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 Beguin

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 *v* 8.0 ± 1.3 g/dL, *P* > .05), but sEpo levels were notably higher in patients with low erythroid activity (1,601 ± 1,542 *v* 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.

IN ADULT HUMANS, erythropoietin (Epo) is primarily made by a single organ, the kidney, outside the bone marrow (BM) and participates in a classic negative feedback control system.[1,2] Hypoxia is the fundamental physiologic stimulus that causes a rapid increase in renal production of erythropoietin through an exponential increase in the number of erythropoietin-producing cells. It is generally accepted that serum Epo (sEpo) concentration is directly related to the rate of renal production. Serum erythropoietin levels are in the range of 5 to 30 mU/mL in normal individuals and increase exponentially as hemoglobin (Hb) or hematocrit (Hct) decreases, unless there is a blunted renal production.[3]

With the availability of commercial immunoassays for sEpo, assessment of endogenous Epo production has become a routine diagnostic procedure. Serum Epo is evaluated in relation to the degree of anemia, and the definition of defective Epo production relies on a low sEpo in comparison to reference patients with similar Hct (or Hb).[3] In the individual patient, the adequacy of endogenous Epo production can be easily assessed through the observed/predicted log (sEpo) ratio (O/P ratio).[4] The O/P ratio is below 1 if the observed value is lower than the predicted one; in reference subjects, the 95% confidence interval ranged from 0.80 to 1.22.[4]

With the only exceptions of prematurity and renal failure, determination of sEpo is mandatory in all anemic patients for deciding treatment with recombinant human Epo (rHuEpo).[5] In fact, it is mainly in patients in whom endogenous Epo levels are inappropriately low for the degree of anemia that administration of rHuEpo can be effective in increasing red blood cell production. In particular, several reports point to the use of a sEpo threshold of ≤ 100 mU/mL for predicting response to rHuEpo in patients with Hb levels < 10 g/dL.[6,7]

Although tissue hypoxia is the fundamental physiologic stimulus that increases renal secretion, a number of clinical observations suggest that other factors might be involved in the regulation of Epo production and/or may influence serum concentration. Abnormally high Epo levels have been reported in patients with aplastic anemia,[8,9] and dramatic changes in serum levels have been described after chemotherapy[10,11] and during vitamin B12 or iron replacement therapy.[12,13] These findings point to an inverse relationship between red blood cell precursor mass and sEpo levels.[14] In this study, we performed studies to evaluate whether the red blood cell precursor mass is an independent determinant of sEpo concentration.

MATERIALS AND METHODS

Patients. This study was designed to evaluate whether the red blood cell precursor mass can directly and independently influence sEpo levels. Therefore, we planned to study: (1) sEpo levels in patients with low versus high erythroid activity; (2) the time course of sEpo in patients undergoing myeloablative therapy for bone marrow transplantation (BMT); and (3) the time course of sEpo in anemic patients with iron deficiency or vitamin B12 deficiency undergoing specific replacement therapy.

Anemic patients with low versus high erythroid activity. To identify any effect of the erythroid marrow activity on sEpo concentration, we selected two groups of anemic patients: one with defective erythroid proliferation and decreased numbers of erythroid precursors (hypopro-

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liferative anemia), the second one with ineffective erythropoiesis and elevated numbers of marrow immature red blood cells (proliferative anemia). The degree of erythroid proliferation was evaluated through the serum transferrin receptor (sTfR) level (see below). Patients with severe aplastic anemia (n = 11), pure red blood cell aplasia (PRCA, n = 7), or mild hypoplastic anemia (n = 9) having values for erythroid proliferation below the normal range (sTfR < 3 mg/L) were included in the hypoproliferative anemia group. Twenty-eight individuals with β-thalassemia intermedia having sTfR levels > 10 mg/Ll were included in the group of anemic patients with high erythroid activity.

