


Soluble form of transferrin receptor-1 level is associated with the age at first diagnosis and the risk of therapeutic intervention and iron overloading in patients with non-transfusion-dependent thalassemia

Paolo Ricchi¹  · Antonella Meloni² · Silvia Costantini¹ · Anna Spasiano¹ · Tiziana Di Matola³ · Alessia Pepe² · Patrizia Cinque¹ · Aldo Filosa¹

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Abstract We retrospectively evaluated the relationship between serum transferrin receptor-1 (sTfR1) and some fundamental events in the life and the management (the age at diagnosis, the age at the first red blood cells transfusion, the age at splenectomy, and the overall need of chelation therapy) of 111 patients with non-transfusion-dependent thalassemia (NTDT) subdivided in four genetic entities: patients with homozygous or compound heterozygous state for β -thalassemia, patients with triplicated α genotype associated with β heterozygosity, patients with deletional HbH, and patients with the combination of a β defect plus a β chain variant. We found that the group with homozygous or compound heterozygous state for β -thalassemia had the highest sTfR1 levels and that the presence of increased sTfR1 levels (>5 times normal) was associated with a complex and severe history of disease requiring splenectomy, occasional red blood cells transfusions, and early start and continuous iron chelation therapy.

The complexity in the management of NTDT patients is an emerging issue due to the wide heterogeneity of clinical behavior. Our data indicate that the measurement of sTfR1

levels, a common laboratory test, could contribute to correctly stratify disease history and the iron chelation strategy in NTDT patients.

Keywords Thalassemia · Biomarkers · Genotype · Erythropoiesis

Introduction

Non-transfusion-dependent thalassemias (NTDT) are a wide cluster of inherited disorders grouped together only by the fact that their phenotypic expression rarely or occasionally requires regular transfusions. However, because of the extensive presence of the disease worldwide, there is both great variety of genotypes and phenotypes [1, 2]. NTDT is a complex disease ranging from individuals who are completely asymptomatic until adult life to patients who require early regular red blood cell (RBC) transfusions for growth failure, skeletal abnormalities, exercise intolerance, or progressive splenomegaly [3, 4]. This heterogeneity of patients with NTDT is an emerging cause of complex management and treatment of the disease. Therefore, management strategies are extremely different and range from simple observation to commitment to splenectomy and regular RBC transfusions, but involve also the use of hemoglobin inducers and iron chelating agents.

Besides, among different genetic entities of NTDT, the lack of a clear genotype–phenotype relationship further complicates this complex and extensive scenario in clinical practice. The severity of the disease depends on the variable degree of peripheral hemolysis, ineffective erythropoiesis, and iron loading [5]. Thus, markers linked to these fundamental pathogenetic events such as non-transferrin-bound iron (NTBI), liver iron concentration (LIC), growth differentiation factor-

✉ Paolo Ricchi
pabloricchi@libero.it

¹ U.O.S.D. Rare Red Blood Cells Diseases, Azienda Ospedaliera di Rilievo Nazionale “A.Cardarelli”, Via A.Cardarelli 9, 80131 Naples, Italy

² Magnetic Resonance Imaging Unit, Fondazione G. Monasterio, CNR-Regione Toscana, Pisa, Italy

³ UOC Clinical Pathology, AORN Monaldi-Cotugno-CTO, Naples, Italy

15 (GDF-15), and fetal hemoglobin (HbF) have been used to score the clinical severity of NTDT patients and could help to manage these patients [6–9]. LIC has been evaluated as a good marker of disease severity in patients naive to iron chelation. Patients with LIC values ≥ 5 mg Fe/g dry are considered at a higher morbidity risk [7]. However, apart from the rare conditions to observe naive patients or to not treat patients, LIC is modifiable by iron chelation therapy and is also quite expensive [10], therefore limiting its use as a biomarker. On the other hand, HbF and GDF15 levels have been tested as potential parameters of morbidity only in patients with β -thalassemia intermedia. Very recently, an exhaustive study comparing a range of biomarkers of iron metabolism and erythropoiesis, accurately explored the pathophysiological processes associated with the expanded erythroid and disordered iron metabolism across several NTDT genetic entities; these authors for the first time discussed several interesting relationships between all parameters [11]. However, as authors stated, less iron-overloaded patients were excluded from this study and the prospective assessment of biomarkers reciprocal trend following iron chelation therapy was lacking. On the other hand, we found that in NTDT, the soluble transferrin receptor-1 (sTfR1), that fully reflects the marrow erythropoietic activity, had not only a striking diagnostic accuracy in predicting the risk of extramedullary hematopoiesis (EMH), but also in scoring disease severity, being homozygous or compound heterozygous state for β -thalassemia the group with the highest rate of complications and highest sTfR1 levels [12, 13].

