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ORIGINAL ARTICLE

A useful relationship between the presence of extramedullary erythropoeisis and the level of the soluble form of the transferrin receptor in a large cohort of adult patients with thalassemia intermedia: a prospective study

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Abstract In thalassemia intermedia (TI), the increase in bone marrow hemopoietic activity frequently leads to extramedullary erythropoeisis (EMH), but its relationship with the soluble form of transferrin receptor (sTfR) which fully reflects the marrow erythropoietic activity, has not yet been explored. From January 2007 to December 2010, all TI patients attending at our center were prospectively enrolled to undergo sTfR assay and MRI or CT (if claustrophobic) scan evaluation for the presence of paraspinal EMH. A total of 59 patients with TI were studied; EMH involved 23 (39%) patients; overall, the concentration of sTfR varied from 2.6 to 20.6 (mean=8.7) mg/L, but in splenectomized group and in unsplenectomized group, it varied from 4.2 to 17.8 (mean±SD=9.86±3.33) mg/L and from 2.6 to 20.6 (mean±SD=7.25±3.9) mg/L, respectively with a statistically significant intergroup difference ($p<0.01$). The cutoff point at 8.6 mg/L using the ROC curve showed a sensitivity of 78.3% and a specificity of 72.2%, in predicting EMH but, in unsplenectomized subgroup, they raised to 100% and 90.9%, respectively. These data showed that in TI the level of sTfR could represent a predictive factor of EMH particularly in patients with spleen.

Keywords Thalassemia · MRI · Soluble transferrin receptor · Extramedullary erythropoeisis, splenectomy · Iron loading

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Introduction

In thalassemia intermedia (TI), ineffective erythropoiesis is the primary pathogenetic event in the development of anemia [1]. As patient's hemoglobin cannot be maintained at a sufficiently high level, an uncontrolled expansion of early erythroid progenitors arises as compensatory response. Such an increase in bone marrow activity frequently leads to expansion of the hematopoietic tissue outside the marrow and to extramedullary erythropoeisis (EMH) [2]. The most frequent position for EMH in thalassemia is paraspinally in the thorax cavity; paraspinal involvement requires particular

attention due to the potential neurologic impairment caused by spinal cord compression. As a consequence, the identification and the early diagnosis of patients affected by paraspinal EMH could prevent also such potential disabling outcome. However, only few series describe in patients with TI the exact occurrence of paraspinal EMH using magnetic resonance imaging (MRI) and not sure risk factors for its presence have yet been found. In particular, its relationship with the level of the soluble form of the transferrin receptor (soluble transferrin receptor, sTfR) that fully reflects the marrow erythropoietic activity [3] has not yet been explored. For this reason, we decided to prospectively evaluate by MRI all our patients affected by TI for the presence of EMH at paraspinal plane and to test their level of sTfR.

Material and methods

From January 2007 to December 2010, all patients affected by TI referring to our unit were prospectively considered. Out of 75 patients, a total of 59 patients agreed to be included in the study and written informed consent was provided by all patients. None of these patients was regularly transfused; however, someone had occasionally received transfusion because of surgery, pregnancy, or concomitant illnesses.

A total of 53 patients with TI were evaluated with MRI for the presence of paraspinal extramedullary hematopoiesis. Six claustrophobic patients underwent computed tomography (CT) examination.

Axial and coronal MRI scans were obtained in all patients using a Philips Eclipse system operating at 1.5 T with a phased array surface coil for spinal imaging. Spin echo pulse sequences were used to obtain T1-weighted (TR: 400, 500 ms, TE: 10, 15 ms), and T2-weighted (TR: 4,500 ms, TE: 108 ms) images with 4-mm slice thickness and 0.4-mm gap. CT scans was performed using a Philips tomoscan.

For each patient, sTfR and hemoglobin (Hb) were titred at least three and ten times, respectively, during the period of the study. sTfR, besides the determination coinciding with the radiological examination, was given throughout the study on two occasions. sTfR was investigated with a commercially available kit using N Latex sTfR and BN II System (Siemens Healthcare Diagnostics) nephelometric technique. Reference ranges were: 0.76–1.76 mg/L.

