



## Serum levels of erythropoietin and soluble transferrin receptor during pregnancy in non- $\beta$ -thalassemic and $\beta$ -thalassemic women

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### ABSTRACT

**Background and Objectives.** In non-thalassaemic women serum erythropoietin (Epo) level increases during pregnancy, whereas that of soluble transferrin receptor (STFR) drops slightly in the first two trimesters to attain the original values in the third trimester. In this study the time-course of these two parameters was explored in  $\beta$ -thalassemic and non- $\beta$ -thalassemic women, both pregnant and not.

**Design and Methods.** Two hundred and fifty-seven women were studied: 64 non- $\beta$ -thalassemic, non-pregnant women made up the reference group, 89 were non- $\beta$ -thalassemic pregnant women, and 104 were  $\beta$ -thalassemic pregnant or non-pregnant women. The full blood count, hemoglobin levels and iron status (serum iron and serum ferritin levels) were explored by traditional methods. Serum Epo and STFR levels were measured with standard commercial kits.

**Results.** In non- $\beta$ -thalassemic women the mean non-pregnant Epo level ( $10.95 \pm 4.7$  mU/mL) increased in the first trimester ( $17.12 \pm 5.18$  mU/mL), was stationary in the second, and increased again in the third ( $31.43 \pm 14.13$  mU/mL). STFR mean value dropped in early pregnancy from  $2.4 \pm 0.72$  mg/L to  $1.78 \pm 0.64$  mg/L, and then returned to the original value ( $2.38 \pm 0.94$  mg/L). In  $\beta$ -thalassemic women the mean non-pregnant Epo level ( $15 \pm 6.56$  mU/mL) was higher than in non- $\beta$ -thal non-pregnant women. During pregnancy it progressively increased to  $35.60 \pm 25.46$  mU/mL. STFR (non-pregnant level  $3.37 \pm 1.07$  mg/L) gradually increased throughout the whole gestation period and by the third trimester its level was markedly higher than that in non- $\beta$ -thal women at the corresponding stage of gestation ( $9.41 \pm 5.39$  mg/L vs  $2.38 \pm 0.94$  mg/L).

**Interpretation and Conclusions.** The STFR level changed to different extents in non- $\beta$ -thal and  $\beta$ -thal women during their pregnancies. In the former STFR markedly decreased in early pregnancy; in the latter it showed no decrease in the first trimester, increased in the second and reached very high values in the third. This change over time is likely to be

the consequence of erythroid bone marrow hyperplasia and hyperactivity, which are usually present in all  $\beta$ -thalassemic patients and in heterozygous carriers as well.

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Key words: thalassemia, pregnancy, Epo, STFR

It has long been known that blood changes occur during pregnancy. During the first trimester the total red blood cell mass diminishes while the volume of plasma increases.<sup>1-4</sup> As from the second trimester, the erythroid mass progressively increases up to 120-125% of normal values (no pregnancy) in the third trimester.<sup>3</sup>

These changes could not be accurately quantified until two plasma factors, namely erythropoietin (Epo) and soluble transferrin receptor (STFR), were demonstrated to reflect erythropoietic activity in the bone marrow and suitable methods were implemented to quantify these factors in pregnant women without the use of radioactive elements *in vivo*.

Anemia and hypoxia appear to be the primary stimuli of *de novo* synthesis of Epo, a glycoprotein produced in the kidneys during adult life and in the liver during fetal life. As a rule, the hypoxia resulting from an anemic condition swiftly triggers a particular regulatory mechanism that increases Epo production. It has been demonstrated<sup>5</sup> that the primary effect of Epo in the bone marrow is to enhance mitotic activity of late erythroid precursors (CFU-E cells) and their immediate successors (proerythroblasts), and to accelerate maturation times. Through this receptor-mediated mechanism Epo stimulates the production of red cells.