In this study, in order to further reinforce the relevance of this marker, we retrospectively evaluated the relationship between sTfR1 and some milestones in the course of disease and in the management of patients with NTDT such as the age at diagnosis, age at the first RBC transfusion, age at splenectomy, and the overall need of iron chelation. Furthermore, in a subgroup of patients, we performed a comparative prospective analysis between sTfR1 and several parameters of iron loading such as serum ferritin and LIC values.

Patients and methods

Study population

All NTDT patients followed at AORN Cardarelli center in Naples, Italy, were evaluated. Patients with hemoglobin S/ β -thalassemia were excluded from the analysis.

A group of our patients was enrolled in the Myocardial Iron Overload in Thalassemia (MIOT) network, constituted by 70 thalassemia centers and nine magnetic resonance imaging (MRI) centers where MRI exams are performed using homogeneous, standardized, and validated procedures [14].

At our center, together with a periodical assessment of liver iron overload by evaluation of biochemical and instrumental

parameters in order to decide when to start iron chelation therapy and monitor its efficacy, NTDT patients have been undergoing since 2007 the measurement of the sTfR1 levels twice a year and far from occasional RBC transfusions.

The study complied with the Declaration of Helsinki. All patients gave written informed consent to the protocol. The study was approved by the Ethics Committee of the Cardarelli Hospital, Napoli.

sTfR1 measurement

sTfR1 was measured with a commercially available kit using N Latex sTfR1 and BN II System (Siemens Healthcare Diagnostics) nephelometric technique. Reference ranges were 0.76–1.76 mg/L.

Iron overload assessment

All patients enrolled in the MIOT network underwent a periodic (every 18 ± 3 months) MRI scan for the assessment of iron overload using a 1.5 T scanner.

The T2* technique was used for iron overload assessment. Its reproducibility and its transferability within the MIOT network had been previously demonstrated [15]. For the heart, a multislice multiecho T2* approach was used. Three parallel short-axis views (basal, medium, and apical) of the left ventricle (LV) were obtained [16–19]. For the liver, a single mid-transverse slice was obtained using a T2* gradient-echo multiecho sequence [18]. T2* images analysis was performed using a custom-written, previously validated software program (HIPPO MIOT®) [18]. The software provided the T2* value on each of 16 LV segments [20]. The global heart T2* value was obtained by averaging all segmental T2* values. The value of 20 ms was used as “conservative” normal value for all 16 segments and for the global T2* value [21]. For the liver, the T2* value was calculated in a large region of interest (ROI) of standard dimension, chosen in a homogeneous area of parenchyma without blood vessels [22]. Care was taken to avoid the ROI placement in the posterior lateral (VII) and medial (VIII) segments, more prone to susceptibility artifacts [23]. Liver T2* values were converted into LIC values using the calibration curve introduced by Wood et al. [24].

Statistical analysis

All data were analyzed using SPSS version 13.0 statistical package. Continuous variables were described as mean \pm standard deviation (SD). Categorical variables were expressed as frequencies and percentages.

The normality of distribution of the parameters was assessed by using the Kolmogorov-Smirnov test.

For continuous values with normal distribution, comparisons between groups were made by independent-samples *t*

test (for 2 groups) or one-way ANOVA (for more than 2 groups). Wilcoxon's signed rank test and Kruskal-Wallis test were applied for continuous values with non-normal distribution. The Bonferroni adjustment was used in all pairwise comparisons.

Correlation analysis was performed using Pearson's test or Spearman's where appropriated.

To determine the best sTfR1 cut-offs for discriminating between two different states, the maximum sum of sensitivity and specificity was calculated from receiver-operating characteristic (ROC) curve analysis.

A 2-tailed probability <0.05 was considered statistically significant.

Results

Patients data

The characteristics of our NTD patients at the time of the last determination of sTfR1 levels are shown in Table 1. The 45.9% of patients had received occasional RBC transfusions while 54.1% was never transfused. Iron chelation therapy had been previously started in 50 patients, due to the presence of either a LIC > 5 mg Fe/g dry weight or serum ferritin levels >800 ng/mL, as established by NTD guidelines [25]. Overall, the levels of sTfR1 varied from 2 to 19 mg/L and were above the normal range in all patients.