Statistical analysis

Statistical analysis was performed using Med Calc software. Results for descriptive statistics were expressed as mean± standard deviation. The Fisher's exact test was used to compare the incidence of different parameters between the two groups of patients. Student's *t* test was used to compare the difference in parametric data. A *p* value below 0.05 was considered as significant. The MedCalc statistical software package was used to drew continuous variable receiver operating characteristic (ROC) analysis

Results

A total of 59 patients with TI were studied—53 (90%) with MRI and 6 (10%) with CT, respectively. As shown in Table 1, the population included 26 males and 33 females with a median age of 39 years (range 15–75). Thirty-three

Table 1 Baseline characteristics of the patients

	Overall (*n*=59)	EMH+(*n*=23)	EMH − (*n*=36)	*p* value
Male/female	26/33	11/12	15/21	0.788883
MRI (+/−)/CT (+/−)	53 (19/34)/6 (4/2)	19 (19/0)/4 (4/0)	34 (0/34)/2 (0/2)	0.196
sTFR (mg/L) mean/SD (range)	8.71/3.8 (2.6–20.6)	11.1/4.1 (4.2–20.6)	7.18/2.66 (2.6–13)	4.55 E−05
Splenectomy (%)	33 (56%)	19 (83%)	14 (38.9)	0.0012
On chelation therapy	34 (58%)	20 (87%)	14 (39%)	0.000353
Median age (years) (range)	39.4 (14.8–75.6)	40.4 (20.6–68.8)	39.2 (14.8–75.6)	0.748
10≤Age≤20	1 (2%)	0 (0%)	1 (3%)	1.00
20<Age≤40	31 (53%)	10 (44%)	21 (58%)	0.296
40<Age≤75	27 (45%)	13 (56%)	14 (39%)	0.284
Hemoglobin (g/dL) mean/SD (range)	9.53/0.97 (7.5–12.1)	9.46/1.05 (7.5–11.6)	9.57/0.92 (8.25–12.1)	0.674
7.5≤Hb ≤9	22 (37.3%)	8 (35%)	14 (39%)	0.79
9<Hb ≤10.5	28 (47.5%)	11 (48%)	17 (47%)	1.00
Hb >10.5	9 (15.2%)	4 (17%)	5 (14%)	0.72

patients (56%) had been splenectomized at least 2 years before the study. Thirty-four (58%) were on chelation therapy because of the presence of pathologic ferritin levels and/or abnormal liver T2* value. Paraspinal EMH was found in 23 (39%) patients. The concentration of sTfR varied from 2.6 to 20.6 (mean=8.71±3.8) mg/L, but in patients with paraspinal EMH and those without it varied from 4.2 to 20.6 (mean±SD=11.1±4.1) mg/L and from 2.6 to 13 (mean±SD=7.18±2.66) mg/L, respectively, with a statistically significant intergroup difference ($p<0.01$). The area under the ROC curve was used to determine if any level of sTfR could be used to differentiate between patients with and without paraspinal EMH. As shown in Fig. 1a, the cutoff point at 8.6 mg/L of sTfR showed a sensitivity of 78.3 and a specificity of 72.2%, in predicting paraspinal EMH in all patients but, considering the unsplenectomized subgroup (Fig. 1b), the sensitivity and the specificity raised to 100% and 90.9%, respectively. It is well worth to note that in two patients with paraspinal EMH, an initial spinal cord compression was observed at MRI scan (data not shown): one was a 40-year-old splenectomized man partially symptomatic (back pain) and the other was a 22-year-old unsplenectomized asymptomatic woman, but both had very high mean levels of sTfR (20.6 and 18 mg/L, respectively).

The Fisher's exact test revealed that paraspinal EMH more frequently ($p=0.00035$) affected patients (87%) on chelation with respect to those not chelated (39%). Similarly, paraspinal EMH more frequently affected splenectomized patients with respect to unsplenectomized ones (83% vs. 38.9%, $p=0.0012$). By contrast, among different age and hemoglobin, subgroups not associated with an increased risk of paraspinal EMH were observed (Table 1). A statistical analysis was performed to determine whether in our series both splenectomy and chelation status were associated with some of the baseline characteristics of the patients.

