

## SOLUBLE TRANSFERRIN RECEPTOR FOLLOWING BONE MARROW TRANSPLANTATION FROM DONORS HETEROZYGOUS FOR $\beta$ THALASSEMIA

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### ABSTRACT

This study analyzes the serum transferrin receptor (sTfR) levels in a series of 184 ex-thalassemic patients with a follow-up of 1 to 9 years after bone marrow transplantation (BMT) for homozygous  $\beta$  thalassemia. A significant inverse correlation between sTfR and Hb levels was observed ( $r = -0.36$ ,  $p < 0.001$ ). Patients who received the marrow from an HLA-identical sibling donor heterozygous for  $\beta$  thalassemia displayed significantly higher levels of sTfR than patients transplanted from a normal sibling donor ( $p < 0.001$ ). A cut-off value of 2600 ng/mL of sTfR was established. Only 3 out of 56 (5%) patients who received the marrow from a normal sibling, reached a sTfR value above the cut-off level, while 64 out of 128 (50%) patients transplanted from a heterozygous sibling donor showed sTfR values  $> 2600$  ng/mL ( $p < 0.001$ ). These results suggest that the level of sTfR helps to identify ex-thalassemic patients with enhanced or normal erythropoietic activity among those transplanted from HLA-identical sibling donors heterozygous for  $\beta$  thalassemia. The physiologic and clinical significance of different patterns of sTfR levels in ex-thalassemic patients with  $\beta$  thalassemia trait deserves to be investigated.

Key words: soluble transferrin receptor,  $\beta$  thalassemia, bone marrow transplantation, ex-thalassemic

Bone marrow transplantation (BMT) in thalassemia is currently performed using an HLA-identical sibling donor, heterozygous for  $\beta$  thalassemia, and results in sustained marrow engraftment.<sup>1</sup> In this case, the homozygous  $\beta$  thalassemic patient acquires the donor's pattern of globin chain synthesis.<sup>2</sup> Only a slight decrease in hemoglobin values with low MCH is observed. The patient, while, cured of the genetic defect, retains the residual signs of organ damage due to iron overload and dysfunction acquired during the pretransplant years. We call such an individual *ex-thalassemic*.<sup>3</sup>

The serum transferrin receptor (sTfR) reflects the rate of erythropoiesis; elevated levels occur in patients with iron deficiency anemia, auto-

immune hemolytic anemia, polycythemia and thalassemia.<sup>4,5</sup>

Patients with heterozygous  $\beta$  thalassemia have increased erythroid marrow activity,<sup>6</sup> and patients transplanted from heterozygous sibling donors would be expected to have enhanced erythropoietic activity.

In order to test this hypothesis we measured the sTfR levels of 184 marrow recipients with well-functioning graft and a follow-up of 1 to 9 years after BMT for homozygous  $\beta$  thalassemia.

One hundred and twenty-eight patients received marrow from an HLA-identical heterozygous sibling donor and 56 from normal a sibling donor (Table 1).

sTfR was measured by a commercially available enzyme immunoassay (EIA) Kit (Amgen

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Table 1. Relevant laboratory measurements of 184 patients transplanted from an HLA-identical sibling donor, normal or heterozygous for  $\beta$  thalassemia.

	heterozygous donor (n=128)	normal donor (n=56)
sTfR (ng/mL)	3113 $\pm$ 1796	1975 $\pm$ 1070
Hb (g/dL)	11.4 $\pm$ 1.4	13.1 $\pm$ 1.5
Hct (%)	35.4 $\pm$ 3.7	38.2 $\pm$ 3.6
MCH (pg)	21.1 $\pm$ 1.1	28.8 $\pm$ 1.8
$\alpha/\beta$ ratio	2.2 $\pm$ 0.2	1.0 $\pm$ 0.1

Values are means $\pm$ SD.

Diagnosics, Oaks, CA; Technogenetics, Italy).

The sensitivity of the EIA was determined by analysis of the variability of the 0 ng/mL standard. The O.D. (450 nm) three standard deviations above the 0 ng/mL standard was determined to be equivalent to 0.2 ng/mL when calculated from the sTfR standard curve. Each sample was run in duplicate; the between-assay variability was 5%.