Transferrin is the plasma protein which delivers iron to erythroid cells through an interaction with specific membrane transferrin receptors, which are particularly numerous in erythroid precursors. During this process, soluble truncated monomers of membrane transferrin receptors are delivered in the serum (STFR) and their level appears to be closely related to the number of erythroid precursors in the bone marrow.<sup>6-9</sup> The two major determinants of the level of STFR are body iron status<sup>10</sup> and bone marrow erythroid expansion and activity.<sup>11</sup> In fact, in patients with iron-deficient anemia the STFR level increases immediately and dramatically, whereas in patients

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with hemochromatosis it is lower than in normal subjects.<sup>12</sup> Moreover, the low STFR levels in patients with erythroid hypoplasia and the very high levels in patients such as the thalassemics, who have severe bone marrow hyperplasia, show that STFR reflects erythroid bone marrow mass and activity. This is confirmed by a very strong correlation that has been ascertained<sup>1,7</sup> between serum TFR levels and ferrokinetic estimates of erythropoiesis (ETU: erythron transferrin uptake). In conclusion, the amount of soluble transferrin receptor in human plasma is a highly reliable indicator of the rate of erythropoiesis.<sup>7-9,13</sup>

The latest methods by which Epo and STFR levels have been measured in pregnant women confirmed the preceding results: STFR and Epo levels are low in the first two trimesters and increase in the third until delivery.<sup>6,14,15</sup> STFR levels are similar in pregnant women in the third trimester and in non-pregnant women.<sup>16</sup> At the beginning of pregnancy STFR is lower but then increases, which indicates an initial phase of reduced erythropoiesis that subsequently increases.<sup>17</sup> It has been observed that, on the whole, STFR levels are higher in 86% of anemic pregnant women and 41% of non-anemic ones.<sup>18</sup>

In this paper we present the results of serum Epo and STFR measurements in pregnant and non-pregnant women and, for the first time (to the best of our knowledge), in heterozygous  $\beta$ -thalassemic women, pregnant and non-pregnant. We also report the correlation between these two factors and the blood profile and hemoglobin concentration of the subjects.

## Design and Methods

### Subjects

(Table 1). Two hundred and fifty seven-women aged 14-47 years were examined: 100 while not pregnant, 157 during pregnancy.

The reference group was formed by 64 non-pregnant  $\beta$ -non-thalassemic women: 22 were not iron deficient (the minimum normal ferritin level in non-pregnant women in our laboratory was 19 ng/mL), and 42 were iron deficient. Of the remaining 193 women, 89 were non- $\beta$ -thal pregnant women in first, second and third trimesters; 104 were  $\beta$ -thal women, 36 of whom were not pregnant and 68 who were in the first, second or third trimester of pregnancy.

### Methods

Red blood cell indices were determined with a Technicon H1 automatic cell counter. Erythrocyte osmotic fragility was evaluated by a single saline solution at 0.36% concentration.<sup>19</sup> Red blood cell morphology was examined on a thin, unstained blood smear. Serum iron and ferritin levels were measured by standard methods. Hemoglobin was studied with the methodology standardized in our laboratories,<sup>20</sup> i.e., by electrophoretic separation of the various hemoglobin fractions on agar gel strips followed by a measurement of the optical density of the hemoglobin bands with a semi-automatic densitometer. In  $\beta$ -thal women with slightly high Hb F levels, this fraction was also measured by radial immunodiffusion using the commercial QUIPlate kit (Helena Lab-

**Table 1.** Hb, Hct, Hb F, iron, ferritin, STFR and EPO serum levels and O/P log (EPO) ratio in non-thal and in  $\beta$ -thal heterozygous women, pregnant or non-pregnant (standard deviation in brackets).

