Blunted Erythropoietin Production and Decreased Erythropoiesis in Early Pregnancy

By Yves Beguin, Gyorgyi Lipscei, Henri Thoumsin, and Georges Fillet

After decreasing in the first trimester of pregnancy, the total red blood cell mass increases in the second and third trimesters to peak at term at about 120% to 125% of nonpregnant values, but how this is brought about by changes in the rate of erythropoiesis is not known. We evaluated erythropoiesis by measuring serum transferrin receptor (TfR) levels in 406 women during normal pregnancy (N = 317), at delivery (N = 63), or in the early postpartum (N = 27). Despite the presence of the placenta and the frequent occurrence of iron deficiency, TfR levels remained low in the first two trimesters and increased in the third trimester and at delivery. To explain why erythropoiesis activity was relatively low in early pregnancy, we also measured serum immunoreactive erythropoietin (Epo) in relation to the degree of anemia. There was a very strong correlation between serum TfR and Epo levels in the entire group (r = 0.59, P < .0001) as well as in each period of pregnancy. Epo levels remained low for the degree of anemia and did not correlate with hematocrit in the first two trimesters, but recovered afterwards. In the early postpartum, Epo production and erythropoiesis were normal. We conclude that: (1) erythropoiesis is decreased in the first part of pregnancy but increases afterwards; and (2) blunted Epo production in early pregnancy could be responsible for that observation.

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that uses recombinant human Epo (rHuEpo) for tracer and standards. Samples are incubated with rabbit anti-Epo serum for 2 hours at room temperature before Epo tracer is added. After overnight incubation, goat antirabbit serum is added. After centrifugation, the unbound tracer is removed by decantation and the pellet is counted. Several samples had to be diluted 1:10. Twelve control samples were run in each assay, with a between-assay coefficient of variation ranging from 10.3% to 14.1%.

*Miscellaneous.* Serum iron and total iron-binding capacity (TIBC) were measured by standard procedures. Serum ferritin was measured by a radioimmunoassay. Statistical methods. Log transformed Epo and TR values were used in statistical analyses. Student's t-tests, with pooled or separated variances as appropriate, were used to compare two groups. Analysis of variance (ANOVA), with Snedecor's F-test or Welch's test as appropriate, was used to compare more than two groups. Two-way analysis of variance was used to assess the effect of iron stores on TIR values within each period studied. Pearson and likelihood-ratio $\chi^2$ tests were used to measure associations in two-way or multiway frequency tables. The $r$ correlation coefficient between two variables was computed in least squares regression equations. The slopes and y-intercepts of the regression lines between log(Epo) and Hct in controls and study subjects were tested for equality among groups by Student's t-tests in an analysis of covariance, using the BMDP IV program of the BMDP Statistical Software (University of California). A multivariate stepwise regression analysis was performed with serum TIR as the dependent variable.

RESULTS

Controls. Mean serum TIR level in 43 normal women was 6,940 ± 1,480 ng/mL (range, 4,300 to 11,450). Thirty-three normal control women had a mean Hct of 39.5% ± 2.1% (M ± SD) and a mean Epo level of 16.4 ± 41 mU/mL, while Epo levels in 41 nonpregnant anemic women ranged from 10 to 237 mU/mL. The following regression (N = 74, $r = -0.88$, $P < .0001$) was obtained between Epo (mU/mL) and Hct (%): log(Epo) = 3.8279 - (0.0662 Hct). Based on this formula, a predicted log(Epo) value was derived for each sample and the O/P ratio of observed/predicted log(Epo) ranged from 0.80 to 1.20. Consequently, O/P ratios in study subjects were considered abnormal if lower than 0.80.

Study subjects. Table 1 displays Hct, iron status, serum TIR and Epo levels, as well as O/P ratios in groups of pregnant women. As compared with controls, Hct decreased in the first and further so in the second and third trimesters, before reincreasing at delivery and on day 7 postpartum ($P < .0001$ for comparison between periods of pregnancy). Transferrin saturation as well as serum ferritin levels decreased throughout pregnancy. In the first trimester, only 8% of women were iron deficient (serum ferritin < 12 µg/L). This figure rapidly increased to 27% in the second and 59% in the third trimester ($P < .0001$).

