Assessment of Heart and Liver Iron Overload in Thalassemia Major Patients Using T2* Magnetic Resonance Imaging

Hamid Farhangi¹ · Zahra Badiei¹ · Hasan Mottaghi Moghaddam¹ ·
Mohammad Reza Keramati²

Received: 1 July 2015 / Accepted: 7 June 2016 / Published online: 15 June 2016
© Indian Society of Haematology & Transfusion Medicine 2016

Abstract Accumulation of excess iron in heart can lead to cardiac dysfunction, which is the most common cause of death in thalassemia major patients. Biopsy is an invasive procedure and therefore not an ideal option to assess iron load. However, standard/usual non-invasive methods, such as ferritin measurement, have some limitations and the results show poor correlations with iron load. Magnetic Response Imaging (MRI-T2*), as a non-invasive and reliable method for iron load assessment in organs such as liver and heart, can be suggested as a favorable alternative. This cross-sectional study was implemented in Thalassemia and Hemophilia Clinic Center (Sarvar) affiliated with Mashhad University of Medical Sciences, Mashhad, Iran, from 2012 to 2013. After the approval of the research protocol by the local ethic committee, laboratory tests, including CBC and serum ferritin, were carried out, and echocardiography and heart and liver MRI-T2* were performed. All statistical analysis was done through SPSS software (version 11.5), using independent sample *t* test and Pearson's correlation coefficient test. A *P* value ≤ 0.05 was considered to be significant. 88 patients with the mean ($\pm$ SD) age of 21.2 (± 5.6) years, (range 11–37 years) were observed. Iron load was assessed using MRI-T2* with the following results: Out of 88 patients, 48.9 % had mild to severe cardiac siderosis, and 75.2 % had mild to severe liver siderosis. We demonstrated a correlation between

liver MRI-T2* and serum ferritin, and heart MRI-T2* and ejection fraction. However, no correlation between liver and heart MRI-T2* was observed. Heart and liver siderosis is a common and serious problem in thalassemia major patients, and MRI-T2* as a sensitive and non-invasive technique can be used for early/timely detection of siderosis and good therapeutic monitoring in these patients.

Keywords Thalassemia · MRI · Iron overload · Ferritin · Liver · Heart

Introduction

Thalassemias are a group of hemoglobin disorders characterized by a deficiency in the synthesis of one or more globin chains. They are the most common genetic disorder worldwide. The overall prevalence of thalassemia across different provinces of Iran is 3–100 per 100,000 people [1, 2]. β -Thalassemia major is a severe form of the disease which causes severe hemolytic anemia in infants and children. Frequent blood transfusion is therefore considered to be the basic treatment for these patients. Despite its lifesaving benefits, blood transfusion can cause further complications, particularly iron overload and hemochromatosis. Iron accumulation in heart leads to cardiac dysfunction, cardiomyopathy and heart failure, which is the most common cause of death in these patients [3]. The liver is also susceptible to early iron deposition. Liver iron overload, if not diagnosed and properly treated, rapidly develops into cirrhosis. In order to prevent hemosiderosis, iron has to be chelated and excreted.

There are several methods to assess iron burden in multitransfused thalassemic patients, including serum iron, transferrin saturation, serum ferritin, liver biopsy

✉ Mohammad Reza Keramati
Keramati@ums.ac.ir

¹ Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

² Faculty of Medicine, Cancer Molecular Pathology Research Center, Emam Reza Hospital, Mashhad University of Medical Sciences, Mashhad, Iran

and Magnetic Resonance Imaging (MRI). However, these patients always show increased serum iron levels, and complete saturation of transferrin in early years of transfusion. Moreover ferritin has a poor correlation with body iron load. Several factors render ferritin an inaccurate marker of iron overload: ferritin, as an acute-phase reactant, increases in cases of chronic infectious diseases, malignancies and/or inflammatory disorders; Moreover, Ascorbate deficiency can decrease the rate of ferritin synthesis and release. Consequently, patients with active hepatitis may have elevated levels of serum ferritin, which bear little or no correlation with iron burden [4, 5].

