledge of the effect of alpha-thalassemia and beta-thalassemia genotype on iron overload in NTDT is limited.

Thalassemia international federation (TIF) recommends that assessment of iron overload in NTDT patients be done by measurement of liver iron concentration (LIC) by magnetic resonance imaging (MRI) at 1–2 years interval [2]. Assessment of LIC is the gold standard for the quantification of total body iron [11]. MRI measurement of LIC is preferred over a liver biopsy as it is non-invasive and can reduce sampling error in case of uneven iron accumulation. However, the method is costly and not widely available. Serial measurement of serum ferritin may be used for the assessment of iron overload in case that MRI is not available. Previous studies have shown that the serum ferritin has a significant positive correlation with LIC as in TDT, but with a lower ratio of serum ferritin to LIC [12–14]. However, a study shows no significant correlation between LIC and serum ferritin in thalassemia intermedia [8].

This study aimed to determine the prevalence of iron overload and its correlation with genotype and other clinical risk factors in patients with NTDT, and to study the correlation of serum ferritin and LIC as measured by MRI. The identified risk factors and serum ferritin level can be used to select the high-risk patients for MRI study where the resources are limited.

Patients and methods

This is a prospective cohort of NTDT patients, age 10–50 years, who attended the Adult and Pediatric Hematology clinics at Chiang Mai University Hospital from September 2013 to May 2014. NTDT was defined as thalassemia disease that received not more than 3 red blood cell transfusion per year in the past 5 years. Patients who had contraindications for MRI and patients with fever or active infections that may interfere with the blood test results were excluded. The study protocol was approved by the institutional research ethics committee. All patients and their parents in case of minorities gave written informed consent.

The medical records were reviewed for thalassemia diagnosis and genotypes, transfusion history, history of splenectomy, comorbidities, and the maximum ferritin level

in the last 5 years. All patients were evaluated for complete blood counts, liver function tests, serum ferritin, and MRI for cardiac T2* and LIC.

Complete blood counts were performed using an automated hematological analyzer (Beckman Coulter A^C.T 5diff, Coulter Corp., Miami, FL, USA). Serum ferritin was measured by a chemiluminescent method (Roche Cobas E601, Roche Diagnostics, Germany) as per the manufacturer's recommendation. Magnetic resonance acquisition was performed using a 1.5-T MR scanner (GE, HDxt, Milwaukee, WI, USA), used 8-channel body array as described previously [15]. For the measurements of myocardial and hepatic iron overload, a T2* gradient-echo multi-echo sequence was used. Both cardiac and hepatic T2* analysis and hepatic iron quantification were done on a workstation (Advantage Window 4.6, GE healthcare) using a commercial analysis software (Star Map 4.0, GE Healthcare). Cardiac iron overload was defined as cardiac T2* <20 ms and hepatic iron overload was defined as LIC ≥5 mg Fe/g dry weight.

Continuous data were reported as mean and standard deviation and categorical data were reported as frequency and percentage. The Chi-square test or Fisher's EXACT test was used to compare between categorical variables and the Student's t test was used to compare between continuous variables as appropriate. Variables that were significantly related with iron overload with the p value of less than 0.05 in the univariate analysis were entered into the multivariate analyses. Multivariate binary logistic regression analysis was used to identify the independent risk factors for hepatic iron overload. Odds ratios (OR) and 95 % confidence intervals (CI) were calculated for all associations that emerged. A p value of less than 0.05 was considered as statistically significant. The correlation coefficient between serum ferritin and LIC was examined. The predictive model between serum ferritin and LIC was constructed using an exponential regression method, running a regression of logarithmic transformation of ferritin on the two-parameter exponential function, $y_i = \beta_1 \beta_2^{x_i} + \epsilon_i$. Statistical analysis was performed by Stata/IC 13.0 for windows (StataCorp LP, College Station, TX, USA).

Results

Ninety-one patients were included in this study. There were 33 (36.3 %) male patients. The mean age was 24.6 ± 8.4 years. Fifty-four (59.3 %) patients had beta-thalassemia disease and 37 (40.7 %) patients had alpha-thalassemia disease. The subtype of thalassemia, the history of transfusion, splenectomy and iron chelation, the laboratory data, and the iron parameters are summarized in Table 1.