N.	Subjects	N. Cases	Hb g/dl	Hct %	Hb F %	Serum iron level $\mu$ g/dl	Serum ferritin level ng/ml	EPO mU/ml	O/P ratio Log (EPO)	STFR mg/l
1a	Reference group: non iron deficient, non $\beta$ thal, non pregnant women	22	13.8 (0.8)	40.9 (2.4)	<1	91 (22.2)	44 (34.5)	10.95 (4.70)	1.00 (0.25)	2.40 (0.72)
1b	Reference group: iron deficient, non $\beta$ thal, non pregnant women	42	8.7 (1.2)	29.5 (3.1)	<1	28.2 (7.9)	5 (2.3)	160.1 (312.6)	0.98 (0.24)	12.8 (8.41)
2	Non $\beta$ thal women pregnant I trimester	47	12.3 (0.7)	36.8 (2.6)	<1	87 (4.9)	52 (44.8)	17.12 (5.18)	0.93 (0.17)	1.78 (0.64)
3	Non $\beta$ thal women pregnant II trimester	31	12.0 (1.1)	35.0 (2.9)	<1	95 (20.7)	44 (33.1)	17.36 (9.46)	0.82 (0.19)	1.83 (1.23)
4	Non $\beta$ thal women pregnant III trimester	11	11.1 (0.9)	32.8 (2.6)	<1	87 (31.3)	18 (9.5)	31.43 (14.13)	0.86 (0.13)	2.38 (0.94)
5	$\beta$ thal women non pregnant	36	10.8 (0.8)	33.7 (2.3)	3 (1.0)	79 (16.1)	55 (46.9)	15.00 (6.56)	0.73 (0.15)	3.37 (1.07)
6	$\beta$ thal women pregnant I trimester	19	10.4 (1.0)	32.2 (2.9)	3 (0.6)	98 (27.9)	44 (21.2)	17.31 (11.36)	0.71 (0.15)	3.44 (1.47)
7	$\beta$ thal women pregnant II trimester	27	9.8 (1.4)	30.4 (4.2)	4 (1.6)	102 (26.4)	36 (30.3)	29.67 (14.36)	0.80 (0.11)	7.92 (8.26)
8	$\beta$ thal women pregnant III trimester	22	9.7 (1.0)	30.4 (3.4)	5 (2.4)	99 (17.1)	38 (32.6)	35.60 (25.46)	0.83 (0.15)	9.41 (5.39)

This table was produced after checking that the distributions of all the parameters were normally distributed.

oratories, USA). Thal and non-thal women with high Hb F levels due to genetic defects ( $\delta\beta$  thal, mutations of the promoter of the AG or GG gene) were excluded from this study. Globin chain synthesis *in vitro* was determined by the reverse HPLC technique.<sup>21</sup> The molecular defects of globin genes were explored by the usual methods.<sup>22,23</sup>

Epo levels were recorded on sera separated from blood samples, and stored at  $-20^{\circ}\text{C}$  until analysis. The commercial RIA kit Epo-Trac (INSTAR Corporation-Stillwater, MN, USA) was used according to the manufacturer's instructions.

STFR levels were estimated in all cases in the same serum samples separated and stored for the Epo examination. The quantitative immunoenzymometric assay of Orion Diagnostic IdeA (Finland) was used. This is based on a non-competitive *sandwich type* assay technique and utilizes monoclonal anti-STFR antibodies.

### Statistical analysis

The statistical significance of the differences found between two groups was calculated by Student's *t*-test. The correlation coefficient between two variables [Hct and log (Epo) or Hct and log (STFR)] was calculated by least squares regression equations.

## Results

### Controls

The 22 non- $\beta$ -thal, non-pregnant, not iron deficient women had hematologic parameters, serum iron and ferritin levels, and mean Epo and STFR values within the normal ranges (Table 1). The 42 non- $\beta$ -thal, non-pregnant women with marked iron-deficient anemia had lower hematologic parameters but higher mean values of Epo and STFR (Table 1).

The following regressions were obtained (Figure 1): log (Epo) =  $4.224 - (0.079 \times \text{Hct})$  ( $n = 64$ ;  $r = -0.80$ ;  $p < 0.001$ ); log (STFR) =  $2.387 - (0.048 \times \text{Hct})$  ( $n = 64$ ;  $r = -0.75$ ;  $p < 0.001$ ).

For each sample the observed logarithmic values of Epo were compared with the values "predicted" by the linear regression observed in the appropriate controls, thus obtaining an O/P (observed/predicted) ratio. This ratio was considered abnormal if lower than 0.80<sup>14</sup>.

### Non- $\beta$ -thalassemic women

**Hb and Hct.** The levels of hemoglobin and hematocrit decreased progressively during pregnancy (Table 1, Figure 2a for Hct). The serum iron level remained within the normal range throughout pregnancy, whereas that of ferritin diminished in the third trimester during which 3 out of the 11 subjects examined showed levels slightly higher than the minimum normal value.