As shown in Table 2, while patients without spleen and on chelation therapy were older as compared with those with spleen and not chelated, hemoglobin level was similar among both subgroups of patients. Differently, the concentration of sTfR varied in splenectomized subgroup and in unsplenectomized subgroup from 4.2 to 17.8 (mean±SD=9.86±3.3) mg/L and from 2.6 to 20.6 (mean±SD=7.25±3.9) mg/L, respectively with a statistically significant intergroup difference ($p<0.01$). The Student's t test revealed also that sTfR, but not hemoglobin level, was statistically significant different ($p<0.01$) between groups of patients in chelation therapy as compared with those not in chelation therapy. The area under the ROC curve was used again to establish if any level of sTfR could be used to differentiate between patients receiving or not receiving chelation therapy. Interestingly, as shown in Fig. 1c, ROC curve analysis suggested that the optimum sTfR level cutoff points was 8.15 mg/L, with sensitivity and specificity of 82.4% and 84%, respectively, in predicting the need of chelation therapy.

Discussion

EMH develops, as a compensatory mechanism, in response to insufficient erythrogenesis and its paraspinal location in TI patients, has been described in 11–15% of cases [4]; however, paraspinal EMH is usually discovered incidentally and its occurrence has not been related to any clinical or biochemical patient's parameter. To early identify patients with paraspinal EMH, it is an important challenge that

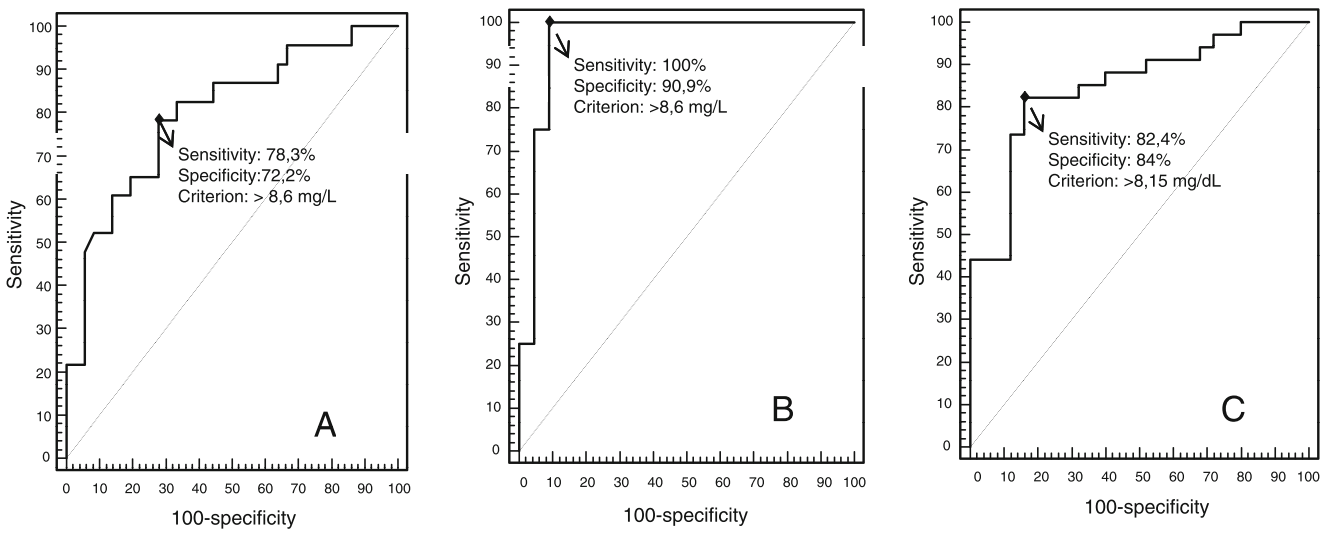

Fig. 1 Graphs show receiver operating characteristic (ROC) curves for sTfR level (in milligrams per liter) and patients with EMH at radiologic scan (**a** represents data from all patients, **b** data only from those with spleen) and patients on chelation therapy (**c**)

Table 2 Statistical analysis of relationship between some of the baseline characteristics of the patients