Liver biopsy is considered to be the reference method for assessing total iron stores in thalassemia major patients. It is invasive, however, and the heterogeneity of iron distribution in liver may lead to inaccurate assessment and serious errors in diagnosis and treatment [6]. Additionally, liver biopsy results are usually poor indicators of cardiac iron load. They may show notable iron loading in liver when cardiac iron burden is, in fact, negligible (the same is true about cardiac biopsy results and their value in assessing liver iron load). Therefore, even with favorable liver biopsy results, cardiac siderosis may develop undiagnosed, only presenting itself with a heart failure [3]. Heart biopsy, as a method of iron load assessment, is invasive; and echocardiography is not sensitive enough to detect early cardiac functional abnormalities/disorders secondary to iron deposition [7].

Magnetic Resonance Imaging (MRI-T2*), as a non-invasive technique, has been considered a reliable method for evaluation of iron deposition in different organs such as liver and heart. The Studies have demonstrated a good correlation between liver iron measured using MRI-T2* and that measured by liver biopsy [6, 8]. This technique can provide a reliable and non-invasive method for early detection of iron overload in body organs and also therapeutic monitoring of chelating drugs. In this study, we compared the diagnostic value of MRI-T2* in assessing cardiac and liver iron load in thalassemia major patients, with that of other usual methods of iron load assessment such as serum ferritin and echocardiography.

Materials and Methods

This cross-sectional study was implemented in Thalassemia and Hemophilia Clinic Center (Sarvar), affiliated with Mashhad University of Medical Sciences, Mashhad, Iran, from 2012 to 2013. Sarvar is the largest Center for diagnosis, registration and therapeutic monitoring of hemophilia and thalassemia patients in Northeast of Iran with about 20 years old. In this center all patients follow

from initiation of diagnosis until death. We only collected data as a cross sectional study in these years.

Up to this time, 300 thalassemia major, and 47 thalassemia intermedia patients have been registered in this center. The research protocol having been approved by the ethical committee of Mashhad University of Medical Sciences, and patients' informed consents obtained, the study was embarked upon. Patients with thalassemia major diagnosis aged nine and above, undergoing iron chelating therapy entered the study. The following cases were excluded: patients with severe heart failure and congenital cardiac anomalies; patients who had been undergoing iron chelating therapy for less than 6 months, and those unwilling to comply with the research protocol.

Patients were divided into two groups based on the type of chelating drug they had received. The first group (50 patients) was taking deferoxamine (desferal) 30–50 mg/kg at least five times per week, and the second group (38 patients) was under desferal and deferiprone (L1) treatment (desferal 30–50 mg/kg three times per week and L1, 75 mg/kg daily). For all patients, the volume of transfused packed red blood cells (mL/kg) in the year preceding the MRI scan; and interval period of transfusion were calculated. Our criterion for transfusion was the hemoglobin threshold of 9 g/dL. Transfusion was administered at the volume of 10 cc/kg.

Laboratory tests including CBC and serum ferritin were measured and echocardiography and heart and liver MRI-T2* were performed. For performing of laboratory tests, before each blood transfusion, two milliliters EDTA-anticoagulated blood for CBC and three milliliters non-anticoagulated blood for serum ferritin measurement were taken. CBC and hemoglobin measurements were analyzed by cell counter (Sysmex KX-21N, Japan) and serum ferritin was measured by radio immune assay method (reference range 15–300 µg/L). Echocardiography was performed for all participants and ejection fraction (EF)—reference range 55–75 %—and fraction shortening (FS)—reference range 28–40 %—were calculated.

MRI-T2* (by Siemens Medical Solution, Erlangen, Germany) was carried out to determine iron burden in liver and heart before appearing clinical signs and symptoms. For liver MRI, a single breath-hold technique using a multiecho gradient echo (8–16 echoes) sequence and for heart a single midpapillary ventricular short-axis slice using a cardiac-gated, segmented and multiecho gradient echo (8 echo) sequence were obtained. In the heart MRI-T2*, conduction time more than 20 ms was considered as normal iron load, between 15 and 20 ms as mild, 10–14 ms as moderate and less than 10 ms as an indicative of severe myocardial siderosis [7]. In the liver MRI, iron load was also divided as normal ($T2^* > 6.3$ ms/ < 2 mg Fe/g dry weight), mild ($T2^* = 2.8$ – 6.3 ms/ 2 – 5 mg Fe/g dry

weight), moderate ($T2^* = 1.4\text{--}2.7$ ms/5–10 mg Fe/g dry weight) and severe ($T2^* = < 1.4$ ms/ > 10 mg Fe/g dry weight) [7, 9].

