Limitations of serum ferritin to predict liver iron concentration responses to deferasirox therapy in patients with transfusion-dependent thalassaemia

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Abstract

Background: In transfusion-dependent anaemias, while absolute serum ferritin levels broadly correlate with liver iron concentration (LIC), relationships between trends in these variables are unclear. These relationships are important because serum ferritin changes are often used to adjust or switch chelation regimens when liver magnetic resonance imaging (MRI) is unavailable. Objectives and methods: This post hoc analysis of the EPIC study compared serum ferritin and LIC in 317 patients with transfusion-dependent thalassaemia before and after 1 yr of deferasirox. Results: Serum ferritin responses (decreases) occurred in 73% of patients, 80% of whom also have decreased LIC. However, 52% of patients without a serum ferritin response did decrease LIC and by &gt;1 mg Fe/g dw (median 3.9) in 77% of cases. Absolute serum ferritin and LIC values correlated significantly only when serum ferritin was &lt;4000 ng/mL (r = 0.59; P &lt; 0.0001) and not at higher levels (&gt;=4000 ng/mL; r = 0.19). Serum ferritin response was accompanied by decreased LIC in 89% and 70% of cases when serum ferritin was &lt;4000 or &gt;=4000 ng/mL, respectively. Conclusions: As serum ferritin non-response was associated with LIC decrease in over half of patients, use of liver MRI may be particularly useful for differentiating true from apparent non-responders to deferasirox based on serum ferritin trends alone.

Key words serum ferritin; liver iron concentration; deferasirox; thalassaemia; chelation

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chelation regimens can be adjusted or modified when necessary. Successful application of serum ferritin for monitoring chelation therapy requires a good understanding of the association between serum ferritin and LIC responses across a wide spectrum of iron burdens, as well as for different chelation regimens. Several studies have reported significant cross-sectional correlations of absolute serum ferritin values with LIC values, measured by a variety of methods (10–13), although the R2 values were often low, which is indicative of the small effect size of serum ferritin. However, relatively little has been reported regarding the predictive utility of serum ferritin trends over time. This may have practical implications because the absence of a serum ferritin response in the first few months of a new chelation regimen could be interpreted as a lack of response with respect to decrease in body iron load, even when patients may be experiencing a reduction in LIC (19).

Serum ferritin levels can be elevated independently of iron levels by factors such as acute or chronic inflammation and by acute or chronic liver damage (9, 20, 21). Serum ferritin levels may also be affected by frequency of blood transfusion (20), iron distribution between hepatocytes and macrophages (22, 23), type of iron chelation therapy used (24) and the duration of chelation therapy (25). Finally, at values between 3000 and 4000 ng/mL, iron-free serum ferritin is secreted by macrophages at levels that are approximately proportional to body iron stores. However, when serum ferritin values are higher than this, an increasing proportion of serum ferritin becomes iron-rich tissue ferritin that has leaked from damaged hepatocytes (26). Thus, the relationship between serum ferritin and LIC, and trends in response to iron chelation therapy, may also differ at high serum ferritin levels, although this has yet to be systematically addressed.

Liver iron concentration determination is less affected by factors unrelated to iron overload than serum ferritin and is a reliable and predictable measure of absolute body iron stores up to levels of approximately 30 mg Fe/g dw (27), as well as changes over time. Body iron stores can be calculated from the LIC: total body iron (mg Fe/kg) = 10.6 × LIC (mg Fe/g dw) (28). Hence, LIC changes over time are frequently used to measure net iron balance during chelation therapy. Magnetic resonance imaging (MRI), when available, is a widely accepted non-invasive method for assessing LIC (29, 30) and offers advantages such as sensitivity to low levels of iron load and high reproducibility (29, 31), although R2 MRI (FerriScan®) becomes less reliable when LIC is above 30 mg Fe/g dw (27). Nevertheless, in some areas of the world, specialist requirements and cost may limit the widespread use of MRI for monitoring iron chelation efficacy. In the absence of access to MRI, the judicious use and interpretation of serum ferritin levels and trajectories become critical to effective patient management.

Therefore, it is important to understand the utility and limitations of serial serum ferritin measurements in prediction of responses to chelation regimens. The Evaluation of Patients’ Iron Chelation with Exjade® (EPIC) study (32) provides a particularly valuable cohort of patients to address these questions as serum ferritin and liver MRI data are available in a large, prospective cohort of patients on a single chelation therapy modality. Therefore, this post hoc analysis of the EPIC study was conducted to gain insight into the relative trajectories of LIC and serum ferritin, across a range of iron burdens, before and after 1 yr of deferasirox treatment in patients with thalassaemia and transfusional iron overload. Specifically, we wanted to aid clearer interpretation of the relationship between changes in serum ferritin and LIC, and to identify factors that predict true versus pseudo non-response to chelation therapy with deferasirox. Ultimately, we aimed to demonstrate how serum ferritin trends can best be used to predict clinical response during treatment with deferasirox and to provide evidence-based practical guidance for patients with transfusion-dependent thalassaemias.