Sequential studies in patients receiving myeloablative therapy or conventional chemotherapy. Twenty patients undergoing allogeneic (n = 14) or autologous (n = 6) BMT were investigated immediately before undergoing myeloablative therapy and on day 0. Previous studies on evolution of erythropoiesis and Epo after BMT[15] showed that sEpo had a peak value on day 0, while sTfR decreased sharply after conditioning to a minimum on day 14. Therefore, for the purpose of this study, we decided to assay sEpo and sTfR before transplant and on day 0. Similar studies were performed in five patients undergoing chemotherapy for non-Hodgkin's lymphoma.

Sequential studies in patients with iron deficiency anemia treated with intravenous (IV) iron saccharate. The five patients with severe iron deficiency anemia had a mean Hb level of 6.4 ± 1.4 g/dL (range, 4.2 to 7.4 g/dL) and a mean serum ferritin of 5 ± 4 μg/L (range, 2 to 10 μg/L). They received IV iron therapy (iron oxide saccharate, Ferrum Hausman, Laboratorien Hausman, St. Gallen, Switzerland). The total amount of iron required was calculated according to the following formula[16]: total dose (mg) = [Hb deficit (g/dL) × estimated blood volume (dL) × 3.4] + 500, where Hb deficit is the difference between 15 and the patient's Hb level, blood volume is estimated according to sex and body surface,[17] 3.4 is the factor converting g Hb to mg iron, and 500 is an arbitrary quantity to allow for restoration of the iron reserve. The daily dose was 100 or 200 mg of iron saccharate: this amount was diluted in 250 mL of normal saline and infused IV over 1 hour.

Case reports. Additional studies were performed in two patients with megaloblastic anemia due to vitamin B12 deficiency or folic acid deficiency and in one patient with pure red blood cell aplasia after autologous BMT for treament of non-Hodgkin's lymphoma.

In the treatment of megaloblastic anemia, vitamin B12 was administered intramuscular (IM) as cyanocobalamin, 500 μg per day; folic acid was administered IM at a dose of 15 mg per day.

The patient with PRCA was previously reported.[18] He was given high doses of rHuEpo (150 U/kg per day subcutaneously [SC], 5 days a week) based on previous observations on the use of Epo in the treatment of PRCA after stem cell transplantation (reviewed in our previous report[18]). During treatment, sEpo was measured on Monday morning, ie, approximately 72 hours after the last SC rHuEpo administration. In normal individuals receiving SC rHuEpo, sEpo increases from basal levels of 10 to 20 mU/mL to peak values of 30 to 40 mU/mL after about 12 hours and then decreases with a half-life of about 24 hours (see review by Cazzola et al[5]).

Hematologic profile. Blood counts were determined with a Coulter Counter Model S (Coulter, Hialeah, FL). Reticulocyte counts were performed with an automated reticulocyte analyzer Sysmex R-3000 (Toa Medical Electronics GmbH, Hamburg, Germany). This system performs reticulocyte analysis using flow cytometry, with an argon laser as the light source. Whole blood specimens stained with a fluorescent dye pass through a sheath flow cell, where fluorescently-labeled cells are irradiated with a laser beam and thus produce forward scatter and fluorescence. The scatter and fluorescence are detected as indicator of the relative cell size and the RNA content, respectively. Reticulocyte count is expressed both as an absolute number per μL and as a percentage of red blood cells. Dividing the reticulocyte area of the scattergram into three sections according to the fluorescent intensity, reticulocytes can then be fractionated into maturity stages: HFR (high fluorescence ratio, immature reticulocytes), MFR (middle fluorescence ratio, intermediate reticulocytes), and LFR (low fluorescence ratio, mature reticulocytes).

Serum erythropoietin assay. Circulating Epo levels were measured by a commercially available radioimmunoassay (Incstar Corp, Sillwater, MN) that uses rHuEpo for tracer and standards.[4] 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(epo) = 3.42 − (0.056 × Hct). For Hct values >40%, the regression equation was: log(epo) = 1.31 − (0.003 × Hct). Based on these equations, the observed/predicted log(epo) 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).