Statistical Analysis

Data were analyzed by SPSS software (version 11.5). First, descriptive and then comparative analyses of variables were performed. The mean of continuous variables with normal distribution were compared, using an independent sample *t* test and a Pearson's correlation coefficient test and variables with non-normal distribution, using non-parametric equivalents, Mann–Whitney and Spearman's tests. A *P* value ≤ 0.05 was considered to be significant.

Results

After application of exclusion criteria, from 300 thalassemia major patients registered in Sarvar Center, 88 entered the study. 50 cases were undergoing deferoxamine treatment, and 38 were under deferoxamine and deferiprone therapy. Among patients, 45 cases were male and 43 cases were female with no significant difference (*P* value < 0.831). Table 1 shows general characteristics of patients. Mean ($\pm$ SD) of serum ferritin in the multiple measurements in this period was 4057 (± 4056) $\mu\text{g/L}$. 22.7 % of patients had ferritin levels less than 1500 $\mu\text{g/L}$

Table 1 General characteristics of thalassemia major patients

Parameter	Mean ($\pm$ SD) (Range)	95 % Confidence interval
Age (years)	21.2 (± 5.6) (11–37)	20–22.4
Weight (kg)	48.6 (± 10.8) (20–76)	46.3–50.9
Height (cm)	152 (± 12.1) (115–180)	149–154
Age of diagnosis (months)	12 (± 10.8) (1–60)	10–14.6
Hemoglobin (g/dL)*	9.58 (± 0.06) (8–11)	9.4–9.7
Blood transfusion interval (days)	21.64 (± 2.84) (14–30)	21–22.2
Serum Ferritin ($\mu\text{g/L}$)*	4057 (± 4056) 54–31,537	3197–4916
Desferal consumption (years)	14.5 (± 4.14) (2–26)	13.5–15.8
Deferiprone and L1 consumption (years)	1.97 (± 0.94) (1–4)	1.66–2.28

* Mean of hemoglobin and ferritin in 1 year

and 57 % showed severely increased ferritin levels (ferritin level > 2500 $\mu\text{g/L}$). Patients were undergoing treatment of desferal for 14.5 (± 4.14) years, and L1 with desferal for 1.97 (± 0.94) years.

Table 2 shows severity of iron load in the heart and liver. As shown in the table, 51.1 % of patients had normal cardiac iron load; 14.8 % showed normal liver iron load, and 75.2 % had mild to severe siderosis. Mean ($\pm$ SD) iron in liver was 6.37 (± 3.22) mg Fe/g dry weight, ranged from 0.31 to 13.9. Out of 22 cases with severe cardiac siderosis, only 8 (36 %) had liver siderosis. Conversely, out of 11 cases with severe liver siderosis, concomitant cardiac siderosis was observed in 8 cases (72 %). Out of the total 88 cases, only in 24 (27 %) the heart and liver siderosis were of similar severity (i.e. normal/normal, mild/mild, moderate/moderate, severe/severe). In 63 cases (73 %) the severity of heart and liver siderosis did not correspond.