**Epo.** The mean Epo value increased (Table 1, Figure 2b) in the first trimester from  $10.95 \pm 4.7$  to  $17.12 \pm 5.18$  mU/mL ( $t = 4.7$ ;  $d.f. = 67$ ;  $p < 0.001$ ), remained stationary during the second, and almost doubled in the third ( $31.43 \pm 14.13$  mU/mL). The time-course of the mean O/P ratio of the log (Epo) showed (Table 1) that this value, though slightly decreased, remained within the normal range 0.81-1.00. The

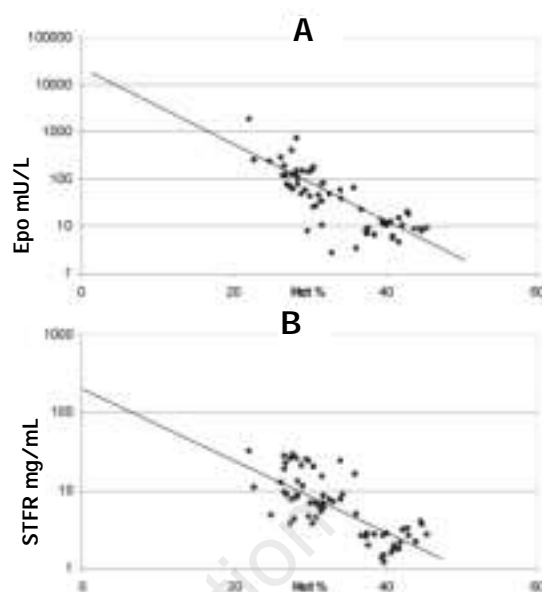


Figure 1. Relationship between Hct and Epo (a) and Hct and STFR (b), in the reference group.

distribution of the O/P ratio log (Epo) in three ranges of values suggested by other authors,<sup>14</sup> that is  $\leq 0.80$ ; 0.81-1.00;  $>1.00$ , showed (Table 2), that the percentage of cases with O/P  $>1.00$  progressively decreased from 50 % in non-pregnant women to 9 % in pregnant women in the third trimester. No inverse correlation between log (Epo) and Hct was observed (Figures 3a, b) during the first and second trimesters ( $r = -0.26$  and  $-0.19$ , respectively), but one was present (Figure 3c) during the third trimester ( $r = 0.70$ ;  $p < 0.05$  in practice with the same slope).

**STFR.** In contrast to Epo, mean STFR level (Table 1 and Figure 2c) dropped considerably during the first trimester [from  $2.40 \pm 0.72$  to  $1.78 \pm 0.64$  mg/L ( $t = 3.60$ ;  $d.f. = 67$ ;  $p < 0.001$ )], remained stationary in the second, and increased again in the third to reach the level of that in non-pregnant women ( $2.38 \pm 0.94$  mg/L).

Table 2. Percent frequencies of three classes of O/P ratios for log (Epo) in non- $\beta$ -thal and  $\beta$ -thal heterozygous women, pregnant and non-pregnant.

Subjects	Cases	EPO		
		$\leq 0.80$	0.81-1.00	$> 1.00$
Non- $\beta$ -thal, non-pregnant	22	27	23	50
Non- $\beta$ -thal, pregnant I trimester	47	21	47	32
Non- $\beta$ -thal, pregnant II trimester	31	52	29	19
Non- $\beta$ -thal, pregnant III trimester	11	27	64	9
$\beta$ -thal, non-pregnant	36	66	28	6
$\beta$ -thal, pregnant I trimester	19	79	16	5
$\beta$ -thal, pregnant II trimester	27	56	40	4
$\beta$ -thal, pregnant III trimester	22	59	23	18