Measurement of sTfR. The amount of circulating transferrin receptor was estimated by an enzyme-linked polyclonal antibody assay, using purified placental receptor-transferrin complexes as a reference standard and rabbit antibodies as described in detail elsewhere.[4] The mean sTfR level in 165 normal control subjects was 5.0 ± 1.1 mg/L, with a normal range from 3 to 7 mg/L.

Data analysis and presentation. Data were stored, analyzed, and reported with the packages STATISTICA/Mac (StatSoft, Tulsa, OK), Exstatix (Select Micro Systems Inc, Yorktown Heights, NY), and DeltaGraph Pro 3 (DeltaPoint Inc, Monterey, CA), all run on a Macintosh Quadra 800 (Apple Computer Inc, Cupertino, CA) personal computer. Results were expressed as mean ± 1 standard deviation (SD) unless otherwise stated. The Student's *t* test and/or the F test (one-way analysis of variance [ANOVA]) were used to evaluate the probability of significant differences between groups. Multivariate ANOVA was used to show any significant difference in the regression of serum sEpo to Hb level in different groups. *P* values less than .05 were considered statistically significant.

As discussed below, the number of erythroid cells in the BM may directly influence the Epo clearance: the higher the erythroid activity, the lower sEpo level. To account for this effect of erythroid activity on sEpo levels, the following correction was made:

$$\text{Corrected sEPo (mU/mL)} = \text{measured sEPo (mU/mL)} \times \frac{\ln \text{sTfr(mg/L)}}{\ln 5\ \text{(mg/L)}}$$

where 5 mg/L is the mean normal value for sTfR, taken as a measure of erythroid activity. For several reasons, including the impossibility of distinguishing between erythroid and nonerythroid TfR at the lowest sTfR levels, an additional empirical correction was introduced: the minimum value for ln sTfR was set to 0.2. Any time the calculated value was < 0.2, it was changed to 0.2.

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, $P \geq .05$) between the 27 patients with low erythroid activity

Table 1. Hb Level, sTfR, and sEpo in Anemic Patients With Low Erythroid Activity (Hypoproliferative Anemia) Versus Anemic Patients With High Erythroid Activity (β-Thalassemia Intermedia)

Condition	Hb Level (g/dL)	sTfR (mg/L)	sEpo (mU/mL)
Hypoproliferative anemia (n = 27)	8.3 ± 1.5 (5.5-10.4)	1.8 ± 0.8 (0.4-2.9)	1,601 ± 1,541 (172-6,030)
β-thalassemia intermedia (n = 28)	8.0 ± 1.3 (5.9-10.6)	23.5 ± 11.4 (10.4-48.6)	235 ± 143 (70-619)
Normal range	12-16 (females) 13.5-17.5 (males)	3.0-7.0	5-30

(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 aplastica anemia or PRCA (Table 1).

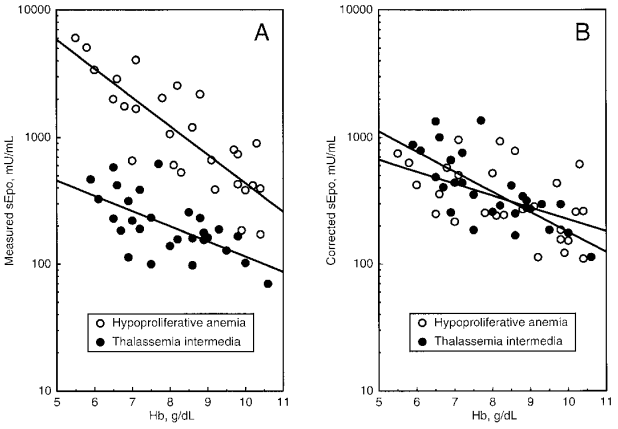

Fig 1. Relationship of sEpo to Hb observed in 27 patients with hypoproliferative anemia having erythroid activity <0.6 times normal (○) versus 28 patients with β-thalassemia intermedia having erythroid activity >2 times normal (●). (A) Relationship of measured sEpo to Hb level. Multivariate ANOVA showed 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 ($P > .05$).