In echocardiography, 78 (88.7 %) patients had normal EF and FS values; abnormal values were observed only in 10 (11.3 %). Out of 10 patients with abnormal EF and FS, normal iron load was observed in 2 (20 %), 1 (10 %) patient showed mild iron overload and 7 (70 %) severe iron overload. Out of 78 patients with normal EF and FS in echocardiography, normal iron load in 43 (55.1 %), mild iron overload in 9 (11.5 %), moderate in 11 (14.1 %) and severe in 15 (19.2 %) patients were observed. As shown by linear regression analysis in Fig. 1, Pearson's correlation coefficient test demonstrated a significant correlation between severity of heart iron load and EF ($r = 0.432$, $P = < 0.001$) and FS ($r = 0.297$, $P = 0.005$). We observed a decrease in EF and FS with increase in severity of iron load (decrease in $T2^*$) in heart. We did not observe any correlation between MRI- $T2^*$ of heart and age ($r = 0.018$, $P = 0.872$), mean of hemoglobin measurements ($r = -0.1$, $P = 0.354$), transfused blood volume ($r = 0.098$, $P = 0.362$) and duration of desferal administration ($r = 0.052$, $P = 0.631$); however, between MRI- $T2^*$ (ms) of heart and mean of ferritin measurements a negative correlation was seen ($r = -0.257$, $P = 0.015$). This showed a direct correlation between serum ferritin concentration and heart iron load.

We did not observe any correlation between liver MRI- $T2^*$ and age ($r = 0.052$, $P = 0.629$), mean of hemoglobin measurements ($r = 0.043$, $P = 0.688$), transfused blood volume ($r = 0.207$, $P = 0.053$), duration of desferal administration ($r = 0.085$, $P = 0.431$) and EF or FS. However, between MRI- $T2^*$ of liver and mean of ferritin values a negative correlation was seen ($r = -0.547$, $P = < 0.001$; Fig. 1). This showed a direct correlation between mean of serum ferritin concentration and liver iron load. Data analysis also did not show a correlation between liver and heart MRI- $T2^*$ ($r = 0.186$, $P = 0.083$; Fig. 1).

Table 2 Severity of iron load in heart and liver of all patients and in the patient group undergoing desferal or combination of desferal and L1 treatments

Iron load	Heart n (%)	Liver n (%)	Heart with desferal (%)	liver with desferal (%)	Heart with desferal and L1 (%)	liver with desferal and L1 (%)
Normal	45 (51.1)	13 (14.8)	50	14	52.6	15.8
Mild	10 (11.4)	17 (19.3)	12	26	10.5	10.5
Moderate	11 (12.5)	47 (53.4)	12	52	13.2	55.3
Severe	22 (25)	11 (12.5)	26	8	23.7	18.4

Fig. 1 **a** and **b** show Correlation between heart MRI-T2* and EF ($r = 0.432$, $P = <0.001$) and FS ($r = 0.297$, $P = 0.005$), **c** shows correlation between liver and heart MRI-T2* ($r = 0.186$, $P = 0.083$) and **d** shows correlation between liver MRI-T2* and mean of ferritin

concentration ($r = -0.547$, $P = <0.001$) in 88 thalassemia major patients demonstrated by linear regression line. Data plotted on double log paper

In desferal group, we observed a correlation between heart MRI-T2* and EF ($r = 0.490$, $P = <0.001$), FS ($r = 0.460$, $P = <0.001$) and serum ferritin ($r = -0.411$, $P = 0.003$). However, we did not observe any correlation

between liver MRI-T2* and heart MRI-T2* ($r = 0.71$, $P = 0.617$). In addition, a negative correlation between liver MRI-T2* and serum ferritin was observed ($r = -0.447$, $P = 0.001$) in this group. In desferal plus L1

group, analysis showed a correlation between heart MRI-T2* and EF ($r = 0.457$, $P = 0.004$), serum ferritin ($r = -0.433$, $P = 0.007$) and liver MRI-T2* ($r = 0.339$, $P = 0.037$). There was also a negative correlation between liver MRI-T2* and serum ferritin.

Echocardiography of patients demonstrated abnormal results in 10 (11.4 %) patients and normal results in 78 (88.6 %) patients. Mean of heart T2* (ms) was lower in patients with abnormal results in echocardiography, compared to patients with normal results ($P = 0.049$). However, mean of liver T2* (ms) did not show any significant differences with echocardiography results ($P = 0.272$).