Methods

Study design and patient population

EPIC was a prospective, multicentre, open-label, 1-yr trial of the efficacy and safety of deferasirox in 1744 patients with transfusion-dependent anaemias (1115 patients with transfusion-dependent thalassaemia), which was conducted between April 2005 and June 2008 across 23 countries (32). In a predefined liver MRI substudy, patients were recruited from 25 sites that had the appropriate apparatus and expertise to perform liver R2-MRI assessments (33). Patients included in this post hoc analysis of EPIC had transfusion-dependent thalassaemia and had both a R2-MRI-assessed LIC measurement and a serum ferritin measurement at study baseline and after 1 yr. The design and patient population of the EPIC study have been described previously (32). In brief, eligible patients were aged ≥2 yr and had transfusional iron overload; deferasirox was started at a dose of 10–30 mg/kg/d. Initial dosing was individualised according to the frequency of blood transfusions, but was adjusted thereafter in line with serum ferritin trends and safety parameters. Written, informed consent was obtained from all patients prior to study participation. The EPIC study conformed to Good Clinical Practice guidelines and the Declaration of Helsinki.

Assessments

This analysis of the EPIC liver MRI substudy evaluated the relationship between serum ferritin and LIC. Serum ferritin levels were assessed at study baseline and every 4 wk thereafter. R2-MRI assessment of LIC was conducted at study baseline and end of study (i.e. after 1 yr of deferasirox) with standard 1.5-Tesla MRI scanners. MRI data were analysed centrally by Inner Vision Biometrics Pty Ltd (Claremont, WA, Australia) as previously reported (34).
Statistical analyses

A receiver operating characteristic (ROC) analysis was conducted to establish the serum ferritin levels that predict an LIC threshold of 20 mg Fe/g dw; analyses were performed for all patients with both LIC and serum ferritin measurements, at baseline (n = 313) and at end of study (n = 311). Summary statistics are provided for serum ferritin and LIC responders (decrease) and non-responders (increase or no change), and for study baseline serum ferritin categories (≥4000 vs. <4000 ng/mL); analyses were based upon patients with both LIC and serum ferritin measurements available at end of study (n = 311). Pearson’s correlation coefficient (r) was used to measure correlations between serum ferritin levels and LIC at study baseline, and change from study baseline to end of study, and presented as scatter plots. Patients were grouped according to study baseline iron burden: LIC <15 or ≥15 mg Fe/g dw and serum ferritin <2000, 2000 to <4000 or ≥4000 ng/mL. These categories were chosen as clinically relevant thresholds and to allow good balance across groups. An additional theoretical consideration was that a greater proportion of serum ferritin derives from damaged hepatocytes at serum ferritin levels >4000 ng/mL (26).

Results

Patient characteristics

Of the 1744 patients comprising the EPIC study population, 317 patients with transfusion-dependent thalassaemia were included in this analysis (Table 1). Of these, 313 and 311 patients had both LIC and serum ferritin measurements available at baseline and end of study, respectively.

The mean age of patients included in this analysis was 20.4 yr. At study baseline, mean ± SD duration of transfusion therapy was 18.3 ± 9.4 yr and most patients (n = 307/317) had received prior chelation therapy. Median (range) serum ferritin level [3675 (462–18 126) ng/mL] and mean ± SD LIC [22.1 ± 12.6 mg Fe/g dw (range 2–55)] were indicative of high body iron burden. Indeed, 140 patients had serum ferritin levels of 4000 ng/mL or more and 196 patients had LIC levels of 15 mg Fe/g dw or more at study baseline (Table 2). Approximately one-quarter of patients had a prior history of hepatitis B and/or C.

Utility of serum ferritin measurements to predict LIC

A ROC analysis was performed to determine a serum ferritin value that predicts an LIC of 20 mg Fe/g dw. The results of that analysis indicated serum ferritin values of 3500 ng/mL at study baseline and 4000 ng/mL at end of study predict this severely high liver iron burden in 83.2% and 72.7% of patients, respectively (Table S1).

