A decisional algorithm to start iron chelation in patients with beta thalassemia

Since the implementation of deferrioxamine for the treatment of thalassemia major, it has been customary to initiate chelation after the first 10-20 transfusions or when serum ferritin reached over 1000 mcg/L.[1] However, in non-chronically-transfused thalassemia patients, ferritin hepatosecretion does not match liver iron accumulation and neither is serum ferritin representative of extrahepatic siderosis.[1-4] These inherent limitations in viewing serum ferritin as a reliable marker of iron overload have led us to consider other plasma factors that might be more pertinent to the etiopathology of iron overload and relevant for initiating treatment and assessing its long-term efficacy.[5] Here, we considered labile plasma iron (LPI), the major redox-active and readily chelatable fraction of non-transferrin-bound iron (NTBI), as a more direct marker of impending tissue iron overload and, therefore, as a potential indicator for the initiation of chelation therapy.[6] We based this consideration on the fact that detectable LPI levels (>0.2 μM) were found only in patients with transferrin saturation over 70%, that its components infiltrate cells by non-iron regulated routes causing labile cell iron to rise to toxic levels, and that they are direct targets of chelation in plasma as in cells.[7-14]

The specific aim of this study was to prospectively evaluate the appearance of LPI in children with thalassemia major and its correlation with the most commonly used parameters for determining the onset of chelation therapy, namely the number of transfusions that patients have undergone and the serum levels of ferritin, but also with the number of grams of administered red blood cells (RBCs) and with the percentage of transferrin saturation (TSAT).

We collected 79 blood samples from 14 patients aged 0.3-4 years; 5 patients were sampled prior to their first transfusion, while the remaining 9 patients, who had already been engaged in transfusion programs and had undergone less than 12 transfusions, had 2 consecutive null LPI levels at initial testing. Blood samples were taken on the day of transfusion, an average 1.3 months apart. During the 16 months of follow up, average available pre-transfusional Hb was 9.4 g/dL (standard deviation (SD) = 0.49), mean blood requirement was 99.3 mL/kg (SD = 57.2), while mean age at first transfusion was 22 months (SD = 12). Serum ferritin was determined by an automated chemiluminescence immunoassay analyzer (IMMULITE 2000®) and TSAT was calculated as the ratio of serum iron to the total iron-binding capacity (transferrin measured by immuno-turbidometry x 1.25, assuming that 1 mg of transferrin links 1.25 μg of iron). LPI was measured by the FeROS kit (Aferrix, Tel Aviv, Israel) adapted for high throughput screening as the more sensitive version, referred to as eLPI, is applicable especially for analyzing samples from polytransfused non-chelated patients.[7]

All statistical procedures were performed using SPSS v.18.0 software (SPSS Inc., Chicago, Illinois, USA) with a critical alpha of 0.05.

For correlation purposes, we calculated the coefficient of determination of linear regression for each of the four tested parameters using single eLPI values. eLPI values at given sampling times correlated highly with TSAT (r^2=0.487; P<0.001) and hardly at all with either the number of transfusions, the amount of transfused RBCs, or serum ferritin (r^2=0.165, P=0.001; r^2=0.160, P=0.001; r^2=0.139, *P*<0.001, respectively) (Figure 1). In previous studies, we had generally considered LPI values below 0.2 as non-significant, largely because of the variability found in LPI and eLPI assays.[10] However, for the present study, we also measured values of 30 normal children and 43 normal adults randomly selected as a cohort of healthy controls, and did not find any detectable (>0 μM) LPI or eLPI. Therefore, for the analyses made in this study, we chose the more restrictive approach (significant when >0 μM).

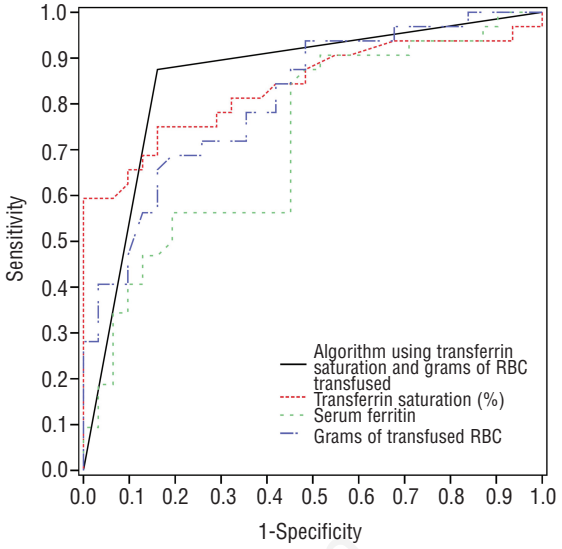

Figure 1. Correlation between single values of eLPI and (a) TSAT: %transferrin saturation, (b) serum ferritin, (c) grams of transfused RBC, (d) number of RBC transfusions.

To decide the basis on which to start chelation therapy, we ranked the measured parameters in terms of their predictive ability towards the appearance of excess plasma iron (i.e. first eLPI non-null value per patient) by using the receiver operating characteristic (ROC) analysis. Area under the curve (AUC) was used to evaluate sensitivity and specificity for each of the parameters or their combination (Figure 2).