Table 1 Clinical and laboratory characteristics of NTDT patients included in the study

Characteristics	NTDT patients ($n = 91$)
Age (years)	24.6 ± 8.4
Male gender (%)	33 (36.3 %)
Type of thalassemia (%)	
Hb E/beta-thalassemia	49 (53.8 %)
Beta-thalassemia intermedia	5 (5.5 %)
Hb H disease	13 (14.3 %)
Hb H/CS disease	20 (22.0 %)
Hb H/CS with Hb E trait	4 (4.4 %)
Cumulative lifetime RBC transfusion (%)	
None	22 (24.2 %)
<10 units	36 (39.5 %)
≥10 units	33 (36.3 %)
Previous splenectomy (%)	27 (29.7 %)
Iron chelators (%)	
None	64 (70.3 %)
Deferiprone	24 (26.4 %)
Desferrioxamine	2 (2.2 %)
Combined deferiprone and desferrioxamine	1 (1.1 %)
Hb level (g/dL)	7.9 ± 1.3
ALT (U/L)	30 ± 27
Serum ferritin level (ng/mL)	767 ± 692
Maximum serum ferritin level in the past 5 years (ng/mL)	1381 ± 1635
Cardiac T2* (ms)	44.5 ± 6.2
LIC by MRI (mg Fe/g dry weight)	8.5 ± 6.7
<5	37 (40.7 %)
5 to <10	20 (22.0 %)
10 to <20	27 (29.7 %)
≥20	7 (7.6 %)

CS Constant Spring, *Hb* hemoglobin, *LIC* liver iron concentration, *RBC* red blood cell

Table 2 shows the clinical and laboratory characteristics of NTDT patients according to hepatic iron overload status. The age, genotype, cumulative lifetime RBC transfusion, previous splenectomy and Hb level were significantly different between the groups with and without hepatic iron overload. Serum alanine aminotransferase and ferritin levels were also different.

The association of clinical and laboratory characteristics and hepatic iron overload by univariate and multivariate analysis are shown in Tables 3 and 4, respectively. By univariate analysis, the age ≥20 years, beta-thalassemia genotype, cumulative lifetime RBC transfusion ≥10 units, previous splenectomy and a Hb level <7 g/dL were significantly associated with hepatic iron overload. By multivariate analysis, only the age ≥20 years, cumulative lifetime RBC transfusion ≥10 units, and a Hb level <7 g/dL were significantly associated with hepatic iron overload.

Figure 1 shows a scatter plot between serum ferritin and LIC. The correlation coefficient (r) between serum ferritin and LIC was 0.60, p value <0.001. The predictive model was then adjusted by running a regression of logarithmic transformation of ferritin on the two-parameter exponential function. The coefficient of determination (R^2) was 0.99, p value <0.001. The predicting value of ferritin was 547 ng/mL for LIC of 5 mg Fe/g dry weight. The sensitivity, specificity, positive predictive value, negative predictive value and kappa analysis for percentage agreement was 92.7, 91.7, 94.4, 89.2, and 92.31 %, respectively.

Discussion

The prevalence of hepatic iron overload as determined by LIC ≥5 mg Fe/g dry weight in NTDT patients from this study is high at 59 %. The prevalence is in agreement with a previous study in a large NTDT population by Taher et al. which reported a prevalence of 63.6 % [16]. The cut-off level of LIC ≥5 mg Fe/g dry weight for hepatic iron overload was used in this study according to the current recommendation that iron chelation should be started when LIC is ≥5 mg Fe/g dry weight in patients with NTDT as iron-related morbidities increase with the LIC level [2, 5, 17]. Our study also shows agreement with previous studies with the absence of cardiac iron overload, even in patients with high LIC level [18, 19].

From univariate analysis, genotype is a significant factor for hepatic iron overload. The prevalence of hepatic iron overload was 76 % in the beta-thalassemia group, 48 % in Hb H/CS disease and none in Hb H disease. The difference was not significant between the beta-thalassemia and Hb H/CS disease groups, but was distinctly seen in Hb H disease that in this study the hepatic iron overload is absent. When adjusted with other risk factors, the genotype showed no significant effect. This is likely because the hepatic iron overload depends more on the disease severity, such as the Hb level and history of transfusion, than the genotype per se.

