Red Blood Cell Precursor Mass as an Independent Determinant of Serum Erythropoietin Level

By Mario Cazzola, Roberta Guarnone, Paola Cerani, Esther Centenara, Andrea Rovati, and Yves Begaun

Serum erythropoietin (sEpo) concentration is primarily related to the rate of renal production and, under the stimulus of hypoxia, increases exponentially as hemoglobin (Hb) decreases. Additional factors, however, appear to influence sEpo, and in this work, we performed studies to evaluate the role of the red blood cell precursor mass. We first compared the relationship of sEpo with Hb in patients with low versus high erythroid activity. The first group included 27 patients with erythroid aplasia or hypoplasia having serum transferrin receptor (sTfR) levels < 3 mg/L (erythroid activity < 0.6 times normal), while the second one included 28 patients with β-thalassemia intermedia having sTfR levels > 10 mg/L (erythroid activity > 2 times normal). There was no difference between the two groups with respect to Hb (8.3 ± 1.6 vs. 8.0 ± 1.3 g/dL, P > .05), but sEpo levels were notably higher in patients with low erythroid activity (1,601 ± 1,542 vs 235 ± 143 mU/mL, P < .001). In fact, multivariate analysis of variance (ANOVA) showed that, at any given Hb level, sEpo was higher in patients with low erythroid activity (P < .0001). Twenty patients undergoing allogeneic or autologous bone marrow transplantation (BMT) were then investigated. A marked increase in sEpo was seen in all cases at the time of marrow aplasia, disproportionately high when compared with the small decrease in Hb level. Sequential studies were also performed in five patients with iron deficiency anemia undergoing intravenous (IV) iron therapy. Within 24 to 72 hours after starting iron treatment, marked decreases in sEpo (up to one log magnitude) were found before any change in Hb level. Similar observations were made in patients with megaloblastic anemia and in a case of pure red blood cell aplasia. These findings point to an inverse relationship between red blood cell precursor mass and sEpo; at any given Hb level, the higher the number of red blood cell precursors, the lower the sEpo concentration. The most likely explanation for this is that sEpo levels are regulated not only by the rate of renal production, but also by the rate of utilization by erythroid cells.

From the Department of Internal Medicine and Medical Therapy, Section of Internal Medicine and Medical Oncology, and the Department of Medicine, Division of Hematology, University of Liège, Liège, Belgium.

Submitted September 2, 1997; accepted October 28, 1997.

Supported by grants from Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Policlinico S. Matteo and Fondazione Ferrata Storti, Pavia, Italy.

Address reprint requests to Mario Cazzola, MD, Internal Medicine and Medical Oncology, Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Policlinico S. Matteo, 27100 Pavia, Italy.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1998 by The American Society of Hematology.
Serum erythropoietin assay. Circulating Epo levels were measured by a commercially available radioimmunoassay (Incatar Corp., Stillwater, MN) that uses HuEpo for tracer and standards. To define Epo levels as appropriate or inappropriate for a given degree of anemia, an exponential regression of sEpo versus Hct was determined in reference subjects (102 normal individuals or patients with iron deficiency anemia, hemolytic anemia, or hypoplastic anemia), and the 95% confidence limits were defined. For Hct values ≤40%, the regression equation was: log(e)(e) = 3.42 – 0.056 × Hct. For Hct values >40%, the regression equation was: log(e)(e) = 1.31 – 0.003 × Hct. Based on these equations, the observed/predicted log(e)(e) ratio (O/P ratio) was derived for each sample. The mean O/P ratio in reference subjects was 1.01 ± 0.11 (95% confidence interval, 0.80 to 1.22).

**Results**

Serum Epo in anemic patients with low versus high erythroid activity. As reported in Table 1, there was no significant difference with respect to Hb level (Student’s t test = 0.97, P = 0.18) between the 27 patients with low erythroid activity and the 27 patients with high erythroid activity.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Hb Level (g/dL)</th>
<th>sTFR (mg/L)</th>
<th>sEpo (mU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypoproliferative</td>
<td>8.3 ± 1.5</td>
<td>1.8 ± 0.8</td>
<td>1.601 ± 1.541</td>
</tr>
<tr>
<td>anemia (n = 27)</td>
<td>(5.5-10.4)</td>
<td>(0.4-2.9)</td>
<td>(172-6,030)</td>
</tr>
<tr>
<td>α-thalassemia inter-</td>
<td>8.0 ± 1.3</td>
<td>23.5 ± 11.4</td>
<td>235 ± 143</td>
</tr>
<tr>
<td>media (n = 28)</td>
<td>(5.9-10.6)</td>
<td>(10.4-48.6)</td>
<td>(70-619)</td>
</tr>
<tr>
<td>Normal range</td>
<td>12-16 (females)</td>
<td>3.0-7.0</td>
<td>5-30</td>
</tr>
<tr>
<td>13.5-17.5 (males)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
(hypoproliferative anemia, sTfR < 3 mg/L) and the 28 individuals with β-thalassemia intermedia and high erythroid activity (sTfR > 10 mg). By contrast, sEpo levels were about one log higher in patients with hypoproliferative anemia (Student’s t test = 4.67, P < .001).