Discussion

Cardiac siderosis and cardiomyopathy is the main cause of death in thalassemia major patients. This complication is reversible by an appropriate iron chelation therapy [10]. We observed a correlation between serum ferritin, and EF and FS; however, taking into account reasons mentioned earlier, the correlation is too weak to support ferritin as a reliable marker of cardiac iron load. As shown in Fig. 1 and also based on other studies [11], cardiac iron load cannot be predicted simply by using liver iron load as an indicator. In addition, echocardiography can demonstrate dysfunction of heart only in patients with advanced disease [12]. We observed abnormal echocardiography results in 11.4 % patients. Additionally, chronic anemia results to cardiac dysfunction by the basal high cardiac output [12]. In line with the results of a study by Fragasso et al. [13] we found a positive correlation between T2* and EF/FS. As mentioned above, compared to ferritin measurement, heart MRI has fewer limitations. Therefore, as a noninvasive method, it can be used to detect iron overload much earlier in patients who need intensive iron chelating therapy, and as a result, decrease mortality of thalassemia patients [14, 15].

Our results demonstrated a substantial prevalence of heart iron overload in regularly transfused patients undergoing chelating therapy (Table 2). Severe myocardial siderosis (T2* time < 10 ms), which if undiagnosed can cause heart failure, was observed in 25 % of patients. MRI-T2*, as a valuable prognostic method can be used to detect myocardial siderosis timely enough to take appropriate preventive measures, such as chelating therapy, and hopefully lower the risk of mortality and morbidity in these patients. Severity and frequency of heart iron overload are different among the various countries. For example a study in China by Yang et al. on MRI-T2* assessment of heart iron overload in thalassemia major patients showed that cardiac siderosis could occur at lower ages than was earlier reported. They suggested that the first assessment of heart

MRI-T2* should be done as early as 6 years of age [16]. In other study by Carpenter et al. [10] for evaluation of cardiac iron load by MRI-T2*, 43.4 % of their patients had a myocardial T2* value less than 20 ms (mild to moderate iron overload) and 20.6 % having a T2* value less than 10 ms (severe iron load). We also observed mild to moderate and severe myocardial iron overload in 23.9 and 25 %, respectively (Table 2). In a study by Merchant et al. for evaluation of heart iron load by MRI on 60 thalassemia patients in India, similar results were observed. In this study, no cardiac siderosis in 50 %, mild to moderate in 33.3 % and severe cardiac siderosis in 16.7 % were observed [17]. One major factor affecting geographical distribution in heart iron overload is volume of transfused blood and efficacy of iron chelation therapy [10]. Our study also like study by Yang et al. showed a correlation between serum ferritin and heart MRI-T2*.

Liver iron concentration by biopsy is the reference method for the measurement of liver iron load; however, it is invasive and not very helpful in detecting iron load in other organs. Additionally, liver cirrhosis and method of biopsy processing could affect the iron concentration of tissue sections. An alternative method of the liver biopsy for the measurement of the liver iron is MRI [12]. Sari-gianni et al. in a systematic review and meta-analysis, published recently, reported that MRI-T2* has a good diagnostic accuracy (sensitivity of 96 % and specificity of 80 %) for detecting patients at risk for iron load complications or requiring titration of chelation drugs. They mentioned this method could safely rule out iron overload (LR−, 0.05); nonetheless, it was moderately useful to confirm a definite diagnosis (LR+, 4.86). They concluded that, given the paucity of good studies on the calculation of diagnostic accuracy of MRI-T2*, further well-designed studies adopting standardized MRI protocols are necessary [18]. Based on our results, 85.2 % of patients showed mild to severe liver iron overload, and 12.5 % had severe liver siderosis (Table 2). Merchant et al. [17] using MRI assessment, also demonstrated liver siderosis in 90 % of thalassemia major patients; however, they observed severe liver siderosis in 53 %. This is a high frequency of severe liver siderosis and may be due to incomplete chelating therapy in these Indian patients.