Table 1 Demographic and study baseline characteristics of the patients from the EPIC study included in this subanalysis

| Characteristics                     | All patients (n = 317) |
|-------------------------------------|------------------------|
| Mean age (range), yr                | 20.4 (2–53)            |
| Male : female, n                    | 148 : 169              |
| Race (Caucasian : Oriental : other), n | 144 : 157 : 16        |
| History of hepatitis B and/or C, n (%) | 91 (28.7)            |
| Splenectomy, n (%)                  | 147 (46.4)             |
| Previous chelation therapy, n (%)   |                        |
| DFO monotherapy                     | 215 (67.6)             |
| DFP monotherapy                     | 3 (0.9)                |
| DFO and/or DFP1                     | 89 (28.0)              |
| Other2                              | 1 (0.3)                |
| None                                | 10 (3.1)               |
| Mean number of transfusion sessions in the year prior to study entry ± SD (n = 316) | 170.1 ± 183.9 |
| Mean total volume of RBCs transfused in the year prior to study entry ± SD, mL/kg (n = 312) | 18.3 ± 9.4 |
| Mean ± SD duration of transfusion therapy, yr (n = 315) |                       |
| Median serum ferritin (range), ng/mL (n = 313) | 3675 (462–18 126) |
| Mean LIC ± SD (range), mg Fe/g dw (n = 313) | 22.1 ± 12.6 (2–55) |

DFO, deferoxamine; DFP, deferiprone; LIC, liver iron concentration; RBC, red blood cell; SD, standard deviation.

1 Patients received DFP and DFO but not necessarily in combination.

2 Other category is not mutually exclusive; patients who received either DFO and/or DFP and other chelation therapies are counted under both categories.

Trajectory of serum ferritin and LIC during chelation therapy

Of the 311 patients analysed after 1 yr, 72.7% (n = 226) were identified as achieving a serum ferritin response (i.e. any decrease in serum ferritin levels after 1 yr) and 27.3% (n = 85) were classified as non-responders (i.e. an increase or no change in serum ferritin levels after 1 yr). LIC decreased in approximately half of serum ferritin non-responders (51.8%; n = 44/85) and in 79.6% of serum ferritin responders (n = 180/226; Fig. 1A). Of the serum ferritin non-responders with an LIC decrease, there was a clinically relevant decrease in LIC of ≥1 mg Fe/g dw in 77% (n = 34/44) of patients [median (range) −3.9 (−18.4 to −1.1) mg Fe/g dw; Fig. 1B]. In general, serum ferritin responders had a lower median transfusional iron intake, although the interquartile ranges overlap [median (interquartile range) 0.3 (0.2–0.4) vs. 0.4 (0.3–0.4) mg/kg/d], and received a higher deferasirox dose [median (interquartile range) 28.1 (22.1–33.6) vs. 23.7 (20.2–29.6) mg/kg/d] compared with serum ferritin non-responders.
Analysis of serum ferritin and LIC response by study baseline serum ferritin level: A serum ferritin response was observed in more patients when study baseline serum ferritin was ≥4000 ng/mL [79.9% (n = 111/139) vs. 66.9% (n = 115/172) < 4000 ng/mL; Fig. 2A]. However, more serum ferritin responders had an LIC response with study baseline serum ferritin <4000 ng/mL [88.7% (n = 102/115)] compared with serum ferritin responders with study baseline serum ferritin ≥4000 ng/mL [70.3% (n = 78/111); Fig. 2B]. The proportion of patients who decreased LIC despite having no serum ferritin response was similar regardless of serum ferritin study baseline category [52.6% (n = 30/57), <4000 ng/mL; 50.0% (n = 14/28), ≥4000 ng/mL; Fig. 2B]. There was little change in median (range) LIC in serum ferritin non-responders after 1 yr regardless of study baseline serum ferritin value [−0.3 (−13.5 to 18.7) for <4000 ng/mL and 0.2 (−18.4 to 19.6) for ≥4000 ng/mL].

Assessment by change in serum ferritin and LIC quadrants indicated that patients without a serum ferritin or LIC response (n = 41) had the lowest study baseline median (range) serum ferritin [2155 (480–9725) ng/mL] and LIC (11.9 (1.8–37.5) mg Fe/g dw) and received a lower median deferasirox dose [23.7 (9.7–36.0) mg/kg/d; Table S1]. Overall, median LIC decrease (mg Fe/g dw) was smaller in patients with study baseline serum ferritin <4000 ng/mL (n = 172) than in those with serum ferritin ≥4000 ng/mL [−2.8 (−38.5 to 18.7) vs. −4.9 (−31.1 to 19.6); n = 139]. Median iron intake was similar between groups (Table S2).