	Overall (n=59)	With spleen (n=26)	Without spleen (n=33)	p value	On chelation (n=34)	Not chelated (n=25)	p value
Male/female	26/33	8/18	18/15	0.112	17/17	9/16	0.304
sTfR mean/SD (range)	8.71/3.8 (2.6–20.6)	7.25/3.9 (2.6–20.6)	9.86/3.3 (4.2–17.8)	0.008	10.5/3.69 (4.2–20.6)	6.27/2.38 (2.6–11)	5.54 E−06
Age median (years) (range)	39.4 (14.8–75.6)	31.7 (14.8–75.6)	42.9 (20.6–70.6)	0.007	41.1 (24.7–70.6)	31.5 (14.8–75.6)	0.020
Hemoglobin (g/dL) mean/SD (range)	9.53/0.97 (7.5–12.1)	9.5/0.79 (8.25–11.1)	9.55/1.11 (7.5–12.1)	0.866	9.6/1.11 (7.5–12.1)	9.4/0.74 (8.25–11)	0.477

would also allow to correctly manage cases with masses within the spinal canal. Although quite expensive, MRI is considered the gold standard for the diagnosis of paraspinal EMH. However, despite the presence of a large number of case reports in TI patients [5–7], because of its rarity, there are no guidelines for establish which patient should undergo MRI assessment also if asymptomatic [8]. Our data showed both that paraspinal EMH affected almost half of our TI patients and that the measurement of sTfR had an attractive diagnostic accuracy in predicting the risk of paraspinal EMH particularly in patients with spleen. Interestingly, the two patients with spinal cord compression had particularly high levels of sTfR; thus, based on our data, the detection of greatly increased level of sTfR could represent an indication for undergo MRI scan because of the suspect of spinal cord compression.

The observed incidence of paraspinal EMH is comparable to that previously reported in a similar Italian study [9] but quite distant from that recently described in the "Optimal care study" where, perhaps, the use of a non MRI-based screening may have underestimated its incidence [2]. On the other hand, ROC curve suggested a level of 8.6 mg/L to be the best cutoff for predicting paraspinal EMH. Considering data in Fig. 1, we hypothesize that the increased amount of sTfR associated with splenectomy may have caused a drop in test sensitivity and specificity (from 100% to 78.3% and from 90.9% to 72.2%, respectively). Further studies are needed to confirm and refine this relation including in the radiologic scan also the evaluation of EMH arising from all other districts of the body [10–13]. However, we found also in our series that the level of sTfR strongly correlated with the need of chelation therapy. These data fully accord with previous observation that the measurement of sTfR may be useful in predicting the risk of iron overload in individual patients with congenital anemia with ineffective erythropoiesis [14].

Accordingly to the previous cited reports [2, 9], our data showed also that splenectomy was a risk factor for the presence of paraspinal EMH further stimulating the speculation on the close association between iron metabolism and erythropoiesis expansion and the impact of splenectomy on their status [15]. In fact, in the past, several reports and clinical observations have suggested that splenectomy in TI can contribute to an increased susceptibility to iron loading [16, 17]. The higher incidence of iron overload-related complications in splenectomized patients have prompted to consider the intact spleen as a reservoir of excess iron with possible scavenging effect on iron-free fractions, including non-transferrin-bound iron [18]. But, in thalassemia intermedia, the enlarged spleen has been indicated also as the site for extramedullary erythropoiesis which likely provides a pool for quiescent immature

erythroid precursors [19]. Furthermore, the levels of sTfR are higher among splenectomized patient with respect to those observed in unsplenectomized ones reflecting an increased ineffective erythropoiesis following splenectomy [20]. Based on these findings, we hypothesize that splenectomy, as established by the increased amounts of sTfR, may have affected the degree of ineffective erythropoiesis thus modifying several known, such as growth differentiation factor 15 (GDF15), and unknown "erythroid regulators" that control both the tendency to develop EMH and iron loading [15, 21]. Although such a relationship appears attractive, we do not know both the onset and the chronology of paraspinal EMH and of iron loading in our patients, and we cannot rule out that previous splenectomy may simply had selected older patients or patients that would have been more prone to develop such complications.

In conclusion, our data, despite not conclusive for developing a risk stratification model for EMH, may suggest to test sTfR in all patients affected by thalassemia intermedia; in fact, its strong association also with the risk to be on chelation therapy, could lead to consider it as a biochemical marker of severity of thalassemia intermedia.

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