According to ROC curve analysis, the appearance of an eLPI positive value is best predicted by TSAT and number of grams of transfused RBC (AUC=0.837, *P*<0.001; AUC=0.810, *P*<0.001, respectively), while the number of RBC transfusions and serum ferritin are worse predictors (AUC=0.758, *P*<0.001; AUC=0.720, *P*=0.003, respectively) (Figure 2).

To check whether a combination of parameters would be more informative and render the prediction more robust towards external elements affecting the values measured, we developed a decisional tree using a Classification and Regression Trees method. This analysis assessed that a combination including number of grams of RBCs and TSAT parameters provides the most accurate prediction of eLPI appearance (AUC=0.857, *P*<0.001) (Figure 2). Effectively, setting optimal thresholds at 1050 grams of RBCs transfused and successively applying a threshold at 90% of TSAT, we correctly predict eLPI values for 84.4% of samplings before eLPI is seen (null observed eLPI value) and 88.2% after (all samplings considered positive as eLPI has already been seen).

Using the same method, we also determined the best predictive threshold for each single parameter: 75% for TSAT (AUC=0.763, *P*<0.001), 1050 grams of transfused

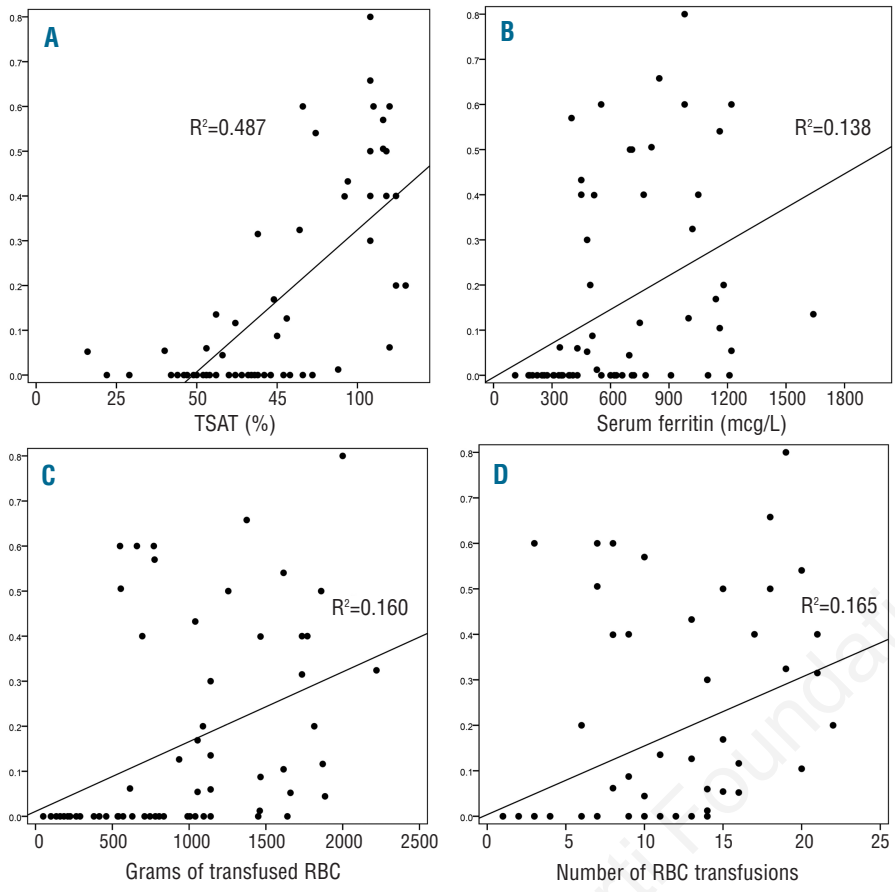

Figure 2. ROC curve for the prediction of eLPI appearance by TSAT, grams of RBC, serum ferritin and the combination of transferrin saturation with amount of RBC transfused.

RBC (AUC=0.747, *P*=0.001) and 400 ng/mL for serum ferritin (AUC=0.679, *P*=0.014), which is significantly lower than that commonly used (1000 ng/mL). The predictor "appearance of a TSAT of 75% or over" (i.e. all samplings considered positive after first TSAT≥75%) was more predictive but still slightly less accurate than combined parameters (AUC=0.830, *P*<0.001).

In conclusion, the parameters and thresholds commonly used to start chelation therapy do not hold true in our cohort. TSAT seems to best predict eLPI single values, but a combination of TSAT with the amount of RBCs transfused predicts eLPI appearance with higher accuracy. Thus, to the extent that eLPI denotes impending iron overload, and within the constraints of this exploratory study, we propose that chelation therapy should be started when more than 1000 grams of RBCs have been transfused or when TSAT is over 90% in patients with less than 1000 grams of RBCs transfused. This needs to be further assessed by larger prospective studies based on repeated measurements of TSAT.

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[5]This work is dedicated to the memory and in honor of Renzo Galanello, who inspired and almost completed the study at the end of an exemplary career as clinician and researcher.

Key words: thalassemia major, iron chelation, start, percentage transferrin saturation, RBC.

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