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original paper

Influence of marrow erythropoietic activity on serum erythropoietin levels after autologous hematopoietic stem cell transplantation

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ABSTRACT

Background and Objective. Serum erythropoietin (sEpo) concentration depends primarily on the rate of renal production in response to hypoxia. However, sEpo levels increase inappropriately after conditioning for autologous stem cell transplantation (ASCT) before progressively returning to adequate levels. We investigated the possible influence of erythropoietic activity on these observations.

Design and Methods. Forty patients undergoing an ASCT, 8 with bone marrow (BMT) and 32 with peripheral blood stem cells (PBSC), were separated into 3 groups. Group 1 was formed of the 8 BMT patients (median time to 1% reticulocytes: 39 days), group 2 of 16 PBSC patients with relatively slow erythroid engraftment (≥ 15 days to 1% reticulocytes, median 19 days) and group 3 of 16 PBSC patients with prompt erythroid recovery (< 15 days to 1% reticulocytes, median 13 days). Marrow erythroid activity was assessed by serum transferrin receptor levels (sTfR). Serum Epo (sEpo) levels were expressed in relation to the degree of anemia as observed/predicted (O/P) ratios of (O/P) log (sEpo).

Results. Serum sTfR levels decreased by more than 50% in all 3 groups after conditioning, reaching their nadir on day 7. Nadir values doubled by day 28 in group 3, day 60 in group 2, but not within 100 days in group 1. O/P sEpo ratios increased inappropriately in all 3 groups after conditioning but then declined at very differing speeds in the 3 groups. In group 1, ratios remained above 1.10 through to day 28 and above 1.00 through to day 42, before leveling off at around 1.00 thereafter. In group 2, ratios remained above 1.00 through to day 14, than decreased to a minimum of 0.89 by day 42 before returning to 1.00 by day 100. In group 3, ratios decreased to 0.84 by day 21 and remained below 0.90 thereafter.

Interpretation and Conclusions. We conclude that sEpo levels are not only influenced by tissue oxygenation but also depend on the mass of erythroid precursors in the bone marrow. This may be the main explanation for the observed changes in sEpo levels during ASCT.

Key words: serum erythropoietin, soluble transferrin receptor, hematopoietic stem cell transplantation, erythropoiesis

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Erythropoietin (Epo) is predominantly produced by the kidney but as there are no pre-formed stores of Epo any increase in the rate of production must be preceded by Epo gene transcription.[1] Epo production is regulated by a feedback mechanism by which the blood oxygen content is maintained at a constant level through the function of a kidney oxygen sensor.[2-4] The major determinant of Epo production is therefore the circulating red cell mass, and serum Epo levels thus increase exponentially as the hemoglobin or hematocrit decreases.[5,6]

The adequacy of serum Epo levels is best assessed by the observed/predicted (O/P) ratio, a value below 1 indicating that Epo production is lower than expected for the degree of anemia.[7] However, a number of clinical observations suggest that the regulation of serum Epo levels depends not only on the rate of Epo production but also on the red cell precursor mass. Abnormally high serum Epo levels have been encountered in patients with marrow aplasia[8-11] and dramatic changes have been observed at the beginning of vitamin B12 or iron therapy before any increase in hemoglobin could occur.[11-13]

In particular, inappropriately high serum Epo levels were observed after intensive chemotherapy.[14,15] These levels were even higher after conditioning before autologous or allogeneic bone marrow transplantation (BMT).[11,15-25] Serum Epo levels usually peaked one week after BMT, before progressively returning to values appropriate for the degree of anemia in autologous BMT recipients.[18,23-25] However, after allogeneic BMT, serum Epo levels rapidly became inadequately low and remained so for several months.[18,22,24,25] This was shown to be the consequence of cyclosporine administration[26,27] but other factors, such as GVHD[18,21,24,25] or CMV infection[18,24] are also involved. Therefore, autologous – but not allogeneic – hematopoietic stem cell transplantation (ASCT) represents an ideal setting for investigating the effect of acute variations in the erythroid precursor mass on serum Epo levels. In this study, we undertook an examination of the temporal relationship between erythropoietic activity and serum Epo levels during autologous transplantation of bone marrow or peripheral blood stem cells with varying speeds of engraftment.