Fifty healthy subjects of both sexes and comparable ages were used as controls. Serum samples were prepared by centrifugation at 4°C for 20 min and stored at -20°C until assayed within 4 weeks.

Statistical analyses were performed using Student's t-test. The  $r$  correlation coefficient between Hb and log sTfR was computed in a least squares regression equation. P values reported are two sided.

Table 1 shows the relevant laboratory parameters of the 184 ex-thalassemic patients studied.

sTfR levels were higher in patients transplanted from heterozygous donors than in patients who received marrow from normal sibling donors ( $p < 0.001$ ).

Hb, Hct, MCH were lower in the heterozygous recipients, reflecting the presence of the  $\beta$  thalassemic trait. The  $\alpha/\beta$  globin chain synthesis ratio in recipients corresponded to the values of their respective heterozygous or normal donors (data not shown).

Figure 1 displays the regression line between sTfR and Hb levels. A significant inverse correlation was observed between these two parameters ( $r = -0.36$ ,  $p < 0.001$ ).

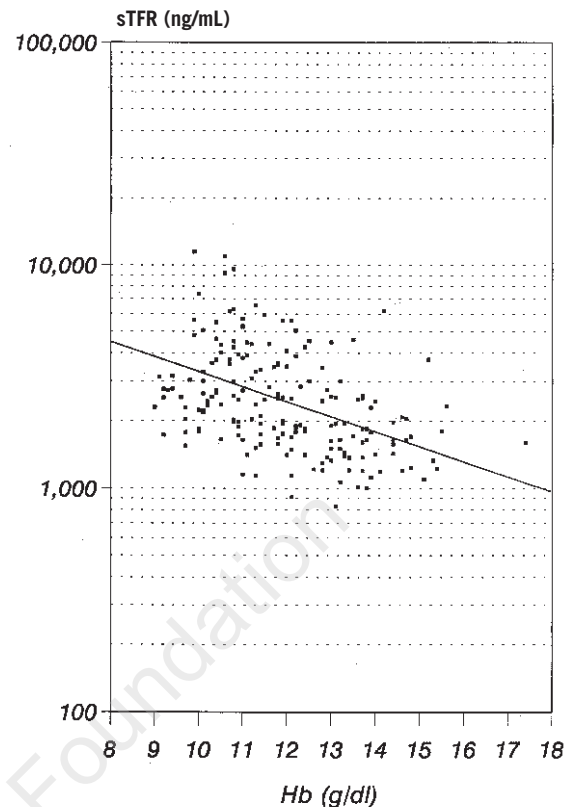


Figure 1. Relationship between Hb and sTfR measurements.

Despite the high  $p$  value, the correlation is relatively weak, probably because it reflects different patterns of erythropoiesis. In fact, patients heterozygous for  $\beta$  thalassemia show various degrees of ineffective erythropoiesis, as is reflected by their Hb decrease and low MCH.

Assuming a cut-off level of 2600 ng/mL, obtained at 1 SD above the mean of the sTfR values of 50 normal volunteers comparable for sex and age, 64 out of 128 (50%) patients who received heterozygous marrow showed sTfR levels  $>2600$  ng/mL, while only 3 of the 56 (5%) patients who received normal marrow showed sTfR levels above the cut-off level ( $p < 0.001$ ) (Figure 2).

Recently, we evaluated sTfR levels every week for 60 days in the sera of 50 patients with homozygous  $\beta$  thalassemia before and after BMT.<sup>7</sup> The findings of this investigation confirmed other observations<sup>8,9</sup> that determination of sTfR levels could be helpful in establishing