Figure 1A displays the relationship of sEpo to Hb observed in the two groups of patients. A significant inverse relationship between Hb and sEpo was found in both patient populations (P < .001 in both groups). However, multivariate ANOVA showed that at any given Hb level, sEpo was higher in patients with low versus high erythroid activity (the multivariate tests Rao’s R and Pillai-Bartlett Trace V were both significant at P < .0001).

Assuming that the erythroid cells in the BM directly influence the Epo clearance rate, we made an empirical correction to remove the effect of variation in erythroid activity on sEpo levels using the formula reported in Materials and Methods.

As shown in Fig 1B, when we reanalyzed the data of Fig 1A using the corrected sEpo instead of the measured sEpo levels, a substantial part of the variation previously observed was abolished. In fact, whereas only 15.8% of the variation in sEpo was explained by variations in Hb level (Fig 1A), these latter variations in Hb level explained 37.5% of the variation in corrected sEpo. In particular, there was no difference (P > .05) between corrected sEpo levels calculated in patients with thalassemia intermedia having high erythroid activity and those calculated in patients with low erythroid activity.

Sequential studies in patients receiving myeloablative therapy or conventional chemotherapy. Twenty patients undergoing allogeneic or autologous BMT were investigated immediately before undergoing myeloablative therapy and on day 0 (Table 2 and Fig 2). Conditioning regimen markedly reduced erythroid activity as shown by the sharp decrease in sTfR (t = 10.40, P < .001). Day 0 values for the circulating receptor were comparable with those of patients with aplastic anemia or PRCA (Table 1).

There was also a mild, although significant decrease in Hb level (t = 2.93, P < .05). However, the marked increment in sEpo (t = 6.66, P < .001) appeared to be disproportionately high when compared with the mild decrease in Hb level (Fig 2). We therefore calculated for each patient the day-0 sEpo concentration expected (or predicted) on the basis of the actual Hb level. As displayed in Fig 2, the predicted day-0 sEpo was significantly lower than the observed one (81 ± 45 mU/mL v 254 ± 141 mU/mL, t = 6.86, P < .001), indicating that factor(s) other than Hb level contributed to the elevation in circulating Epo level.

Similar findings were observed in five patients with non-Hodgkin’s lymphoma undergoing conventional chemotherapy (Fig 3). A marked increase in serum Epo was seen in all cases after 8 days, before any significant decrease in Hb was observed; this was associated with a parallel decrease in sTfR.

---

**Table 2.** Hb, sTfR, and sEpo in 20 Patients Undergoing Allogeneic (n = 14) or Autologous (n = 6) BMT

<table>
<thead>
<tr>
<th>Time</th>
<th>Hb (g/dL)</th>
<th>sTfR (mg/L)</th>
<th>sEpo (mU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pretransplant</td>
<td>9.1 ± 1.2</td>
<td>6.0 ± 1.6</td>
<td>66 ± 37</td>
</tr>
<tr>
<td>Day 0</td>
<td>8.6 ± 1.2</td>
<td>2.1 ± 0.5</td>
<td>254 ± 141</td>
</tr>
</tbody>
</table>

---

**Fig 1.** Relationship of sEpo to Hb observed in 27 patients with hypoproliferative anemia having erythroid activity < 0.6 times normal (C) versus 28 patients with β-thalassemia intermedia having erythroid activity > 2 times normal (D). (A) Relationship of measured sEpo to Hb level. Multivariate ANOVA showed that, at any given Hb level, sEpo was higher in patients with low versus those with high erythroid activity (P < .0001). (B) Relationship of corrected sEpo to Hb level. Data are those of (A), but corrected sEpo levels have been used instead of the measured ones. Multivariate ANOVA showed no significant difference between the relationship in patients with low erythroid activity and that in subjects with high erythroid activity (P > .05).