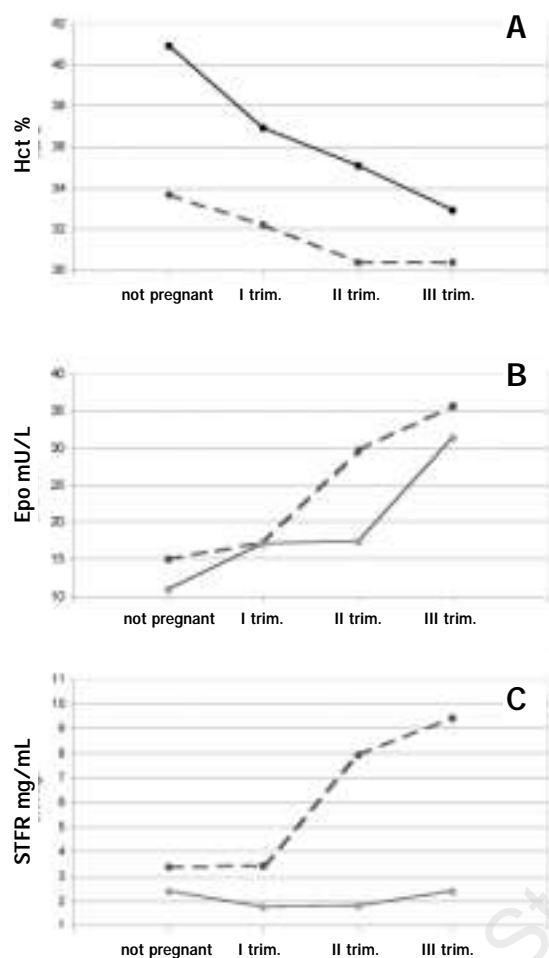


Figure 2. Time-course of Hct (a), Epo (b) and STFR (c) mean values during pregnancy (— = non-thal; --- =  $\beta$ -thal).

#### Heterozygous $\beta$ -thal carrier women

**Hb and Hct.** All  $\beta$ -thal heterozygous women showed the hematologic, hemoglobin (Table 1) and globin synthesis profiles (not reported here) typical of marked  $\beta$ -thalassemia. Hb and Hct mean values were lower than those of the non-pregnant non-thal women ( $10.8 \pm 0.8$  and  $33.7 \pm 2.3$  g/dL as compared to  $13.8 \pm 0.8$  and  $40.9 \pm 2.4$ ) at highly significant levels (for Hb:  $t = 13.7$  and  $p < 0.001$ ; for Hct:  $t = 11.4$  and  $p < 0.001$ ). However, the percent decrease of Hb and Hct in the  $\beta$ -thal non-pregnant women was lower than that in the non-thal women. In the former group, in fact, the decrease was 10.2 % for Hb and 9.8 % for Hct; in the latter group it was 19.6% for both the parameters.

A small amount of Hb F (2-3%) was present in all  $\beta$ -thal women. The prevalent molecular  $\beta$ -thalassemic defect was mutation  $\beta^{39}C \rightarrow T$ . The serum iron level rose during pregnancy (Table 1), while ferritin decreased from  $55 \pm 46.9$  to  $38 \pm 32.6$  ng/mL. In 18% of cases ferritin was low, but always higher than the minimum control value of 10 ng/mL.

**Epo.** Mean serum Epo level (Table 1, Figure 2b) was significantly higher in non-pregnant  $\beta$ -thal women than in the non-pregnant non- $\beta$ -thal women ( $15.00 \pm 6.56$  vs  $10.95 \pm 4.70$  MU/mL:  $t = 2.52$ ; d.f. = 56;  $p > 0.05$ ). It progressively increased during the course of pregnancy: in the second trimester it was twice as high as in non- $\beta$ -thal women ( $29.67 \pm 14.36$  vs  $17.36 \pm 9.46$ ) and in the third trimester it attained values slightly higher than those of non-thal women ( $35.60 \pm 25.46$  vs  $31.43 \pm 14.13$ :  $t = 0.50$ ; d.f. = 31;  $p > 0.05$ ). The distribution of the values of O/P ratio for log (Epo) in the three aforesaid ranges was characterized (Table 2) by 59% of cases with an O/P  $< 0.80$  for  $\beta$ -thal women in the third trimester, as compared with 27% for non- $\beta$ -thal women.

As shown in Figure 3, no correlation between log (Epo) and Hct was observed in first-trimester pregnant women (d), but an inverse correlation was certainly present ( $r = -0.7$ ;  $p < 0.001$ ) in the second trimester (e) – though with a slope approximately half of that of control subjects ( $b = -0.035$  vs.  $b = -0.074$ ) – which did not increase further [indeed it may also decrease ( $b = -0.025$  vs.  $b = -0.035$ ;  $r = -0.34$ ) during the last trimester (f).