TIR levels were decreased in early pregnancy, normalized in the first part of the third trimester, and increased slightly beyond normal values in late pregnancy, at delivery, and in the early postpartum ($P < .001$). The evolution of Hct, TIR, Epo, and O/P ratio throughout pregnancy is illustrated in Fig 1. The fraction of cases with reduced erythropoiesis (TIR < 4,300 µg/L) was 34%, 48%, 16%, 10%, and only 1%, respectively, during weeks 5 through 12, 13 through 20, 21 through 28, 29 through 32, 33 through 36, and after week 37 ($P < .0001$). The effect of iron status on serum TIR levels was analyzed in two-way analysis of variance (Table 2). Within each trimester of pregnancy, women with deficient iron status (serum ferritin < 12 µg/L) had higher TIR levels than women with marginal status (ferritin 12 to 19 µg/mL) and even more so than those with normal iron stores (ferritin ≥ 20 µg/mL). Both time of pregnancy and iron status were significant determinants of TIR levels ($P < .0001$) and there was no interaction between the two.

Epo levels were higher during pregnancy (31.4 ± 20.5 mU/mL), at delivery (33.9 ± 22.3 mU/mL), and on day 7 postpartum (35.1 ± 34.7 mU/mL) than in normal women ($P < .001$). Epo levels increased steadily (Fig 1) throughout pregnancy and into third trimester values ($P < .001$), but remained relatively stable among third trimester, delivery, and early postpartum values (not significant [NS]). The mean O/P ratio was significantly reduced in the first two trimesters ($P < .001$) but returned to normal values in the third trimester, at delivery, and on day 7 postpartum. Epo levels were relatively low for the degree of anemia (O/P ratio < 0.80) in 10% of the cases in the third trimester, and 25% of the cases in the first two trimesters ($P < .01$). In the 9 to 16 week period (Fig 1), the percentage of cases with abnormal O/P ratios peaked to 34%.

Figure 2 displays regression lines between Epo levels and Hct in groups of pregnant women as compared with control women. The inverse linear relationship between log(Epo) and Hct was not significant in the first ($r = -0.01$) and second ($r = -0.16$) trimesters, was present but with a reduced slope ($P < .001$ for difference in slopes) during the third trimester ($r = -0.41$, $P < .0001$), and was normal

### Table 1. Hct, Iron Status, Erythropoiesis, and Serum Epo Values in Controls and Pregnant Women

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>1st Trimester</th>
<th>2nd Trimester</th>
<th>3rd Trimester</th>
<th>Delivery</th>
<th>Postpartum</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>43</td>
<td>53</td>
<td>108</td>
<td>156</td>
<td>110</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Hct (%)</td>
<td>39.5 ± 2.1</td>
<td>36.3 ± 3.4</td>
<td>34.4 ± 2.4</td>
<td>34.2 ± 3.0</td>
<td>36.8 ± 3.2</td>
<td>36.1 ± 4.0</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Tf saturation (%)</td>
<td>29 ± 12</td>
<td>44 ± 30</td>
<td>26 ± 24</td>
<td>14 ± 14</td>
<td>26 ± 16</td>
<td>16 ± 13</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Ferritin (µg/L)</td>
<td>33 ± 20</td>
<td>5,350 ± 2,360</td>
<td>5,130 ± 1,480</td>
<td>7,170 ± 2,990</td>
<td>9,200 ± 3,420</td>
<td>8,380 ± 3,220</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>TIR (µg/L)</td>
<td>3,420 &lt;.0001</td>
<td>29 ± 12</td>
<td>44 ± 30</td>
<td>26 ± 24</td>
<td>14 ± 14</td>
<td>26 ± 16</td>
<td></td>
</tr>
<tr>
<td>Epo (mU/mL)</td>
<td>16.4 ± 4.1</td>
<td>19.1 ± 6.2</td>
<td>28.4 ± 15.5</td>
<td>37.7 ± 24.2</td>
<td>33.9 ± 22.3</td>
<td>36.1 ± 34.7</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>O/P ratio</td>
<td>1.00 ± 0.10</td>
<td>0.91 ± 0.17</td>
<td>0.92 ± 0.14</td>
<td>0.98 ± 0.14</td>
<td>1.04 ± 0.21</td>
<td>1.00 ± 0.16</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