The identified significant risk factors for hepatic iron overload from our study were the age ≥20 years, Hb level <7 g/dL and the cumulative lifetime transfusion ≥10 units. Our results confirm with several previous studies that serum ferritin and LIC increase with age [12, 20–22]. NTDT patients accumulate iron over time because of chronic hemolytic anemia and ineffective erythropoiesis leading to an increase in growth differentiation factor 15 (GDF-15), hypoxia-inducible transcription factor (HIF), a decreased hepcidin and therefore increased gastrointestinal absorption and iron deposition in the liver [6, 23–25]. The

Table 2 Clinical and laboratory characteristics of NTDT patients according to hepatic iron overload status

Characteristics	LIC ≥5 mg Fe/g dry weight ($n = 54$)	LIC <5 mg Fe/g dry weight ($n = 37$)	p value
Age (years)	27.3 ± 7.7	20.5 ± 7.8	<0.001
Male gender (%)	17 (31.5 %)	16 (43.2 %)	0.252
Type of thalassemia (%)			<0.001
Hb E/beta-thalassemia and beta-thalassemia intermedia	43 (79.6 %)	13 (35.2 %)	
Hb H disease	0	12 (32.4 %)	
Hb H/CS disease with or without Hb E trait	11 (20.4 %)	12 (32.4 %)	
Cumulative lifetime RBC transfusion (%)			<0.001
None	2 (3.7 %)	20 (54.1 %)	
<10 units	20 (37.0 %)	16 (43.2 %)	
≥10 units	32(59.3 %)	1 (2.7 %)	
Previous splenectomy (%)	24 (44.4 %)	3 (8.1 %)	<0.001
Hb level (g/dL)	7.4 ± 1.2	8.7 ± 1.4	<0.001
ALT (U/L)	39 ± 29	18 ± 18	<0.001
Serum ferritin level (ng/mL)	1086 ± 731	300 ± 180	<0.001
Maximum serum ferritin level in the past 5 years (ng/mL)	2061 ± 1824	390 ± 283	<0.001

ALT alanine aminotransferase, *CS* Constant Spring, *Hb* hemoglobin, *RBC* red blood cell

Table 3 Association of clinical risk factors and hepatic iron overload by univariate analysis

Characteristics	Odds ratio	95 % confidence interval	p value
Age ≥20 years old	7.2	2.8–18.8	<0.001
Beta-thalassemia	7.2	2.8–18.6	<0.001
Cumulative lifetime RBC transfusion ≥10 units	52.4	6.7–410.8	<0.001
Previous splenectomy	9.1	2.5–33.2	0.001
Hb level <7 g/dL	3.8	1.3–11.2	0.017

Hb hemoglobin, *RBC* red blood cell

Table 4 Association of clinical and laboratory characteristics and hepatic iron overload by multivariate analysis

Characteristics	Adjusted odds ratio	95 % confidence interval	p value
Age ≥20 years old	30.2	4.5–203	<0.001
Beta-thalassemia	3.2	0.8–13.0	0.104
Cumulative lifetime RBC transfusion≥ 10 units	53.6	3.2–884	0.005
Previous splenectomy	4.7	0.5–42.8	0.165
Hb level <7 g/dL	6.3	1.01–39.5	0.049

Hb hemoglobin, *RBC* red blood cell

pathogenesis also emphasizes the important role of lower Hb level on iron overload. The current TIF guideline recommends an assessment for iron overload by either MRI or serum ferritin for NTDT patients starting from 10 years of age. Our study adds that the older patients with lower

baseline Hb level will particularly benefit from iron overload assessment.

Transfusional iron is the main source of iron overload in TDT. The current TIF guideline recommends measuring the ferritin level after 10–20 transfusions have been given.
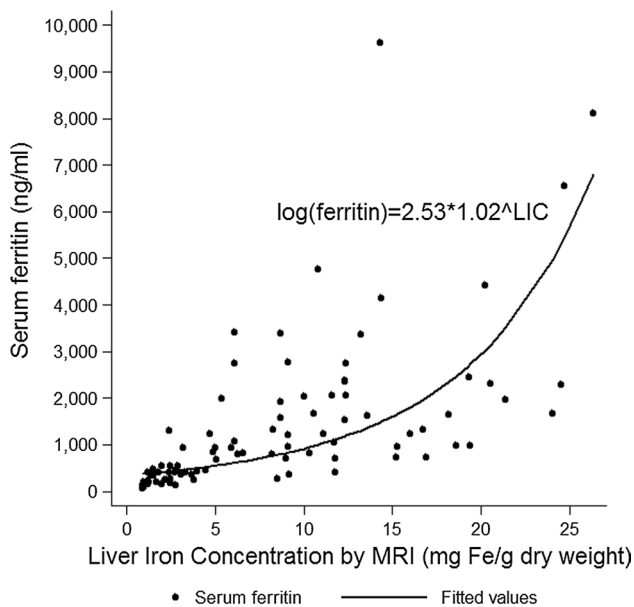
$$\log(\text{ferritin}) = 2.53 \times 1.02^{\text{LIC}}$$