MRI LIC values were available for 64 patients and were significantly higher in iron chelated than in non-iron chelated patients (5.48 ± 6.04 vs 2.86 ± 2.69 mg/g dw; $P = 0.002$). All patients showed a normal global heart T2* value (mean value

Table 1 Main characteristics of patients with non-transfusion-dependent thalassemia in correspondence of the last measurement of sTfR1 levels

	111 patients
Sex (M/F)	49/62
Age (years)	41.14 ± 16.05
Age at diagnosis (years)	20.28 ± 17.10
History of transfusion, N (%)	51 (45.9)
Genotypes, N (%):	
homozygosity or compound heterozygosity for beta-thalassemia	44 (39.6)
combination of β defect plus β chain variant	8 (7.2)
HbH	19 (17.1)
triplicated α genotype associated with β heterozygosity	36 (32.4)
incomplete genotype with only a mutation detected	4 (3.6)
Splenectomy, N (%)	39 (35.1)
Active chelation therapy, N (%)	50 (45)

40.75 ± 4.86 ms) and no correlation was detected between sTfR1 values and cardiac iron.

sTfR1 values were comparable between males and females (7.4 ± 3.6 mg/L vs 6.6 ± 3.6 mg/L, $P = 0.262$).

Forty-four patients showed homozygosity or compound heterozygosity for beta-thalassemia, 8 had the combination of a β defect plus a β chain variant (C, Lepore-BW, Neapolis), 19 had HbH disease, 36 had triplicated α genotype associated with β heterozygosity, and 4 had an incomplete genotype with only a mutation detected. Patients with homozygous or compound heterozygous state for β-thalassemia had significantly higher levels of sTfR1 than HbH patients and patients with triplicated α genotype associated with β heterozygosity (Fig. 1).

Correlation between sTfR1 values and milestones of management: RBC transfusion, splenectomy, and iron chelation therapy

sTfR1 values were negatively correlated to age at diagnosis ($R = -0.462$, $P < 0.0001$).

A strong negative correlation was found between sTfR1 values and age at first RBC transfusion ($R = -0.703$, $P < 0.0001$). Patients which had received at least one RBC transfusion had significantly higher sTfR1 levels (Fig. 2a). Using ROC curve analysis, a sTfR1 greater than 5.3 mg/L was able to differentiate the patients with a previous history of occasional RBC transfusions from those non-transfused with acceptable sensitivity and specificity (74.5 and 61.7%, respectively) and with an area under the curve (AUC) of 0.733 ($P = 0.001$).

sTfR1 values were significantly higher in splenectomized patients (Fig. 2b). Mean age at splenectomy was

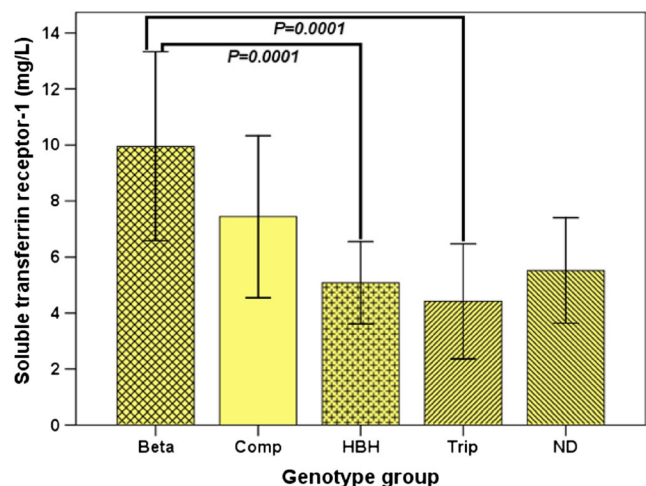


Fig. 1 Comparison of sTfR1 levels among the different genetic groups: Beta (β/β compounds), Comp (β variant/β defect), HbH, Trip (triplicated α/β) and ND (not determined, i.e., second allele undetermined)