P values are given for comparison between groups of pregnant women.
ERYSRHOPOIESIS IN PREGNANCY

Fig 1. Evolution of Hct, TIR levels, Epo levels, and Epo O/P ratios (Mean ± SEM) throughout pregnancy, as compared with normal control values.

(slopes and y-intercepts not significantly different from controls) in the early postpartum (r = - .74, P < .0001). The relationship was also normal in the 28 women sampled beyond week 37 (r = - .70, P < .0001).

There was a very strong correlation between serum Epo and TIR levels in the entire group of pregnant women (r = .59, P < .0001), as well as in the first (r = .48, P < .0001), second (r = .56, P < .0001), and third (r = .54, P < .0001) trimesters, at time of delivery (r = .56, P < .0001), or on day 7 postpartum (r = .48, P < .0001). There was a striking parallelism between Epo O/P ratio and TIR levels throughout pregnancy (Fig 1). TIR also correlated negatively with parameters of iron status such as SeFe, transferrin saturation, and serum ferritin (P < .001), but this was mostly observed in the third trimester. The relationship between Hct and TIR levels was not significant in the first (r = -.04) and second (r = -.14) trimesters, and was weak in the third trimester (r = -.26, P < .01). In multivariate stepwise regression analysis (r = .68), serum Epo contributed the most to the prediction of serum TIR (change in r²: .35), transferrin saturation (change in r²: .08), and ferritin (change in r²: .02) somehow enhanced precision, while Hct and week of pregnancy did not add significance.

DISCUSSION

Pregnancy causes considerable alterations in plasma volume and RBC mass. There is little change in plasma volume before week 16, but then a steady increase takes place to 120% of nonpregnant values by week 20, to 140% by week 30, and to 150% at term. Although the RBC mass peaks at term to about 120% to 125% of nonpregnant control values, little is known about its evolution throughout pregnancy. Most studies have derived calculations of the RBC mass from measurements of plasma volume using Evans blue dye. Such indirect measurements are not recommended because whole-body Hct may vary widely, particularly in situations such as splenomegaly and pregnancy, where considerable redistribution of blood volume and blood flow takes place. In the one study using appropriate methodology for the serial measurement of total RBC mass, there was a decrease in the first trimester of pregnancy, followed by a gradual return to nonpregnant values only by week 30, and a further increase in late pregnancy.

It has been shown that the RBC lifespan remains unchanged during pregnancy. Modifications in the total RBC mass should then be accounted for by variations in the rate of erythropoiesis. No quantitative measurement of erythropoiesis is available from the literature, except near term, because of the prohibitive need to use radioiron.

Table 2. Effect of Iron Status on Serum TIR Levels According to Time of Pregnancy

<table>
<thead>
<tr>
<th>Time of Pregnancy</th>
<th>1st Trimester</th>
<th>2nd Trimester</th>
<th>3rd Trimester</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deficient</td>
<td>7.590 ± 4.810*</td>
<td>5.680 ± 1.400</td>
<td>8.260 ± 3.260</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Marginal</td>
<td>5.490 ± 2.000</td>
<td>5.870 ± 1.640</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>5.040 ± 1.490</td>
<td>4.570 ± 1.040</td>
<td>5.460 ± 1.710</td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td></td>
<td></td>
<td></td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

*Deficient and marginal iron status combined (N = 7).
Methodology has been recently introduced for the measurement of serum TfR levels as a valuable alternative to ferrokinetics for the quantitative assessment of erythropoiesis.\(^7,^9\) Using this assay in the present study, we found that erythropoiesis was decreased in early pregnancy, normalized in the first part of the third trimester, and was moderately stimulated in late pregnancy, at delivery, and in the early postpartum. These data are consistent with the changes of RBC mass observed throughout pregnancy.\(^4\) Others\(^7,^9\) have measured TfR levels without other parameters of erythropoiesis in a small group of women, and observed increased levels in late pregnancy, but no change in the first part of pregnancy. The nature and standardization of the material measured in that assay is not well established\(^8\) and direct comparison is difficult.