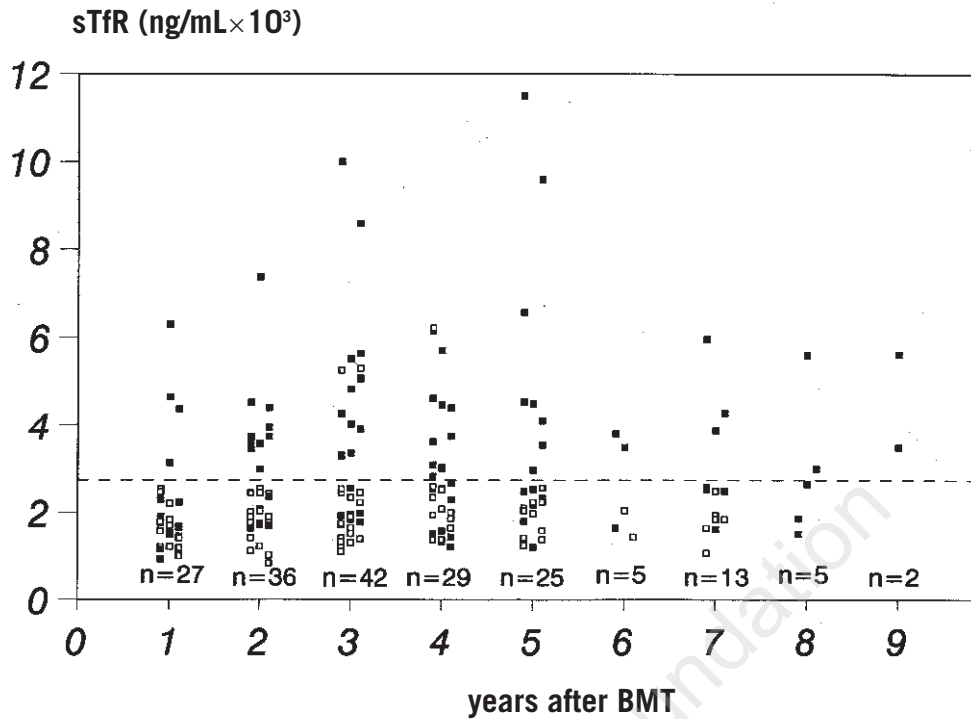


Figure 2. sTfR concentrations of 184 patients 1 to 9 years after BMT from an HLA-identical sibling donor, normal (□) or heterozygous (■) for  $\beta$  thalassemia. The dashed line indicates the mean of sTfR values  $\pm$  1 SD (2600 ng/mL), as determined by sTfR analysis of 50 normal volunteers.

the expansion of the erythron after BMT, when donor erythropoiesis recovers. No differences in sTfR values were observed between patients transplanted from heterozygous and from normal HLA-identical sibling donors.

However, those results might be impaired by the supportive therapy instituted soon after BMT. In the present study, analysis of sTfR levels in long-term transplanted patients, off therapy for six months to nine years, allowed us to identify patients whose sTfR values were not influenced by any therapeutic treatment. We emphasize that 64 out of 128 (50%) ex-thalassemics transplanted from a sibling donor heterozygous for  $\beta$  thalassemia displayed sTfR levels above the cut-off value of 2600 ng/mL. This finding suggests that increased erythropoiesis, albeit in part ineffective, is responsible for the sTfR elevation; alternatively, one could postulate that cellular apoptosis within the erythroblasts of transplanted patients with  $\beta$  thalassemia

trait<sup>10</sup> is responsible for the release of sTfR into the blood stream. High or low sTfR values may simply represent different degrees of erythropoietic activity. Perhaps effective erythropoiesis generates a greater rise in sTfR than does ineffective erythropoiesis.

On the other hand, all patients in this series showed various degrees of iron overload;<sup>11</sup> the influence of iron status on sTfR deserves clarification, since an inverse relationship between iron status and sTfR levels was found in the rat.<sup>12</sup> Finally, decreased erythropoietic activity, as reflected by low sTfR levels may be due to changes in erythropoietin production after BMT.<sup>13</sup> At present, the physiologic and clinical significance of different patterns of sTfR levels in patients with homozygous  $\beta$  thalassemia transplanted from heterozygous donors is uncertain.

sTfR measurement appears to be a simple and rapid method of identifying patients with

enhanced erythropoietic activity after BMT for thalassemia. Further studies are needed to elucidate the wide variability of sTfR levels in ex-thalassemic patients transplanted from an HLA-identical sibling donor heterozygous for  $\beta$  thalassemia.

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