Materials and Methods

Patients

We studied a total of 40 patients, 20 women and 20 men, aged 6 to 61 yrs, undergoing an ASCT for high-risk malignancies. There were 4 patients with Hodgkin's disease, 13 with non-Hodgkin's lymphoma, 7 with multiple myeloma, 2 with acute lymphocytic leukemia, 1 with acute myelogenous leukemia, 8 with breast cancer and 5 with other miscellaneous solid tumors. Conditioning before transplantation consisted of various combinations of high-dose chemotherapy with (n=7) or without (n=33) total body irradiation (TBI). No patient experienced severe liver toxicity. Eight patients were transplanted with autologous bone marrow (BMT) and 32 with autologous peripheral blood stem cells (PBSC). Post-transplant G-CSF (5 µg/kg) was given to 28 PBSC patients but to none of the BMT patients. All patients survived beyond day 100. Eight of them died later, 6 of their malignant disease, 1 of sepsis and 1 of a second cancer. Patients were separated into 3 groups on the basis of their speed of erythroid engraftment. Group 1 consisted of the 8 BMT patients (median time to 1% reticulocytes 39 days), group 2 of 16 PBSC patients with relatively slow erythroid engraftment (≥ 15 days to 1% reticulocytes, median 19 days) and group 3 of 16 PBSC patients with prompt erythroid recovery (< 15 days to 1% reticulocytes, median 13 days).

Laboratory analyses

Complete blood counts were determined in a Technicon H2 cell counter (Bayer, Tarrytown, NJ, USA). Percentages of reticulocytes were obtained by an automated cytofluorometric method.[28] Serum soluble transferrin receptor (sTfR), a quantitative measure of total erythropoietic activity, was measured by an ELISA in which each sample was run in triplicate.[7,29] Mean±SD in 165 normal subjects was 5,000±1,050 ng/mL. Serum erythropoietin (Epo) levels were measured by a commercially available radioimmunoassay (Incstar Corp., Stillwater, MN, USA). Based on regression equations obtained in appropriate reference subjects between Hct on the one hand and log (Epo) on the other, predicted log (Epo) values were derived for each Hct and observed/predicted (O/P) ratios of O/P Epo values were calculated.[7] The 95% confidence limits for O/P Epo in reference subjects were 0.80-1.20.[7]

Statistical methods

Student's t-tests, with pooled or separated variances as appropriate, were used to compare two groups. Analyses of variance (ANOVA), with Snedecor's F-test or Welch's test as appropriate, were used to compare more than two groups. Times to hematopoietic recovery were studied by life table analyses and Wilcoxon rank tests were used for comparison between groups. Statistical analyses were done using Microsoft Excel (Microsoft Corp, Redmont, WA, USA) and Graphpad Prism (Graphpad Software, San Diego, CA, USA) programs.

Results

The speed of engraftment was significantly different among the 3 groups. By definition, erythroid engraftment (as assessed by time to reach 1% reticulocytes) was slower in group 2 than in group 3, and even more so in BMT patients (Figure 1). The speed of platelet engraftment showed the same differences between the 3 groups (Figure 2). Neutrophil recovery was slightly faster in group 3 than in group 2, but was significantly delayed in group 1 (Figure 3).

Figure 4 shows the evolution of mean Hct values in the 3 groups, confirming the faster erythroid engraftment in group 3 compared to group 2. Hct values in

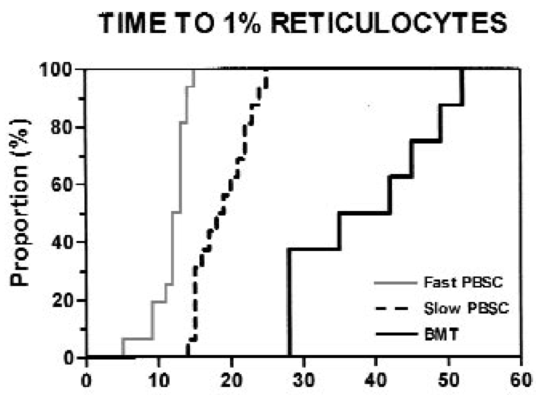

Figure 1. Time to reach 1% reticulocytes in group 1 (BMT), group 2 (PBSC with slow erythroid engraftment) and group 3 (PBSC with rapid erythroid engraftment) (p<0.0001).