Our results did not demonstrate a correlation between liver and heart iron load assessed by MRI-T2*. Results from a study by Deborah Chirnomas et al. [9] on 66 transfusion dependent anemia patients, also using MRI-T2* as an assessment method, did not show such a correlation either. This result is also consistent with those obtained by other studies [11, 17, 19]. In another study, Deborah Chirnomas et al. [9] found a poor correlation between liver and heart iron load assessed by MRI-T2* in patients with transfusion dependent anemia. Poor

association between liver and heart iron load may be due to the differences in cellular iron transport and deposition in these organs [9]. Our results, in line with those obtained by these studies, showed that assessment of liver iron level alone is not sufficient for chelation adjustments and for the good therapeutic monitoring of iron load, both liver and heart MRI are advised.

Although serum ferritin is not a reliable marker of iron load, complications of iron overload can be notably avoided if ferritin levels are kept less than 1500 µg/L. Risk of developing complications rises significantly in patients with serum ferritin levels of 2500 µg/L or above [5]. 57 % of our patients had ferritin levels equal to or above this value. Our results also confirm the correlation between serum ferritin and liver and heart iron concentration reported in other studies (Fig. 1) [5]. However, some studies did not show a correlation between serum ferritin and cardiac iron overload [17]. In addition, according to MRI results (Table 2), 65.9 % of our patients had moderate to severe liver siderosis that was comparable with patients with severe increase in serum ferritin.

Iron overload is the main cause of mortality in thalassemia major patients; therefore, iron chelating therapy is a critical step in their management. Nichols-Vinueza et al. [20] in a study on MRI-T2* as a method to assess liver and heart iron load in thalassemia major patients, showed that annual MRI-T2* assessments are helpful to determine appropriate changes in chelating therapy dosing. Kolnagou et al. in a study compared the effectiveness of desferal treatment and desferal combined with L1, using liver MRI-T2* and serum ferritin as assessment methods. The results, in line with those obtained by other studies [21], confirmed combination therapy as the more effective treatment [22]. However, we did not observe any correlation between heart and liver MRI assessment results and the two treatment groups. This might be due to the shorter period of combination therapy administered to our patients.

Mean age and age range of our patients (Table 1) were lower than thalassemia major patients studied by Merchant et al. (mean age: 17, age range: 6–26) [17]. This could be an indication of our better management of complications, iron load in particular, in our patients. Like Merchant et al. we did not observe any correlation between age, transfused blood volume and type of chelation therapy with cardiac or liver siderosis.

Conclusion

Our results show that, in spite of chelating therapy and good monitoring, heart and liver siderosis is still a common and seriously persistent problem, accounting for a considerable number of deaths in thalassemia major patients.

Therefore, its early detection by a sensitive and noninvasive method such as MRI-T2*, combined with appropriate chelating therapy, can significantly lower the mortality rate in these patients.

Acknowledgments This study was extracted from a thesis by Dr. Saber Mohammadzadeh Rezaei, and would not have been possible without the encouragement and generous financial support of the Vice President of Research at Mashhad University of Medical Sciences. Hereby, we would like to express our heartfelt thanks to them and all those to whose kind assistance this study is indebted.