Relationship between serum ferritin levels and LIC by study baseline iron burden

In total, 313 of 317 patients had both serum ferritin and LIC determined at study baseline; 311 patients had both measurements at end of study. In these patients, results of the correlation analyses between serum ferritin levels and LIC before and after 1 yr of deferasirox treatment (i.e. study baseline and end of study) are summarised by iron burden in Table 2. A higher positive correlation was observed between serum ferritin and LIC at study baseline, and for change from study baseline, when study baseline serum ferritin was <4000 ng/mL (r = 0.59 and r = 0.51, respectively) than when study baseline serum ferritin was ≥4000 ng/mL (r = 0.19 and r = 0.37, respectively; Figure S1A,B). Similarly, a higher positive correlation was observed between serum ferritin and LIC at study baseline, and for change from study baseline, when study baseline LIC was <15 ng/mL (r = 0.53 and r = 0.62, respectively) than when study baseline LIC was ≥15 ng/mL (r = 0.36 and r = 0.29, respectively; Figure S1C,D).

The relationship between serum ferritin and LIC was evaluated further across a range of study baseline iron burden categories. At study baseline, there was a moderate (r = 0.37–0.48) and statistically significant positive relationship between LIC and serum ferritin levels in patients with LIC <15 mg Fe/g dw and serum ferritin <4000 ng/mL (P ≤ 0.005). However, a weak correlation was observed in patients with serum ferritin ≥4000 ng/mL, regardless of LIC (r = −0.14 and r = 0.09; P > 0.3; Table 2).

The mean changes from study baseline for serum ferritin and LIC generally correlated more strongly than at study baseline across iron burden categories (Table 2). Correlation between change from study baseline in LIC and serum ferritin was strongest in patients with LIC <15 mg Fe/g dw and serum ferritin <4000 ng/mL, although a moderate positive relationship was evident even at the highest level of iron burden (LIC ≥15 mg Fe/g dw and serum ferritin ≥4000ng/mL, r = 0.36, P < 0.0001; Table 2).

Table 2 Correlation coefficients (r) between serum ferritin levels and LIC at study baseline and between serum ferritin and LIC mean change from study baseline

| LIC baseline iron burden (mg Fe/g dw) | Serum ferritin (ng/mL) | Correlation coefficient between serum ferritin levels and LIC | Mean change from study baseline |
|-------------------------------------|------------------------|-------------------------------------------------------------|--------------------------------|
|                                      |                        | Study baseline                                              |                                 |
|                                      |                        | n   | r    | P-value | r    | P-value |
| All <4000                           |                        | 172 | 0.59 | <0.0001 | 0.51 | <0.0001 |
| All ≥4000                           |                        | 139 | 0.19 | 0.0248  | 0.37 | <0.0001 |
| <15 ≤4000                           | All                    | 115 | 0.53 | <0.0001 | 0.62 | <0.0001 |
| ≥15 ≥4000                           | All                    | 196 | 0.36 | <0.0001 | 0.29 | <0.0001 |
| <15 <2000                           | 2000 to <4000          | 55  | 0.37 | 0.0005  | 0.56 | <0.0001 |
| ≥4000                               |                        | 49  | 0.48 | 0.0005  | 0.67 | <0.0001 |
| <15 ≥2000                           | 2000 to <4000          | 11  | −0.14 | 0.69 | 0.62 | 0.04 |
| ≥4000                               |                        | 12  | 0.55 | 0.07    | 0.32 | 0.32 |
| ≥4000                               |                        | 56  | 0.06 | 0.64    | 0.41 | 0.002 |
| ≥4000                               |                        | 128 | 0.09 | 0.33    | 0.36 | <0.0001 |

dw, dry weight; LIC, liver iron concentration.

1Based upon patients with both LIC and serum ferritin measurements available at study baseline (n = 173, serum ferritin <4000 ng/mL and n = 140, serum ferritin ≥4000 ng/mL).
Discussion

Serum ferritin measurements are frequently used to monitor patients with transfusional iron overload, particularly in the absence of access to MRI for LIC assessment. Despite the widespread acceptance of serum ferritin as a surrogate marker of iron burden, few studies have investigated the relationships between changes in LIC and changes in serum ferritin during iron chelation therapy. Indeed, this large-scale, post hoc analysis of data from patients with transfusion-dependent thalassaemia in the 1-yr, prospective EPIC study is the first to address these relationships in patients treated with a single chelation modality.