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

Time	Hb (g/dL)	sTfR (mg/L)	sEpo (mU/mL)
Pretransplant	9.1 ± 1.2	6.0 ± 1.6	66 ± 37
Day 0	8.6 ± 1.2	2.1 ± 0.5	254 ± 141

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.

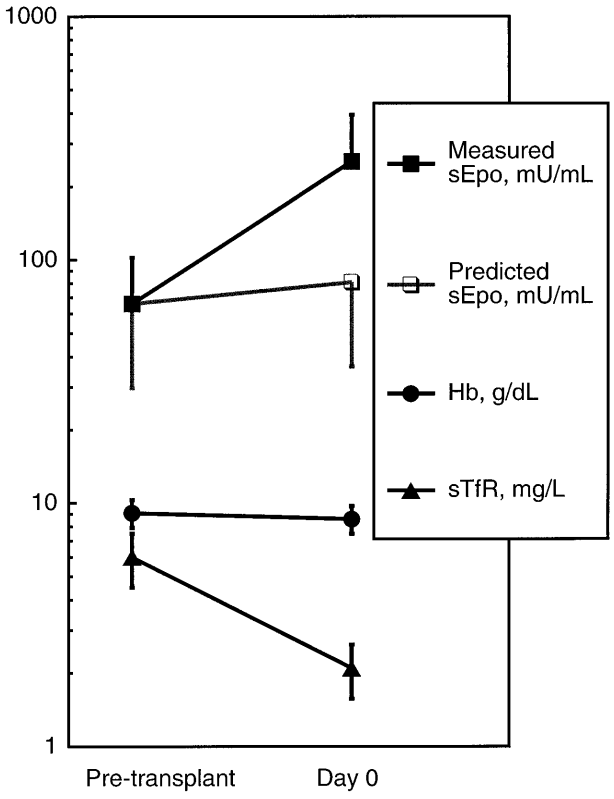

Fig 2. Time course of Hb level, sEpo, and circulating transferrin receptor in 20 patients undergoing BMT. Data are mean values ± 1 SD. Observed values before myeloablative therapy and those on day 0 are shown. Predicted sEpo values were calculated on the basis of the patient's Hct using the equation derived from regression analysis as previously described.[4]

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 despicted 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.