Fig. 1 The scatter plot between serum ferritin and liver iron concentration. The predictive model was constructed by running a regression of logarithmic transformation of ferritin on the two-parameter exponential function

Our study shows that the cumulative lifetime transfusion of more than 10 units of RBC is a risk for hepatic iron overload in NTDT. Although the NTDT patients are not regularly transfused, the records of cumulative transfusion are important and should be periodically reviewed to identify the patients at risk. Of note, there was a large variation of the number of cumulative red blood cell transfusion in this study. Some patients received frequent transfusions in their childhood, and became non-transfusion-dependent after splenectomy. Therefore, the 95 % confidence interval of the adjusted odds ratio of the cumulative red blood cell transfusion was large.

Our study shows two unique characteristics of the correlation of serum ferritin and LIC as measured by MRI in NTDT patients. Firstly, the serum ferritin to LIC ratio is lower than that seen in TDT patients. This is in agreement with the previous large studies [12–14]. The ferritin cut-off point at the LIC of 5 mg Fe/g dry weight is ~550 ng/mL, lower than 800 ng/mL from the previous study [5, 12]. Secondly, the predictive model between serum ferritin and LIC shows a nonlinear correlation. The model shows that at higher LIC, the serum ferritin varies in a larger range. Interpretation of the response to treatment by serum ferritin alone at higher LIC needs to take this into account. The finding of nonlinear correlation between serum ferritin and LIC was also shown in pediatric patients with sickle cell disease who received chronic blood transfusion. The serum ferritin was shown to be associated with iron overload and liver injury [26].

In conclusion, there is a high prevalence of hepatic iron overload in NTDT patients. The risk factors are increasing age ≥ 20 years, lower Hb level <7 g/dL and cumulative lifetime transfusion ≥ 10 units. Genotype does not show the significant association with iron overload which implies that iron overload is related more to the severity of the disease. The ratio of serum ferritin to LIC in NTDT is lower than that seen in TDT. The nonlinear relationship between serum ferritin and LIC should be considered when interpreting the treatment response especially when the LIC is high.

References

1. Weatherall DJ. The definition and epidemiology of non-transfusion-dependent thalassemia. Blood Rev. 2012;26(Suppl 1):S3–6.
2. Taher A, Vichinsky E, Musallam K, Cappellini MD. Viprakasit V. In: Weatherall D, editor. Guidelines for the management of non transfusion dependent thalassaemia (NTDT). Nicosia: Team up Creations; 2013.
3. Taher AT, Musallam KM, Karimi M, El-Beshlawy A, Belhoul K, Daar S, Saned MS, El-Chafic AH, Fasulo MR, Cappellini MD. Overview on practices in thalassemia intermedia management aiming for lowering complication rates across a region of endemicity: the OPTIMAL CARE study. Blood. 2010;115:1886–92.
4. Musallam KM, Cappellini MD, Wood JC, Taher AT. Iron overload in non-transfusion-dependent thalassemia: a clinical perspective. Blood Rev. 2012;26(Suppl 1):S16–9.
5. Taher AT, Viprakasit V, Musallam KM, Cappellini MD. Treating iron overload in patients with non-transfusion-dependent thalassemia. Am J Hematol. 2013;88:409–15.
6. Taher AT, Musallam KM, Cappellini MD, Weatherall DJ. Optimal management of beta thalassaemia intermedia. Br J Haematol. 2011;152:512–23.
7. Tanno T, Miller JL. Iron loading and overloading due to ineffective erythropoiesis. Adv Hematol. 2010;2010:358283. doi:10.1155/2010/358283.
8. Origa R, Galanello R, Ganz T, Giagu N, Maccioni L, Faa G, Nemeth E. Liver iron concentrations and urinary hepcidin in beta-thalassemia. Haematologica. 2007;92:583–8.
9. Lemmens-Zygulska M, Eigel A, Helbig B, Sanguansermsri T, Horst J, Flatz G. Prevalence of alpha-thalassemias in northern Thailand. Hum Genet. 1996;98:345–7.
10. Teawtrakul N, Ungprasert P, Pussadhamma B, Prayalaw P, Fucharoen S, Jetsrisuparb A, et al. Effect of genotype on pulmonary hypertension risk in patients with thalassemia. Eur J Haematol. 2014;92:429–34.