In this analysis, a serum ferritin response predicted an LIC response in 80% of patients with transfusion-dependent thalassaemia treated with deferasirox for 1 yr. Thus, only 20% of patients with a serum ferritin response did not respond in terms of LIC. By contrast, in patients without a serum ferritin response, but with an LIC response experienced a clinically meaningful reduction in LIC. dw, dry weight; IQR, interquartile range; LIC, liver iron concentration.
response is equally likely to indicate an LIC decrease as no LIC decrease. Overall, these results show that all patients should receive LIC determination by MRI as serum ferritin trends can be misleading. Only where resources for LIC measurement are severely limited should follow-up LIC measurement be targeted to patients in whom serum ferritin is not responding; subsequent demonstration of an LIC decrease in these patients would, therefore, indicate treatment effectiveness and unnecessary changes in chelation dose or regimen could be avoided. Such therapeutic modifications could then be targeted only to patients who fail to demonstrate both a serum ferritin and an LIC decrease.

A recent retrospective cohort study conducted in 134 patients with transfusion-dependent anaemia (primarily sickle-cell disease and thalassaemia) over a period of up to 9 yr showed that the change in serum ferritin and change in LIC in patients with thalassaemia was concordant 46% of the time (13). Despite patients receiving several different chelation regimens, which may affect the relationship between serum ferritin and LIC (24), and different analytical methods, Puliyel et al. (13) showed that overall the change in serum ferritin was in the same direction as the change in LIC 74% of the time, which is similar to the 80% of patients we observed with both a serum ferritin and an LIC response.

A second aspect of our study was to determine how the relationship between changes in serum ferritin and changes in LIC was affected by study baseline serum ferritin values. This is of practical importance because clinicians need to know whether serum ferritin changes are more or less reliable as an indicator of body iron removal when baseline serum ferritin levels are high. A ROC analysis was undertaken for LIC and serum ferritin that indicated serum ferritin values of between 3500 and 4000 ng/mL were appropriate to predict severe liver iron burden of at least 20 mg Fe/g dw, although these data should be interpreted with caution because of the low sensitivity of serum ferritin and LIC at high values. Nonetheless, in addition to the fact that a greater proportion of serum ferritin derives from damaged hepatocytes at serum ferritin levels >4000 ng/mL (26), this justified our analyses of serum ferritin and LIC trends according to serum ferritin categories of <4000 or ≥4000 ng/mL at study baseline.

Our findings showed that, generally, the relationship between absolute serum ferritin and LIC values at study baseline was not significant for serum ferritin values of ≥4000 ng/mL, or LIC values of ≥15 mg Fe/g dw. By contrast, the relationship between change in LIC and change in serum ferritin was significant at both high and low baseline serum ferritin values, although this was stronger when baseline serum ferritin was lower. Despite the relatively poor utility of high serum ferritin values to predict absolute LIC, a downward trend in serum ferritin indicates LIC response in 70% of patients with serum ferritin ≥4000 ng/mL. Moreover, as with the whole patient cohort, a lack of serum ferritin response is a poor differentiator for LIC response versus non-response at high serum ferritin levels. Therefore, when MRI resources are severely limited, measurement can be prioritised for patients without a serum ferritin response, irrespective of baseline serum ferritin values.

A number of other observations are notable from this study. More than 60% of patients had previously received deferoxamine (Table 1). Data from both preclinical studies (37) and clinical observations (24) suggest deferoxamine accesses liver and macrophage iron pools differently to deferasirox, which in turn may alter the relationship between LIC and serum ferritin, and between changes in these parameters over time. Additionally, it has been suggested that, during the initial months of deferasirox therapy, serum ferritin response may lag behind the LIC response (38). Such an effect would occur if hepatocellular iron, which is rapidly targeted in vitro by deferasirox (39), were targeted by deferasirox faster than macrophage (Kupffer cell) iron, which is the major contributor to serum ferritin levels (26, 38). It would be of interest to examine this relationship over shorter time periods to address whether serum ferritin trends in the first 3–6 months of treatment with deferasirox reflect early LIC responses. However, this study shows that after 1 yr of deferasirox, changes in serum ferritin reflect changes in LIC and the relationship between the two variables is not significantly altered, in most cases. Additionally, these results suggest that serum ferritin levels may better predict liver iron levels once patients have been receiving effective chelation therapy for at least 1 yr and iron burden is lower. However, it must be noted that there are many other variables that may impact upon treatment with deferasirox, including treatment adherence, which is particularly relevant outside of the clinical trial setting.

A further implication of this analysis is that iron intake rate and chelation dose should be routinely examined in patients in whom serum ferritin and/or LIC are not responding. Of note, deferasirox dose was suboptimal in all patients, but particularly in those without a serum ferritin or LIC response; these patients received lower deferasirox doses and had a higher transfusional iron intake compared with patients who experienced a response. These insights highlight the
necessity of tailoring dose to a patient’s individual needs and ensuring appropriate management of iron overload.