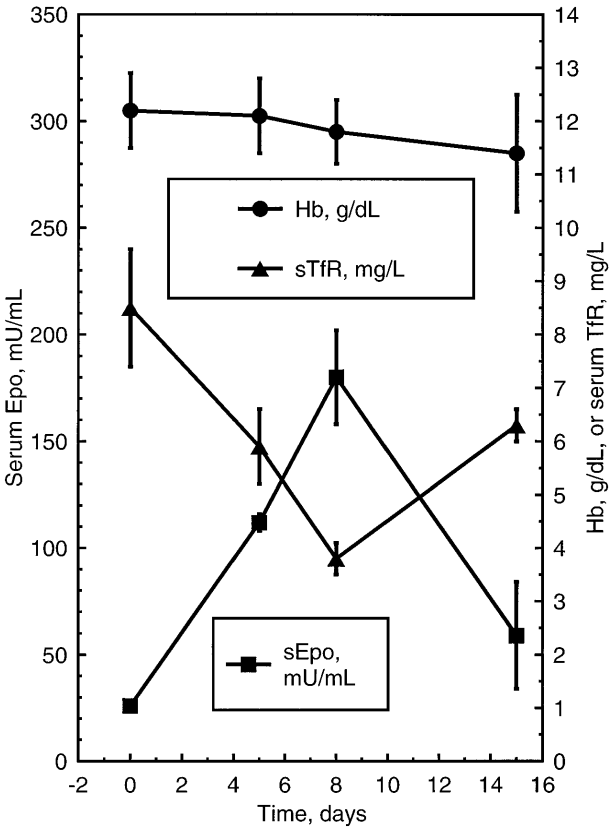

Fig 3. Time course of Hb level, sEpo, and circulating transferrin receptor in five patients with non-Hodgkin's lymphoma undergoing conventional chemotherapy (CHOP regimen). Data are mean values ± 1 SEM. One way ANOVA showed that Hb level did not change significantly during the observation period ($P > .05$), whereas both the decrease in circulating transferrin receptor (sTfR, F = 6.02, $P < .01$) and the mirror increase in sEpo (F = 14.54, $P < .001$) were found to be significant changes.

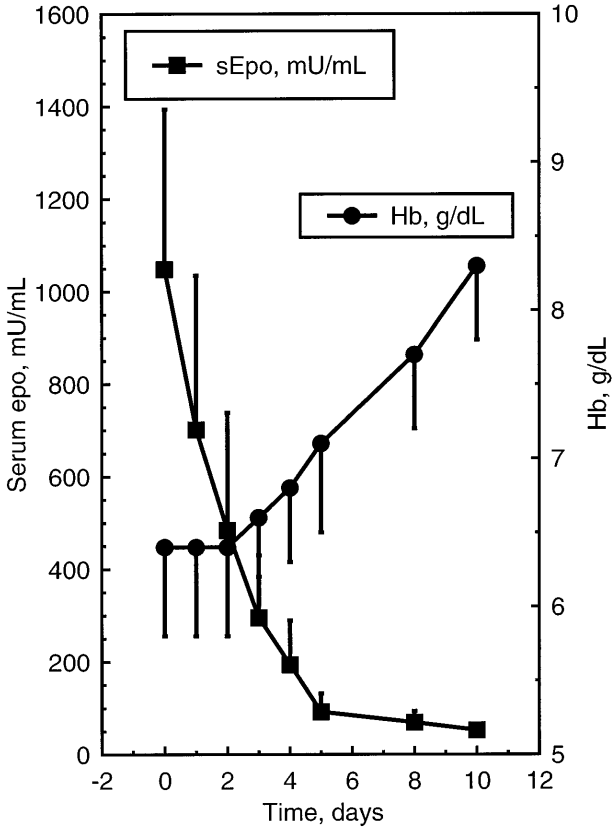

Fig 4. Time course of Hb level and sEpo in five patients with iron deficiency anemia treated with IV iron saccharate from day 0. Data are mean values ± SEM. Within 48 hours, sEpo fell from 1,049 ± 772 mU/mL to 485 ± 567 mU/mL ($P < .01$), whereas Hb level did not change significantly (6.4 ± 1.4 *v* 6.4 ± 1.2, $P > .057$).

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,[18] 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.[19,20] However, a number of additional pathophysiopathologic factors, including inflammatory cytokines[21] and plasma viscosity,[22] 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[8,9] 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,[23] sickle cell anemia,[24] thalassemia,[25] and megaloblastic anemia[26] 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.[10,11,27-30] 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.