11. Angelucci E, Brittenham GM, McLaren CE, Ripalti M, Baronciani D, Giardini C, Polchi P, Lucarelli G. Hepatic iron concentration and total body iron stores in thalassemia major. N Engl J Med. 2000;343:327–31.

12. Taher AT, Porter JB, Viprakasit V, Kattamis A, Chuncharunee S, Sutcharitchan P, et al. Defining serum ferritin thresholds to predict clinically relevant liver iron concentrations for guiding deferasirox therapy when MRI is unavailable in patients with non-transfusion-dependent thalassaemia. Br J Haematol. 2015;168:284–90.

13. Taher A, El Rassi F, Isma'eel H, Koussa S, Inati A, Cappellini MD. Correlation of liver iron concentration determined by R2 magnetic resonance imaging with serum ferritin in patients with thalassemia intermedia. Haematologica. 2008;93:1584–6.

14. Pakbaz Z, Fischer R, Fung E, Nielsen P, Harmatz P, Vichinsky E. 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;49:329–32.

15. Inthawong K, Charoenkwan P, Silvilairat S, Tantiworawit A, Phrommintikul A, Choeyprasert W, Natesirinilkul R, Siwasomboon C, Visrutaratna P, Srichairatanakool S, Chattipakorn N, Sanguansermsri T. Pulmonary hypertension in non-transfusion-dependent thalassemia: correlation with clinical parameters, liver iron concentration, and non-transferrin-bound iron. Hematology. 2015;20:610–7.

16. Taher AT, Porter J, Viprakasit V, Kattamis A, Chuncharunee S, Sutcharitchan P, et al. Deferasirox reduces iron overload significantly in nontransfusion-dependent thalassemia: 1-year results from a prospective, randomized, double-blind, placebo-controlled study. Blood. 2012;120:970–7.

17. Musallam KM, Cappellini MD, Wood JC, Motta I, Graziadei G, Tamim H, Taher AT. Elevated liver iron concentration is a marker of increased morbidity in patients with β thalassemia intermedia. Haematologica. 2011;96:1605–12.

18. Mavrogeni S, Gotsis E, Ladis V, Berdousis E, Verganelakis D, Toulas P, Cokkinos DV. Magnetic resonance evaluation of liver and myocardial iron deposition in thalassemia intermedia and b-thalassemia major. Int J Cardiovasc Imaging. 2008;24:849–54.

19. Taher AT, Musallam KM, Wood JC, Cappellini MD. Magnetic resonance evaluation of hepatic and myocardial iron deposition in transfusion-independent thalassemia intermedia compared to regularly transfused thalassemia major patients. Am J Hematol. 2010;85:288–90.

20. Chen FE, Ooi C, Ha SY, Cheung BM, Todd D, Liang R, Chan TK, Chan V. Genetic and clinical features of hemoglobin H disease in Chinese patients. N Engl J Med. 2000;343:544–50.

21. Taher AT, Musallam KM, El-Beshlawy A, Karimi M, Daar S, Belhoul K, Saned MS, Graziadei G, Cappellini MD. Age-related complications in treatment-naive patients with thalassaemia intermedia. Br J Haematol. 2010;150:486–9.

22. Mishra AK, Tiwari A. Iron overload in beta thalassaemia major and intermedia patients. Maedica (Buchar). 2013;8:328–32.

23. Musallam KM, Taher AT, Duca L, Cesaretti C, Halawi R, Cappellini MD. Levels of growth differentiation factor-15 are high and correlate with clinical severity in transfusion-independent patients with beta thalassemia intermedia. Blood Cells Mol Dis. 2011;47:232–4.

24. Fiorelli G, Fargion S, Piperno A, Battafarano N, Cappellini MD. Iron metabolism in thalassemia intermedia. Haematologica. 1990;75(Suppl 5):89–95.

25. Musallam KM, Rivella S, Vichinsky E, Rachmilewitz EA. Non-transfusion-dependent thalassemias. Haematologica. 2013;98:833–44.

26. Adamkiewicz TV, Abboud MR, Paley C, Olivieri N, Kirby-Allen M, Vichinsky E, 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;114:4632–8.