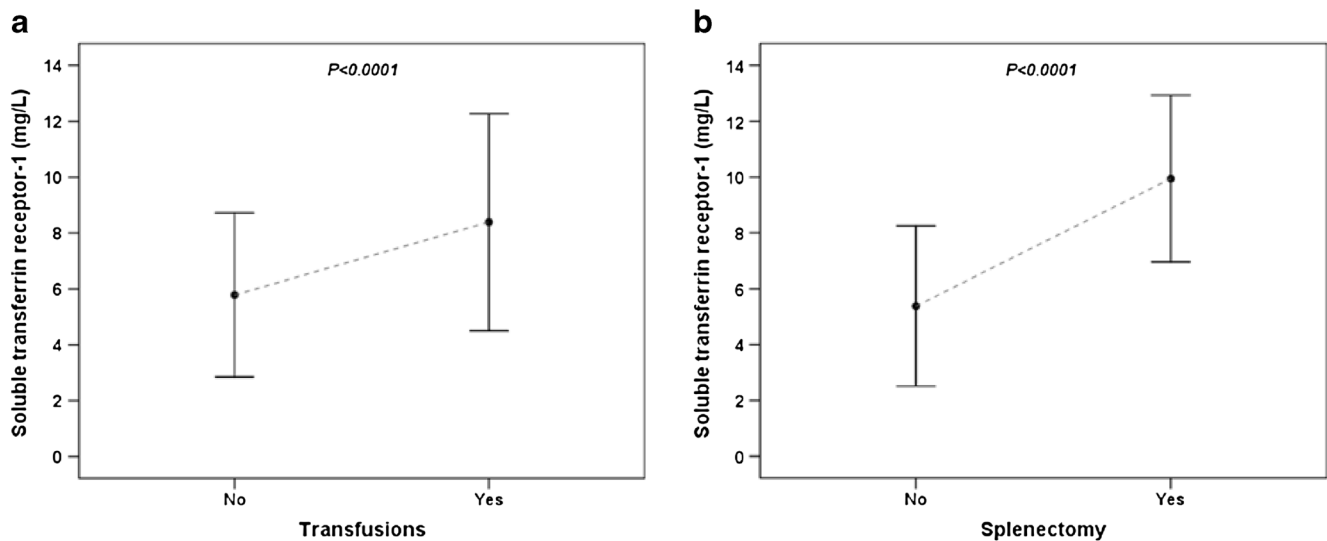


Fig. 2 Comparison of sTfR1 levels between patients without and with prior transfusions (a) and between patients with and without the spleen (b)

21.86 ± 13.90 years and sTfR1 values were negatively related to age at splenectomy ($R = -0.328$, $P = 0.044$). In unsplenectomized patients, the mean spleen diameter was 152.03 ± 29.08 mm and a significant positive correlation was found between sTfR1 values and spleen diameter ($R = 0.572$; $P < 0.0001$).

sTfR1 values were negatively correlated to age at starting iron chelation therapy ($R = -0.564$, $P = 0.044$). The relationship between the iron chelation history and sTfR1 levels was accurately explored in the 56 patients with a past and/or current iron chelation therapy which were further subdivided in: (a) patients iron chelated in the past, but not at the time of the sTfR1 determination (six), (b) patients who underwent a recent, continuous treatment (thirty-one), and (c) patients who required such a treatment in the past, but still needs a treatment for persistently and /or recurrent abnormal parameters of iron loading (nineteen). As shown in Fig. 3, patients who have never iron chelated before showed significantly lower sTfR1 values than patients under iron chelation therapy.

Correlation between sTfR1 values and iron overload parameters

Overall, sTfR1 values were significantly correlated with serum ferritin levels ($R = 0.321$, $P < 0.0001$), also in the group of never iron chelated patients ($R = 0.423$, $P = 0.001$).

Using ROC curve analysis, a sTfR1 value of 8.2 mg/L was found to be the best threshold for discriminating the presence of serum ferritin levels >800 ng/ml with a sensitivity of 69% and a specificity of 74%; the AUC was 0.67 ($P = 0.046$).

Conversely, there was no correlation between sTfR1 and LIC levels (last determination) ($R = 0.070$, $P = 0.583$), likely because most of the patients who underwent MRI had been under iron chelation therapy.

sTfR1 levels and iron overload parameters in prospective assessment.

For 39 patients, a previous evaluation of sTfR1 levels and all described iron overload parameters were available (mean time between two measurements 3.65 ± 2.13 years). This group encompasses two groups of patients: (a) patients (17, 43%) which were under a maintenance iron chelation therapy (ie, alternate days or three times/week of iron chelation or daily at reduced doses) aiming at stabilization of normal ferritin and LIC levels previously reached and (b) patients (22, 57%) which were receiving daily and/or conventional dose of iron chelation therapy to reduce iron overload. A statistically