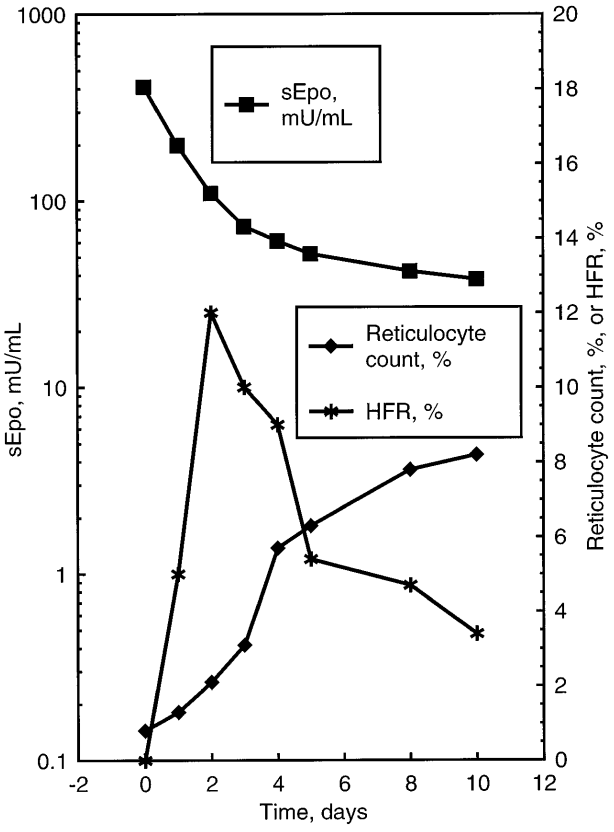

Fig 5. Time course of sEpo, reticulocyte count, and HFR in a patient with iron deficiency anemia treated with IV iron saccharate from day 0. HFR, ie, the most immature reticulocytes, expressed as % of total reticulocytes.

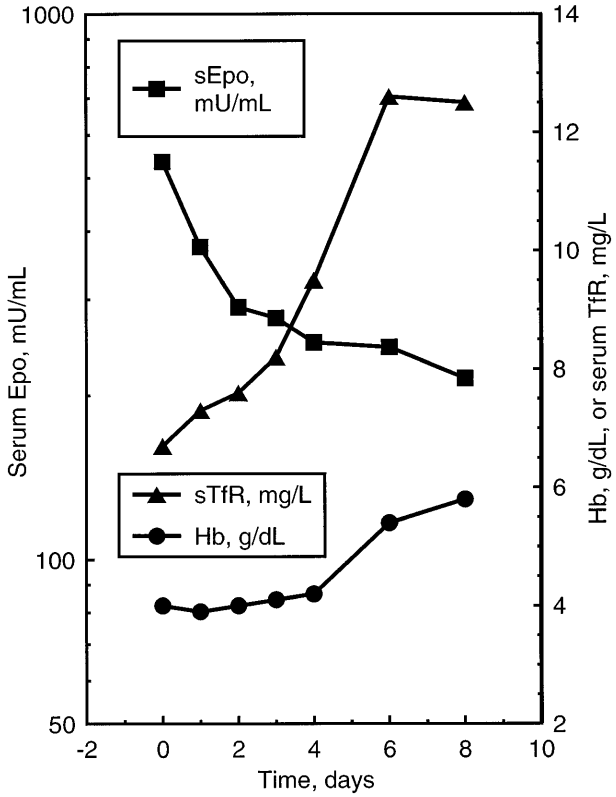

Fig 6. Time course of Hb level, sEpo, and sTfR in a patient with megaloblastic anemia due to vitamin B12 deficiency treated with vitamin B12 (IM as cyanocobalamin, 500 μg per day). A marked decrease in serum Epo was seen after the first injection and before any increase in Hb level. There was a parallel increase in serum transferrin receptor, indicating a rapid expansion of the erythroid marrow during the first days of treatment.