Management of deferasirox dose at low serum ferritin values is increasingly becoming an area of interest. Current drug labelling for deferasirox suggests stopping treatment when serum ferritin values reach 500 ng/mL (40). However, gradual downward dose adjustment, without dose interruption, has been proposed as a preferable approach to achieve a ‘soft landing’, with a reduced possibility of over chelation; this has been shown in patients treated with a combination of deferiprone and deferoxamine (41), or deferiprone and deferasirox (42). Prospective studies are required to characterise how low serum ferritin values can be most safely achieved without over chelation. In the context of this paper, which highlights the caveats of serum ferritin for determining trends in LIC, measurement of LIC by MRI is likely to be important in patients with low serum ferritin values to guide deferasirox dose reduction or discontinuation.

In conclusion, this study clarifies how LIC determination can be used to aid interpretation of serum ferritin trends in patients with transfusion-dependent thalassaemia receiving deferasirox treatment. The chelator dose typically needs to be adjusted for each individual patient; both absolute levels and overall trends in serum ferritin are used to guide dose adjustments. However, this analysis demonstrates that, where possible, MRI determination of LIC should be offered for all transfused patients because of the limitations of using serum ferritin alone. This is particularly important in the absence of a downward trend in serum ferritin, because roughly equal proportions of patients will be responding, or not, in terms of LIC. Interpretation of serum ferritin trends can be particularly challenging at very high serum ferritin levels. However, these findings show that a clear downward trend in serum ferritin is a reasonable indicator of a decrease in LIC (and hence body iron) in 80% of cases, at both high and low baseline serum ferritin levels. Based on the data presented here, when the availability of MRI is limited, LIC determination by MRI could be prioritised for patients without a serum ferritin response. Dose adjustment, or change of regimen, could then be performed in patients without a downward trend in LIC. As the patient population had a high degree of iron overload, it would be valuable to examine the relationship between serum ferritin and LIC trends as patients approach lower, near-‘normal’ serum ferritin levels, so that doses can be adjusted appropriately to achieve a ‘soft landing’ and to minimise the risk of over chelation.

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Author contributions

JBP, ME, AT, YA, SHL, PS and AEB served as investigators on this trial, screening and enrolling patients and contributed to data interpretation. AEA contributed to data analysis and interpretation. JH served as the study analysis statistician. All authors reviewed, provided their comments on this manuscript and approved the final manuscript.

Conflict of interest

JBP reports consultancy, receiving research funding and honoraria from Novartis; consultancy and honoraria from Shire; and consultancy for Celgene. JBP is supported by the NIHR University College London Hospitals Biomedical Research Centre (BRC). AT reports receiving research funding and honoraria from Novartis. YA reports receiving research funding from Novartis, being a member of an advisory committee and participating in a Novartis speakers’ bureau. PS reports receiving research funding from Novartis. ME, AEB and SHL declare that they have no conflict of interest to disclose. AEA is a full-time employee of Novartis Pharma AG. JH is a full-time employee of Novartis Pharmaceuticals.

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References

1. Takatoku M, Uchiyama T, Okamoto S, et al. Retrospective nationwide survey of Japanese patients with transfusion-dependent MDS and aplastic anemia highlights the negative impact of iron overload on morbidity/mortality. *Eur J Haematol* 2007;78:487–94.
2. Hershko C. Pathogenesis and management of iron toxicity in thalassaemia. *Ann N Y Acad Sci* 2010;1202:1–9.
3. Borgna-Pignatti C, Rugolotto S, De Stefano P, et al. Survival and complications in patients with thalassemia major treated with transfusion and deferoxamine. *Haematologica* 2004;89:1187–93.
4. Belhoul KM, Bakir ML, Saned MS, Kadhim AM, Musallam KM, Taher AT. Serum ferritin levels and endocrinopathy in medically treated patients with beta thalassemia major. *Ann Hematol* 2012;91:1107–14.
5. Brittenham GM, Griffith PM, Nienhuis AW, McLaren CE, Young NS, Tucker EE, Allen CJ, Farrell DE, Harris JW. Efficacy of deferoxamine in preventing complications of iron overload in patients with thalassemia major. *N Engl J Med* 1994;331:567–73.

6. Fischer R, Harmatz PR. Non-invasive assessment of tissue iron overload. *Hematology Am Soc Hematol Educ Program* 2009:215–21.