References

1. Abolghasemi H (2007) Thalassemia in Iran—epidemiology, prevention, and management. *J Pediatr Hematol Oncol* 29:233–238
2. Keramati MR, Sadeghian MH, Ayatollahi H, Younes Y (2013) Screening of hemoglobin disorders in referral cases to the hospital's laboratory in northeast Iran. *Int J Hematol Oncol* 23(2):138–142
3. Baksi AJ, Pennell DJ (2014) Randomized controlled trials of iron chelators for the treatment of cardiac siderosis in thalassaemia major. *Front Pharmacol* 5:217
4. Borgna-Pignatti C, Galanello R (2009) Thalassemias and related disorders: quantitative disorders of hemoglobin synthesis. In: Wintrobe MM, Greer JP (eds) *Wintrobe's clinical hematology*, vol 2, 12th edn. Lippincott Williams & Wilkins, Philadelphia, pp 1336–1342
5. Telfer PT, Prestcott E, Holden S, Walker M, Hoffbrand AV, Wonke B (2000) Hepatic iron concentration combined with long-term monitoring of serum ferritin to predict complications of iron overload in thalassaemia major. *Br J Haematol* 110(4):971–977
6. Gianesin B, Zefiro D, Musso M, Rosa A, Bruzzone C, Balocco M et al (2012) Measurement of liver iron overload: noninvasive calibration of MRI-R2* by magnetic iron detector susceptometer. *Magn Reson Med* 67(6):1782–1786
7. Barzin M, Kowsarian M, Akhlaghpour S, Jalalian R, Taremi M (2012) Correlation of cardiac MRI T2* with echocardiography in thalassemia major. *Eur Rev Med Pharmacol Sci* 16(2):254–260
8. Carson SM, Martin MB (2014) Effective iron chelation practice for patients with beta-thalassemia major. *Clin J Oncol Nurs* 18(1):102–111
9. Deborah Chirnomas S, Geukes-Foppen M, Barry K, Braunstein J, Kalish LA, Neufeld EJ et al (2008) Practical implications of liver and heart iron load assessment by T2*-MRI in children and adults with transfusion-dependent anemias. *Am J Hematol* 83(10):781–783
10. Carpenter JP, Roughton M, Pennell DJ (2013) International survey of T2* cardiovascular magnetic resonance in beta-thalassemia major. *Haematologica* 98(9):1368–1374
11. Estey MP, Cohen AH, Colantonio DA, Chan MK, Marvasti TB, Randell E et al (2013) CLSI-based transference of the CALIPER database of pediatric reference intervals from Abbott to Beckman, Ortho, Roche and Siemens Clinical Chemistry Assays: direct validation using reference samples from the CALIPER cohort. *Clin Biochem* 46(13–14):1197–1219
12. Brittenham GM (2015) Reference method for measurement of the hepatic iron concentration. *Am J Hematol* 90(2):85–86
13. Fragasso A, Cincio A, Mannarella C, Gaudiano C, Scarciolla O, Ottonello C et al (2011) Myocardial iron overload assessed by magnetic resonance imaging (MRI)T2* in multi-transfused patients with thalassemia and acquired anemias. *Eur J Intern Med* 22(1):62–65

14. He T (2014) Cardiovascular magnetic resonance T2* for tissue iron assessment in the heart. *Quant Imaging Med Surg* 4(5):407–412
15. Kirk P, Roughton M, Porter JB, Walker JM, Tanner MA, Patel J et al (2009) Cardiac T2* magnetic resonance for prediction of cardiac complications in thalassemia major. *Circulation* 120(20):1961–1968
16. Yang G, Liu R, Peng P, Long L, Zhang X, Yang W et al (2014) How early can myocardial iron overload occur in beta thalassemia major? *PLoS ONE* 9(1):e85379
17. Merchant R, Joshi A, Ahmed J, Krishnan P, Jankharia B (2011) Evaluation of cardiac iron load by cardiac magnetic resonance in thalassemia. *Indian Pediatr* 48(9):697–701
18. Sarigianni M, Liakos A, Vlachaki E, Paschos P, Athanasiadou E, Montori VM et al (2015) Accuracy of magnetic resonance imaging in diagnosis of liver iron overload: a systematic review and meta-analysis. *Clin Gastroenterol Hepatol* 13(1):55–63
19. Teixeira C, Barbot J, Freitas MI (2015) From blood film to the diagnosis of rare hereditary disorders. *Br J Haematol* 168(3):315
20. Nichols-Vinueza DX, White MT, Powell AJ, Banka P, Neufeld EJ (2014) MRI guided iron assessment and oral chelator use improve iron status in thalassemia major patients. *Am J Hematol* 89(7):684–688
21. Telfer PT, Warburton F, Christou S, Hadjigavriel M, Sitarou M, Kolnagou A et al (2009) Improved survival in thalassemia major patients on switching from desferrioxamine to combined chelation therapy with desferrioxamine and deferiprone. *Haematologica* 94(12):1777–1778
22. Kolnagou A, Economides C, Eracleous E, Kontoghiorghes GJ (2008) Long term comparative studies in thalassemia patients treated with deferoxamine or a deferoxamine/deferiprone combination. Identification of effective chelation therapy protocols. *Hemoglobin* 32(1–2):41–47