**STFR.** Even in the absence of pregnancy the mean STFR value (Table 1, Figure 2c) was higher in  $\beta$ -thal than in non- $\beta$ -thal women ( $3.37 \pm 1.07$  vs.  $2.40 \pm 0.72$  mg/L). It did not decrease at all in the first trimester and then increased up to very high levels in the second and even more in the third trimester. In this last phase the difference between  $\beta$ -thal and non- $\beta$ -thal women was remarkably high:  $9.41 \pm 5.39$  vs.  $2.38 \pm 0.94$  mg/L ( $t = 4.26$ ; d.f. = 51;  $p < 0.001$ ).

#### Discussion

The comparison of the changes in hematologic parameters (Hb and Hct) in  $\beta$ -thal and non- $\beta$ -thal women during pregnancy shows (Figure 2a for Hct) that – apart from the typically lower values for thal women even in the absence of pregnancy – the levels progressively decrease in both groups, but to a lesser extent in  $\beta$ -thal women (Table 1). This trend should not be attributed to an iron deficit since most subjects in both groups had mean to normal values, and only about 20% of them had values slightly higher than the normal minimum, notwithstanding the decreased mean ferritin value.

As far as Epo and STFR are concerned, our results confirm the trend indicated by other authors for non- $\beta$ -thal pregnant women.<sup>6, 14-17</sup> There is a progressive increase of the Epo level (Table 1 and 2, Figure 2b), but lower than expected on the basis of hemoglobin and Hct levels, i.e., the subject's degree of anemia. In fact the percentage of cases with O/P log Epo  $> 1.00$  decreases in the course of pregnancy from 50% to 9% (Table 2). The STFR level decreases significantly in early pregnancy and only in the most advanced stages does it reach the level of non-pregnant, non- $\beta$ -thal women (Table 1, Figure 2c).

As already suggested by other authors, this pattern is likely to be the expression of erythropoiesis in the bone marrow, an activity which at the beginning of pregnancy does not fulfil the requirements of the new condition.

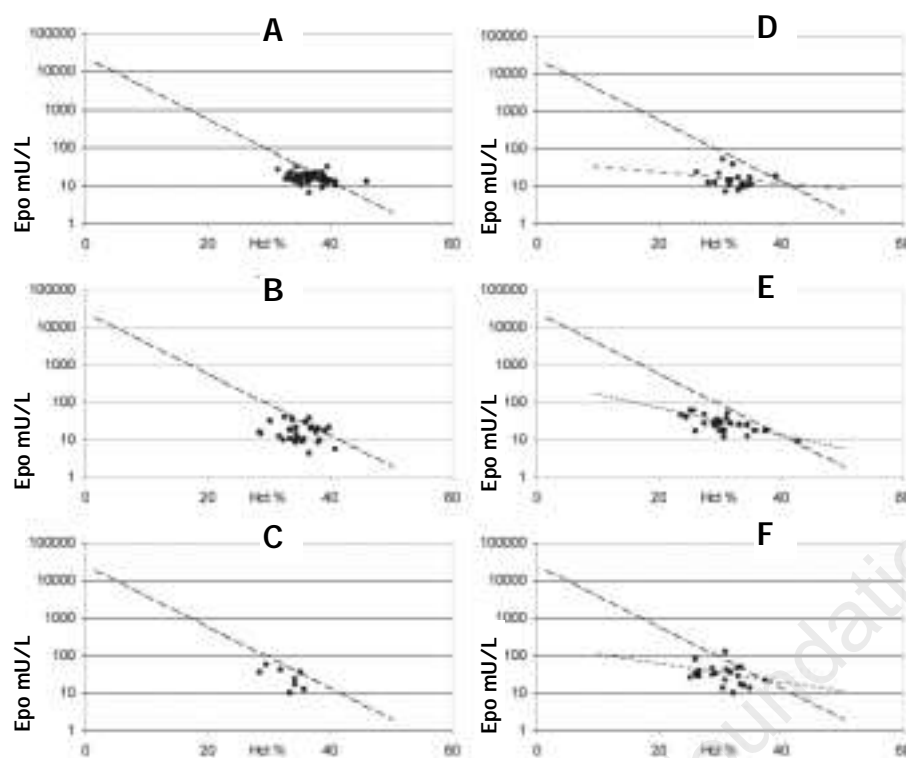


Figure 3. Relationship between Epo and Hct levels in the 1st, 2nd and 3rd trimesters of pregnancy in non-thal women (a, b, c) and  $\beta$ -thal women (d, e, f). The hatched line is the linear regression between Epo and Hct (Figure 1a).