7. Olivieri NF, Brittenham GM. Iron-chelating therapy and the treatment of thalassemia. *Blood* 1997;89:739–61.

8. Thalassaemia International Federation. *Guidelines for the Clinical Management of Thalassaemia*, 2nd rev. edn. 2008. http://www.thalassaemia.org.cy/wp-content/uploads/pdf/educational-programmes/Publications/Guidelines%20-%202008%20/Thalassaemia%20Guidelines%20ENGLISH.pdf.

9. Kohgo Y, Ikuta K, Ohtake T, Torimoto Y, Kato J. Body iron metabolism and pathophysiology of iron overload. *Int J Hematol* 2008;88:7–15.

10. Olivieri NF, Brittenham GM, Matsui D, Berkovich M, Blendis LM, Cameron RG, McClelland RA, Liu PP, Templeton DM, Koren G. Iron-chelation therapy with oral deferiprone in patients with thalassemia major. *N Engl J Med* 1995;332:918–22.

11. Cappellini MD, Cohen A, Piga A, et al. A phase 3 study of deferasirox (ICL670), a once-daily oral iron chelator, in patients with beta-thalassemia. *Blood* 2006;107:3455–62.

12. Porter J, Galanello R, Saglio G, et al. Relative response of patients with myelodysplastic syndromes and other transfusion-dependent anaemias to deferasirox (ICL670): a 1-yr prospective study. *Eur J Haematol* 2008;80:168–76.

13. Pulyel M, Sposto R, Berdoukas VA, Hofstra TC, Nord A, Carson S, Wood J, Coates TD. Ferritin trends do not predict changes in total body iron in patients with transfusional iron overload. *Am J Hematol* 2014;89:391–4.

14. Anderson LJ, Holden S, Davis B, et al. Cardiovascular T2-star (T2*) magnetic resonance for the early diagnosis of myocardial iron overload. *Eur Heart J* 2001;22:2171–9.

15. Eghbali A, Taherhamadi H, Shahbazi M, Bagheri E, Brahami L. Association between serum ferritin level, cardiac and hepatic T2-star MRI in patients with major beta-thalassemia. *Iran J Ped Hematol Oncol* 2014;4:17–21.

16. Majd Z, Haghpanah S, Ajami GH, Matin S, Namazi H, Bardestani M, Karimi M. Serum ferritin levels correlation with heart and liver MRI and LIC in patients with transfusion-dependent thalassemia. *Iran Red Crescent Med J* 2015;17:e24959.

17. Ho P, Tay L, Lindeman R, Catley L, Bowden D. Australian guidelines for the assessment of iron overload and iron chelation in transfusion-dependent thalassemia major, sickle cell disease and other congenital anaemias. *Intern Med J* 2011;41:516–24.

18. Angelucci E, Barosi G, Camaschella C, Cappellini MD, Cazzola M, Galanello R, Marchetti M, Piga A, Tura S. Italian Society of Hematology practice guidelines for the management of iron overload in thalassemia major and related disorders. *Haematologica* 2008;93:741–52.

19. Cappellini MD, Cohen A, Porter J, Taher A, Viprakasit V. Guidelines for the management of transfusion dependent thalassaemia (TDT). *Thalassaemia International Federation* 2014; No. 20:3rd edn.

20. 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.

21. Othlof AW, Sijens PE, Kreeftenberg HG, Kappert P, Irwan R, van der Jagt EL, Oudkerk M. Relation between serum ferritin levels and iron levels under the influence of iron chelation therapy. *Int J Hematol* 2010;92:583–8.

22. Origa R, Galanello R, Ganz T, Giagi N, Maccioni L, Faa G, Nemeth E. Liver iron concentrations and urinary hepcidin in β-thalassemia. *Haematologica* 2007;92:686–94.

23. 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.

24. Ang AL, Shah FT, Davis BA, Thomas A, Murugachandran G, Kumuradevan J, Garbowski MW, Porter JB. Deferiprone is associated with lower serum ferritin (SF) relative to liver iron concentration (LIC) than deferoxamine and deferasirox- implications for clinical practice. *Blood* 2010;116:abst 4246.

25. Fischer R, Longo F, Nielsen P, Engelhardt R, Hider RC, Piga A. Monitoring long-term efficacy of iron chelation therapy by deferiprone and desferrioxamine in patients with β-thalassemia major: application of SQUID biomagnetic liver susceptometry. *Br J Haematol* 2003;121:938–48.

26. Worwood M, Cragg SJ, Jacobs A, McLaren C, Ricketts C, Economidou J. Binding of serum ferritin to concanavalin A: patients with homozygous beta thalassaemia and transfusional iron overload. *Br J Haematol* 1980;46:409–16.