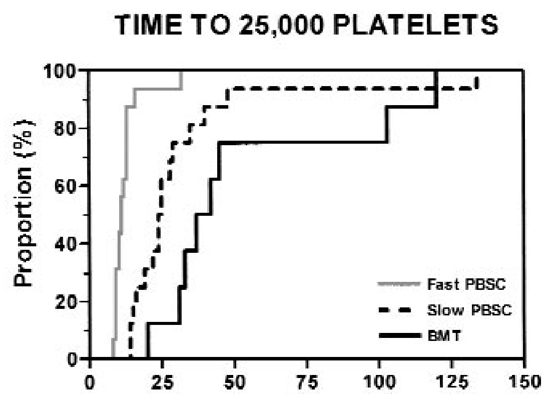

Figure 2. Time to reach 25,000 platelets without further transfusion in group 1 (BMT), group 2 (PBSC with slow erythroid engraftment) and group 3 (PBSC with rapid erythroid engraftment) (p<0.0001).

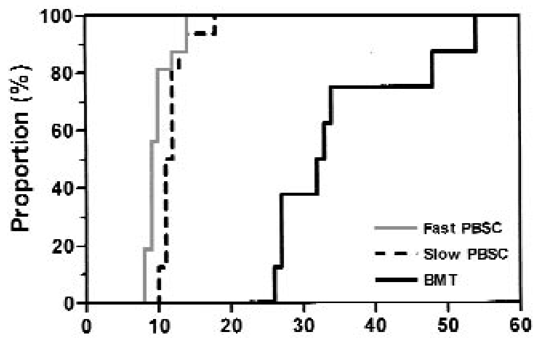

Figure 3. Time to reach 500 neutrophils in group 1 (BMT), group 2 (PBSC with slow erythroid engraftment) and group 3 (PBSC with rapid erythroid engraftment) (p<0.0001).

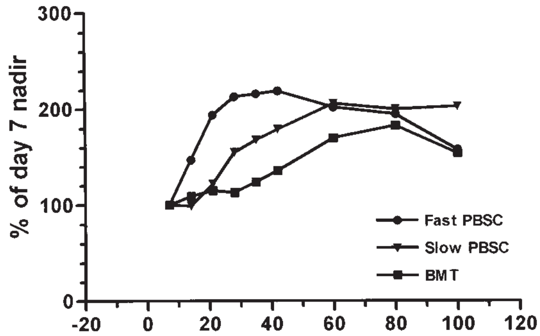

Figure 5. Mean serum sTfR levels, expressed as percentages of day 7 nadir values, in group 1 (BMT), group 2 (PBSC with slow erythroid engraftment) and group 3 (PBSC with rapid erythroid engraftment) up to day 100 post-transplant.

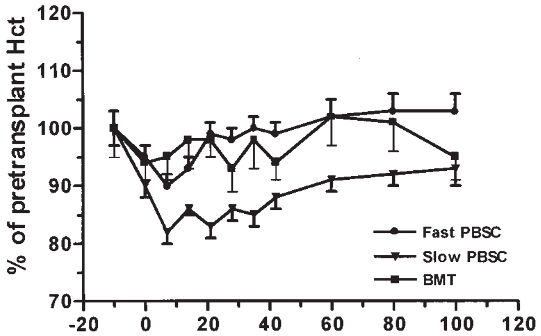

Figure 4. Mean Hct values, expressed as percentages of pre-transplant Hct, in group 1 (BMT), group 2 (PBSC with slow erythroid engraftment) and group 3 (PBSC with rapid erythroid engraftment) up to day 100 post-transplant.

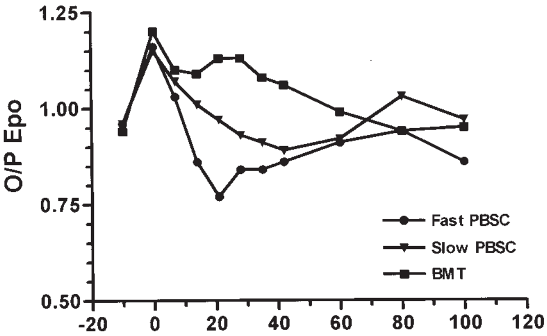

Figure 6. Median O/P Epo ratios in group 1 (BMT), group 2 (PBSC with slow erythroid engraftment) and group 3 (PBSC with rapid erythroid engraftment) up to day 100 post-transplant.