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,[31] cobalamin deficieny does not raise Epo level per se, but only to the extent that it produces anemia.[32] 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.[18] 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

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 al[33] 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 al[34] 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 inadequate 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

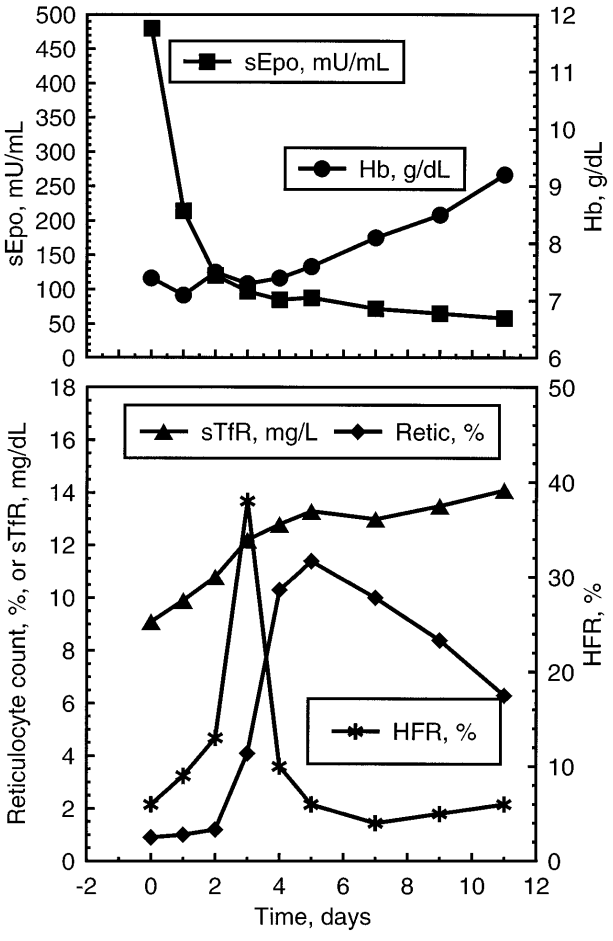

Fig 7. Time course of Hb level and sEpo (upper panel) and of sTfR, reticulocyte count and HFR (lower panel) in a patient with megaloblastic anemia due to folate deficiency treated with folic acid (15 mg per day IM). A marked decrease in serum Epo was seen after the first injection and before any increase in Hb level. There was a parallel increase in HFR, sTfR, and reticulocyte count, indicating a rapid expansion of the erythroid marrow during the first days of treatment.

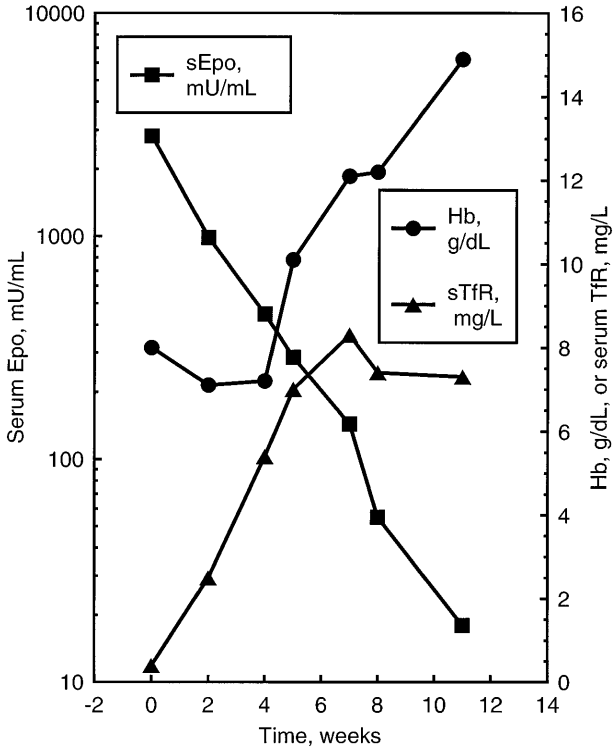

Fig 8. Time course of Hb level, sEpo, and sTfR in a patient with PRCA responding to treatment. The patient was given rHuEpo at an initial dose of 150 U/kg per day SC, 5 days a week; dosage was reduced to three weekly administrations when Hb level achieved 12 g/dL and treatment was discontinued after 8 weeks. Serum Epo started to decrease as erythroid marrow activity reappeared, before any change in Hb level.

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.

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