**Fig 2.** Time course of Hb level, sEpo, and circulating transferrin receptor in 20 patients undergoing BMT. Data are mean values ± 1 SD. Observed values before myeloablative therapy and those on day 0 are shown. Predicted sEpo values were calculated on the basis of the patient’s Hct using the equation derived from regression analysis as previously described.
Sequential studies in patients with iron deficiency anemia treated with IV iron saccharate. Five patients with severe iron deficiency anemia (mean Hb, 6.4 ± 1.4 g/dL) were studied immediately before and during IV iron therapy. Data of these sequential studies are depicted in Fig 4. Within 24 to 72 hours after starting iron treatment, marked decreases in sEpo were observed (up to one log magnitude) before any change in Hb level.

Because both the expression of transferrin receptors on erythroid cells and the soluble receptor level are influenced by the body iron status, the measurement of sTfR could not be used in these patients to evaluate the erythroid activity. However, in one patient, we were able to monitor the reticulocyte response to IV iron. Figure 5 shows that the reticulocyte count and, in particular, the percentage of immature reticulocytes (HFR), increased sharply after starting IV iron, and this was paralleled by a mirror decrease in sEpo.

Case reports: megaloblastic anemia and PRCA. Two patients with megaloblastic anemia were studied (Figs 6 and 7). In both cases, replacement therapy with vitamin B12 or folate induced a sharp decrease in sEpo in the first few days before any change in Hb level. Such decreases were paralleled by increases in sTfR, and in one case (Fig 7), also of immature reticulocytes (HFR), indicating that ineffective erythropoiesis was replaced by effective erythropoiesis with a subsequent expansion of the red blood cell precursor mass.

Of particular interest was the patient with PRCA after peripheral stem cell transplantation (Fig 8). His sTfR was 0.4 mg/L, indicating the complete absence of any erythroid activity: this amount of TfR, in fact, is contributed by nonerythroid tissues. As previously reported, this patient responded to rHuEpo therapy despite the elevated sEpo (2820 mU/mL). For 4 weeks, there was no increase in Hb level; however, sTfR started to increase after 2 weeks, and there was a parallel decrease in sEpo despite exogenous Epo administration, suggesting increased use by an expanding erythroid precursor mass.

DISCUSSION

Renal Epo production is typically regulated by a transcriptional feedback mechanism where hypoxia plays a crucial role. However, a number of additional pathophysiological factors, including inflammatory cytokines and plasma viscosity, may independently affect the renal response to hypoxia. Epo catabolism is largely unknown and it is not clear whether sEpo levels are determined only by the production rate or rather reflect a balance between this and consumption by erythroid cell use.

The observation that serum Epo levels in aplastic anemia are higher than those in iron deficiency anemia suggests that use
by erythroid precursors may be an important factor in determining serum concentrations. Unexpectedly low sEpo levels have been previously found in patients with refractory anemia, sickle cell anemia, thalassemia, and megaloblastic anemia indicating that erythroid hyperplasia may involve a faster clearance of Epo.

In the initial part of this study, we have clearly shown that the sEpo level in aplastic anemia (erythroid activity < 0.6 times normal) is much higher than the level in thalassemia intermedia (erythroid activity > 2 times normal) at the same hemoglobin concentration (Fig 1A). This may either suggest that the clearance of Epo is much faster in thalassemia than in marrow failure, or alternatively that the renal production is to some extent higher in the latter condition.

To establish any relationship between erythropoiesis and sEpo, several investigators studied patients receiving myelosuppressive treatments. Overall, patients treated with chemotherapy were found to have a temporary, but prominent, increase in sEpo titers without a concomitant change in Hb concentration. However, different interpretations were provided for the observed marked sEpo increase before the decrease in Hb after treatment with cytostatic drugs. Possible explanations included: (1) cytotoxic therapy causes direct injury to Epo-producing cells in the kidney in a manner that mimics hypoxia; (2) BM inhibition triggers an unknown stimulus for Epo production; (3) a decreased mass of erythroid precursors disrupts the usual Epo degradation pathway, reduced Epo use resulting in prolonged sEpo lifespan and concentration; (4) cytotoxic drugs enhance Epo mRNA stability with a consequent increase in protein synthesis.