In pregnant  $\beta$ -thal women Epo levels are increased (Table 1, Figure 2b) in the second and third trimesters, but they are much higher than those of non- $\beta$ -thal women only in the second. The high percentage (59%) of O/P for Epo < 0.80 in the third trimester (Table 2) shows that in  $\beta$ -thal women Epo production is lower than needed in pregnancy.

Upon comparison of Epo levels in  $\beta$ -thal women in the third trimester with those of non- $\beta$ -thal non-pregnant and those of non-thal highly iron-deficient anemic women (Table 1), it is clear that in the three groups Epo levels rise as a function of the degree of anemia, with a marked leap only in the case of severe anemia. In fact, in healthy controls with a mean Hb level of 13.8 g/dL, mean Epo is 10.9 mU/mL; in third-trimester pregnant thal women with mean Hb = 9.7 g/dL, Epo is 35.6 mU/mL; in patients with sideropenic anemia and a mean Hb of 8.7 g/dL Epo rises to 160 mU/mL. Within the sideropenic group, the twenty-five most anemic women (Hb < 9 g/dL, mean 7.9 g/dL) even had a mean Epo value of 252 mU/mL. This trend clearly indicates the close correlation between Epo production and Hb, and demonstrates that the Hb of  $\beta$ -thal women is not reduced sufficiently to trigger strong production of Epo, the level of which thus remains slightly higher than normal.

The most important finding is the trend in STFR concentration in  $\beta$ -thal women during pregnancy. Whilst in non- $\beta$ -thal women STFR levels decrease significantly in the first trimester, remain stationary in the second to reach the original values in the third, in  $\beta$ -thal pregnant women STFR is already dramatically high in the second trimester and continues to

increase in the third (Table 1, Figure 2c). Concurrently, the decreases in Hb and Hct are slighter than in non- $\beta$ -thal pregnant women: Hb decreases by 12.2% during pregnancy vs. 19.6% in non- $\beta$ -thal women. Once again, the phenomenon should not be associated with a sideropenic condition since none of the women had ferritin values below the normal threshold: it was slightly higher in 18% of cases, mean to high in 82% of cases.

This trend can reasonably be interpreted as being correlated to hyperplasia and hyperactivity of the erythropoietic tissue in the bone marrow, a typical condition in thalassemia major<sup>24</sup> and, to a lesser degree, also in simple thalassemic heterozygosis. In fact, in healthy carriers there is hyperplasia of the bone marrow which only affects the erythroid series, and which is characterized by a high prevalence of normo-erythroblasts.<sup>25</sup> The abundance of erythroid elements – the richest in transferrin receptors – may account for the strong increase in STFR in  $\beta$ -thal pregnant women and its absence in non- $\beta$ -thal women.

In conclusion, there is evidence enough to believe that the different trend of Epo and STFR in  $\beta$ -thal and non- $\beta$ -thal women during pregnancy is associated with the biological mechanisms responsible for the production of these factors. Hypoxia and anemia increase the production of Epo but in  $\beta$ -thal women the degree of anemia is not marked enough to trigger a strong production of this factor. Increased erythropoietic activity in the bone marrow, constant in  $\beta$ -thal women in order to respond better to anemia, is responsible for the greater production of STFR.

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IB – conception and design, analysis and interpretation of data; GM – critical review; FM – statistical analysis of the data; MM and CDA – collection of the samples; BG – haematologic analysis; PP – Epo and STFR assays. We would like to thank the staff of the Association: Drs. Maria Lerone, Donatella Ponzini, Enrica Foglietta, Maria Pia Cappabianca, Antonio Amato, Paola Grisanti, Paola Di Biagio and Mrs. Brunella Sidorini, Silvana Rinaldi, Nadia D'Arcangeli, for their technical contribution and Mr. Marco Ballante for his valuable secretarial assistance.

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### Potential implications for clinical practice

- ◆ The measurement of STFR levels during early pregnancy in  $\beta$ -thalassemic women may give an indication of the degree of increase of marrow erythropoietic activity and may signal the need for therapeutic intervention.

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