There are theoretical interferences potentially precluding the use of serum TfR levels as a measurement of erythropoiesis during pregnancy. First, the placenta is very rich in receptors quite similar to the erythroid TfR, allowing its use as immunogen and standard in the present assay.\(^10,^16\) If placental TfR contributed significantly to circulating TfR levels, they would have caused an apparent overestimation of erythropoiesis in pregnancy, but this was obviously not the case. Second, functional iron deficiency beyond depletion of iron stores has been shown to produce an increase in serum TfR levels.\(^8,^9,^29\) The effect of iron stores on TfR levels in each trimester was even more decreased, while iron deficient women had normal levels. Therefore, iron deficiency does not appear to interfere significantly with the conclusion that the rate of erythropoiesis is low in pregnancy.

Plasma expansion could cause an apparent reduction in the serum concentration of TfR by diluting the total number of soluble receptors in a larger volume. Correcting for plasma volume would give mean receptor levels of about 5,290, 5,710, and 7,930 pg/L for women with normal iron stores, respectively, in the first, second, and third trimester. These levels are still well below expected values, particularly in view of levels in patients with immune hemolytic anemia or hemoglobin H disease, where a similar degree of anemia (Hct 35% \pm 5%) induced considerably higher rates of erythropoiesis.\(^9\)

We attempted to identify factors responsible for decreased erythropoiesis in pregnancy. In multivariate stepwise regression analysis, serum Epo contributed the most to the prediction of serum TfR. The correlation between serum TfR and Epo was strong in each period of pregnancy. Epo levels are best expressed in relation to the Hct.\(^6\) We found an excellent correlation between Epo levels and Hct in 74 women with various degrees of anemia. Therefore, Epo levels measured in pregnant subjects could be related to predicted values calculated from the Hct. Absolute Epo levels increased throughout pregnancy and were in agreement with the Hct in the third trimester, at delivery, and in the early postpartum, but in the first two trimesters they were below predicted levels in the vast majority and low relatively to the Hct in 25% of the cases. The inverse linear relationship between log(Epo) and Hct, absent in the first trimester, progressively returned to normal as pregnancy advanced (Fig 2).

Several physiologic adaptations to pregnancy may augment oxygen supply to the kidney sensor, thus depressing Epo release in the first trimester. Erythrocyte 2,3-diphosphoglycerate increases early in pregnancy, producing a shift in the oxygen dissociation curve to the right.\(^31\) In the first trimester, the renal blood flow is considerably increased,\(^25\) but in the second half of pregnancy, a larger part of the expanded cardiac output is directed to the utero-placental and cutaneous circulations.\(^29\) These alterations in oxygen supply to the kidney could in part explain variations in Epo production rate throughout pregnancy and the early postpartum. Modifications in the endocrine status, such as the production of human placental lactogen, have also been suggested to possibly influence changes in Epo production and release during pregnancy.\(^12,^13\)

We conclude that serum Epo levels, though increased over nonpregnant values, remain relatively low for the degree of anemia in the first part of pregnancy but return progressively to predicted levels thereafter. These changes in Epo production could explain the slowdown in erythropoietic activity observed in the first two trimesters and its normalization later in pregnancy. It should be emphasized that these alterations in the rate of erythropoiesis are adaptive changes that would certainly not require therapeutic intervention, such as the administration of rHuEpo.

REFERENCES

Blunted erythropoietin production and decreased erythropoiesis in early pregnancy [see comments]

Y Beguin, G Lipscei, H Thoumsin and G Fillet