Our studies after myelosuppressive therapy (Figs 2 and 3) definitely show an inverse relationship between erythroid activity (as indicated by sTfR) and sEpo. Such relationship is further reinforced by observations in patients with iron deficiency, megaloblastic anemia, and PRCA (Figs 4 through 8). Although it has been suggested that iron deprivation increases Epo production, cobalamin deficiency does not raise Epo level per se, but only to the extent that it produces anemia. It is not clear why the erythroid marrow of our patient with PRCA did not respond to endogenous Epo and responded to exogenous rHuEpo (Fig 8). We cannot rule out that the erythroid response was spontaneous and unrelated to rHuEpo, but at least three other similar cases have been reported. Endogenous Epo production might have been defective in this patient despite the elevated sEpo levels if one assumes that these levels essentially reflected a very low utilization rate by the few erythroid cells existing in the BM.

Overall, our findings point to an inverse relationship between red blood cell precursor mass and sEpo level: the higher the number of red blood cell precursors, the lower the sEpo level. There are four possible explanations for this relationship: (1) sEpo levels are independently regulated by the rate of hormone

![Graph](attachment:image1)

**Fig 5.** Time course of sEpo, reticulocyte count, and HFR in a patient with iron deficiency anemia treated with IV iron saccharate from day 0. HFR, ie, the most immature reticulocytes, expressed as % of total reticulocytes.

![Graph](attachment:image2)

**Fig 6.** Time course of Hb level, sEpo, and sTfR in a patient with megaloblastic anemia due to vitamin B12 deficiency treated with vitamin B12 (IM as cyanocobalamin, 500 μg per day). A marked decrease in serum Epo was seen after the first injection and before any increase in Hb level. There was a parallel increase in serum transferrin receptor, indicating a rapid expansion of the erythroid marrow during the first days of treatment.
use by erythroid cells through Epo receptors; (2) erythroid marrow hypoplasia triggers a stimulus for Epo synthesis; (3) erythroid marrow expansion inhibits renal production; and (4) Epo excretion by the kidneys is directly influenced by erythroid activity.

Two reports argue against the model of regulation by the utilization rate. Piroso et al33 studied Epo lifespan in rats with hypoplastic and hyperplastic BMs. They found no significant difference and concluded that it is unlikely that erythroid activity determines sEpo lifespan and catabolism. Using a mouse model, Lezón et al34 have found an inverse relationship between the rate of stimulated Epo production and erythropoietic marrow activity. They concluded that decreases in sEpo levels during periods of rapidly increasing erythropoiesis are the reflection of a decrease in the rate of production rather than an increase in the rate of utilization by expanding erythroid cells.

Although the above direct studies failed to show evidence for increased utilization when the erythroid precursor mass is expanded, a large body of evidence points to a role by the utilization rate in the regulation of circulating levels of hematopoietic growth factors. In particular, thrombopoietin levels appear to be primarily regulated through absorption and metabolism by both megakaryocytes and platelets.35 Our findings indicate that the rate of utilization by erythroid cells acts as an independent determinant of sEpo, this latter being a balance between the rate of renal production and the rate of erythroid consumption. This interpretation may be too simplistic, as other factors linking erythron to renal production likely exist. Indeed, we have previously reported elevated sEpo levels in compensated hereditary spherocytosis, a condition defined by decreased red blood cell lifespan without anemia.36 Products of red blood cell destruction may not only exert a distinct stimulatory effect on BM,37,38 but also influence Epo production.

From a practical point of view, we have recently proposed that treatment with rHuEpo should be started only after an adequate erythropoietin production has been documented, eg, by showing sEpo levels < 100 mU/mL in patients with Hb values < 10 g/dL.5 According to the present study, when using sEpo for this purpose, it might be necessary to take into account the patient’s erythroid activity. For example, patients with erythroid hypoplasia may present sEpo values > 100 mU/mL due to the small erythroid cell mass and still be responsive to rHuEpo treatment.18 We are not suggesting the adoption of the empirical correction for sEpo reported in Fig 1B, but consideration of this point in the clinical reasoning of the patient-oriented approach to the use of rHuEpo.5 In this reasoning, it
should be taken into account that apparently normal sEpo levels in patients with hypoproliferative anemia may reflect an inadequate production combined with reduced utilization rate and, conversely, that inappropriately low levels in patients with proliferative anemia can be simply due to an accelerated hormone consumption.

REFERENCES

34. Lezón C, Alippi AM, Barceló AC, Martínez MP, Conti MI, Bozzini CE: Depression of stimulated erythropoietin production in mice with enhanced erythropoiesis. Haematologica 80:491, 1995
35. Nagata Y, Shozaki Y, Nagahisa H, Natasawa T, Abe T, Todokoro K: Serum thrombopoietin level is not regulated by transcription but by the total counts of both megakaryocytes and platelets during thrombocytopenia and thrombocytosis. Thromb Haemost 77:808, 1997