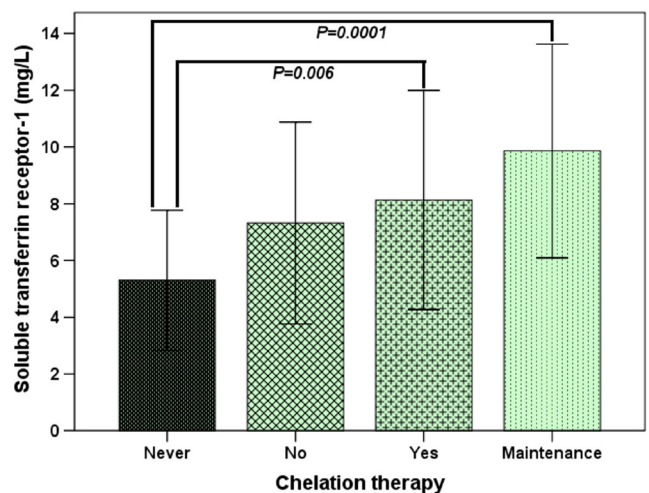


Fig. 3 Comparison of sTfR1 levels among the groups identified on the basis of the iron chelation history: *Never* (patients who have never received chelation before), *No* (patients iron chelated in the past, but not at the time of the sTfR1 determination), *Yes* (patients who underwent a recent, continuous treatment), and *Maintenance* (patients who required iron chelation treatment in the past, but still need treatment for persistently and /or recurrent abnormal parameters of iron loading)

significant decrease in ferritin levels was observed from the first to the second determination (*from* 625.74 ± 5251.1 to 489.21 ± 509.63 ng/ml, $P = 0.030$), in presence of unchanged sTfR1 levels (*from* 8.68 ± 3.36 to 8.42 ± 3.36 mg/L, $P = 0.097$) and LIC values (*from* 5.12 ± 3.91 to 4.97 ± 5.81 mg/g dw, $P = 0.096$). Considering separately the two groups, a significant decrease in serum ferritin levels was found in the group under conventional therapy (from 813 ± 621 to 572 ± 643 ng/ml, $P = 0.046$) but not in the group under maintenance iron chelation therapy (from 384 ± 196 to 382 ± 230 ng/ml, $P = 0.977$).

Discussion

In the present study, evaluating a slightly larger population of NTDT and including scantily iron-overloaded patients, we confirmed our previously published data [13] showing that homozygous or compound heterozygous state for β -thalassemia patients had the highest sTfR1 levels. The presence of increased sTfR1 levels (>5 times normal) was associated with a severe history of disease requiring splenectomy, occasional RBC transfusion, early age at diagnosis, early start, and continuous chelation therapy. Furthermore, sTfR1 levels strongly inversely correlated with age at first transfusion. Accordingly, in the study of Porter and co-workers, sTfR1 levels progressively increased in patients who received more transfusions, but the relationship between sTfR1 and splenectomy was not explored [11].

It has been well established that in patients with NTDT, as well as other iron-loading anemias, iron overload develops as a consequence of increased iron absorption [26, 27], which averages 0.011 mg/kg/day in the absence of transfusion therapy [28]; occasional transfusions also may add supplementary iron accumulation. Overall, our data on never iron chelated and chelated patients seem to suggest that sTfR1, as a good marker of expanded erythropoiesis, well reflects the amount of iron absorption in the explored NTDT subgroups. These data again well accord with seminal observation of Cazzola and co-workers who first found that sTfR1 levels were related to the degree of iron overload in 20 patients with thalassemia intermedia and that serum ferritin values above 1000 ng/ml were found only in patients with sTfR1 levels >30 mg/L, i.e., > 6 times normal [29].

These data, despite carrying the limit to be retrospective, suggest that the measurement of sTfR1 levels, may help to correctly stratify the disease history in our explored subtypes of NTDT and further support its use as a general marker of disease severity in these patients. On the other hand, all our sTfR1 measurements exceeded the normal range (0.76–1.76 mg/L); we can therefore hypothesize that, in cases where clinical, hematological and electrophoretic features suggest the diagnosis of NTDT, sTfR1 measurement with 2 mg/l as

cut-off could be used as a “screening test” to differentiate between patients with the thalassemia carrier status from those with co-heredance of classic mutations in the beta globin gene and silent, rare or unrecognized globin defect. However, further studies are needed to verify its sensitivity and specificity for this purpose.

Furthermore, our prospective data showed that sTfR1 is unchanged with iron chelation therapy when ferritin is clearly and significantly decreased; this very interesting evidence suggests that iron chelation may not positively affect erythropoiesis in NTDT patients. However, this may also be a consequence of insufficient time or dose in light of unchanged LIC and further studies involving naive and/or more heavily iron-overloaded patients should be conducted to test again this evidence.

Although the data presented herein cannot yet assist practically in making decisions on clinical management of NTDT, it may help to personalize general and iron overload follow management suggesting, perhaps from age at diagnosis, the risk of undergoing splenectomy and transfusions and of becoming iron overloaded early in life. Further studies are needed to prospectively verify these findings in larger series of untreated patients and in patients with different genetic entities of NTDT such as those with hemoglobin E/ β -thalassemia and non-deletional HbH.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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