27. Garbowski MW, Carpenter JP, Smith G, Roughton M, Alam MH, He T, Pennell DJ, Porter JB. Biopsy-based calibration of T2* magnetic resonance for estimation of liver iron concentration and comparison with R2 Ferriscan. *J Cardiovasc Magn Reson* 2014;16:40.

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

29. St Pierre TG, El-Beshlawy A, Elalfy M, Al JA, Al ZK, Daar S, Habr D, Kriemler-Krahn U, Taher A. Multicenter validation of spin-density projection-assisted R2-MRI for the noninvasive measurement of liver iron concentration. *Magn Reson Med* 2014;71:2215–23.

30. Wood JC, Ghufr N. Magnetic resonance imaging assessment of excess iron in thalassemia, sickle cell disease and other iron overload diseases. *Hemoglobin* 2008;32:85–96.

31. Westwood M, Anderson LJ, Pennell DJ. Treatment of cardiac iron overload in thalassemia major. *Haematologica* 2003;88:481–2.
32. Cappellini MD, Porter JB, El-Beshlawy A, et al. Tailoring iron chelation by iron intake and serum ferritin: the prospective multicenter EPIC study of deferasirox in 1744 patients with various transfusion-dependent anemias. Haematologica 2010;95:557–66.

33. Porter JB, Elalfy MS, Taher AT, Aydinok Y, Chan LL, Lee SH, Sutcharitchan P, Habr D, Martin N, El-Beshlawy A. Efficacy and safety of deferasirox at low and high iron burdens: results from the EPIC magnetic resonance imaging substudy. Ann Hematol 2013;92:211–9.

34. St Pierre TG, Clark PR, Chua-anusorn W. Single spin-echo proton transverse relaxometry of iron-loaded liver. NMR Biomed 2004;17:446–58.

35. St Pierre TG, Clark PR, Chua-anusorn W, Fleming AJ, Jeffrey GP, Olynyk JK, Pootrakul P, Robins E, Lindeman R. Noninvasive measurement and imaging of liver iron concentrations using proton magnetic resonance. Blood 2005;105:855–61.

36. Angelucci E, Muretto P, Nicolucci A, et al. Effects of iron overload and hepatitis C virus positivity in determining progression of liver fibrosis in thalassemia following bone marrow transplantation. Blood 2002;100:17–21.

37. Hershko C, Konijn AM, Nick HP, Breuer W, Cabantchik ZI, Link G. ICL670A: a new synthetic oral chelator: evaluation in hypertransfused rats with selective radiolabeled probes of hepatocellular and reticuloendothelial iron stores and in iron-loaded rat heart cells in culture. Blood 2001;97:1115–22.

38. Porter JB, Shah FT. Iron overload in thalassemia and related conditions: therapeutic goals and assessment of response to chelation therapies. Hematol Oncol Clin North Am 2010;24:1109–30.

39. Glickstein H, El RB, Shvartsman M, Cabantchik ZI. Intracellular labile iron pools as direct targets of iron chelators: a fluorescence study of chelator action in living cells. Blood 2005;106:3242–50.

40. Novartis Pharmaceuticals. EXJADE® (deferasirox) US Prescribing Information, 2015. http://www.pharma.us.novartis.com/product/pi/pdf/exjade.pdf.

41. Farmaki K, Tzoumari I, Pappa C, Chouliaras G, Berdoukas V. Normalisation of total body iron load with very intensive combined chelation reverses cardiac and endocrine complications of thalassaemia major. Br J Haematol 2010;148:466–75.

42. Voskaridou E, Komninaka V, Karavas A, Terpos E, Akianidis V, Christoulas D. Combination therapy of deferasirox and deferoxamine shows significant improvements in markers of iron overload in a patient with beta-thalassemia major and severe iron burden. Transfusion 2014;54:646–9.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Figure S1.** Relationship between serum ferritin and LIC at study baseline* (A, C) and change from study baseline (B, D) by study baseline iron burden categories of serum ferritin <4000 or ≥4000 ng/mL (A, B) and LIC <15 or ≥15 mg Fe/g dw (C, D). A higher positive correlation was observed between serum ferritin and LIC at baseline (A), and for change from baseline (B), when baseline serum ferritin was <4000 ng/mL (grey) than when baseline serum ferritin was ≥4000 ng (black). A higher positive correlation was observed between serum ferritin and LIC at baseline (C), and for change from baseline (D), when baseline LIC was <15 ng/mL (grey) than when baseline LIC was ≥15 ng/mL (black).

**Table S1.** ROC analysis to evaluate serum ferritin levels that predict an LIC threshold of ≥20 mg Fe/g dw.

**Table S2.** Transfusional iron intake and deferasirox dose by serum ferritin and LIC response.