BMT patients were relatively higher because the transfusion trigger in these patients was a Hct value below 30% instead of 27% for PBSC patients. Serum sTfR levels decreased by more than 50% in all 3 groups after conditioning to reach their nadir on day 7. Nadir values doubled by day 28 in group 3, day 60 in group 2, but not within 100 days in group 1 (Figure 5). Before conditioning, O/P Epo ratios were similar in the 3 groups (0.94, 0.96, and 0.96, in groups 1, 2 and 3, respectively). After conditioning all 3 groups showed an abnormal elevation of O/P Epo ratios to 1.20, 1.16 and 1.15, respectively (ns). After transplantation, O/P Epo ratios declined at very different speeds in the 3 groups (Figure 6). In group 1, ratios remained above 1.10 through to day 28 and above 1.00 through to day 42, before leveling off at around 1.00 thereafter. In group 2, ratios remained above 1.00 through to day 14, than decreased to a minimum of 0.89 by day 42 before returning to 1.00 by day 100. In group 3, ratios decreased to 0.84 by day 21 and remained below 0.90 thereafter.

Discussion

Serum Epo levels may vary considerably.[5,6,30] Levels are usually between 10 and 20 mU/mL in normal subjects, may decrease somewhat in primary polycythemia, but increase exponentially when an anemia develops below an Hct of 30-35%. Therefore, a serum Epo value must always be evaluated in relation to the degree of anemia, for instance through the O/P ratio.[7] Epo levels inappropriately low for the degree of anemia are encountered not only in renal failure,[31] but also in a number of other conditions, particularly in the anemia of chronic disorders,[32] including the anemia associated with cancer.[33] Inappropriately high serum Epo levels are often observed in secondary polycythemia, a feature permitting its diagnostic separation from primary polycythemia.[34] Such inappropriately high levels are also encountered in patients with aplastic anemia (erythropoietic activity < 0.6 times normal) compared to subjects with iron deficiency anemia (erythroid activity relatively normal) or thalassemia intermedia (erythroid activity > 2 times normal).[8-11]

High serum Epo levels are also observed transiently after intensive chemotherapy, whether followed by bone marrow transplantation or not, without concomitant changes in hemoglobin or hematocrit.[11,14-25] As shown again in our study, the peak Epo values are observed 7 days after transplantation, i.e. about 14 days after the start of the conditioning regimen, at the time of the nadir of erythropoietic activity. As there are no preformed stores of Epo, this cannot be due to a sudden release of Epo by the kidney mediated by cytostatic drugs. Some other speculations have been offered as explanations for this phenomenon.[20] Cytotoxic therapy could cause a direct injury to the Epo-producing cells mimicking hypoxia. The blood flow to the kidney and/or liver could be altered so as to expose Epo-producing cells to hypoxia. As protein synthesis and gene transcription are necessary for the normal degradation of Epo mRNA, it is also possible that cytotoxic therapy could enhance Epo mRNA stability. However, some experimental data contradict these hypotheses. Cobalt-induced Epo production was markedly suppressed 18-36 hours after the infusion of the alkylating agents chlorambucil or thiotepa through dog kidneys isolated *in situ*.[35] In cultures of the hepatoma cell line HepG2, vincristine as well as the RNA synthesis-inhibiting drugs daunorubicin, cyclophosphamide, ifosfamide, and CDDP dose-dependently decreased Epo production, while the DNA synthesis-inhibiting drugs methotrexate and cytosine-arabinoside did not have inhibitory properties.[36,37] These results suggest that chemotherapeutic agents do not stimulate Epo production locally.

These findings thus point to an inverse relationship between marrow erythropoietic activity and serum Epo levels: the higher the number of erythroid precursors, the lower the serum Epo value. As Epo exerts its action on target cells after binding to a specific Epo receptor,[38] it is tempting to speculate that serum Epo levels may partly depend on the rate of Epo utilization by Epo receptor-bearing cells, primarily erythroid precursors.[11,39] Therefore, severe myelosuppression following the conditioning regimen could disrupt the usual Epo degradation pathway and provoke a surge of serum Epo concentration through prolonged Epo life span. Similarly, marrow recovery after ASCT would restore Epo utilization by erythroid cells, thus progressively returning Epo levels to a range appropriate for the degree of anemia. Furthermore, the duration of this correction phase would depend on the speed of engraftment: the faster the erythroid recovery, the shorter the time necessary for a complete return to adequate Epo levels. Indeed, our patients with particularly intense erythropoietic activity (group 3) even exhibited somewhat decreased Epo levels during their recovery phase. All these surmises are well illustrated by the mirror evolutions of serum Epo (Figure 6) and sTfR (Figure 5) levels in our 3 study groups.

The idea that marrow utilization influences serum Epo levels was initially based on the observation that radiation-induced marrow hypoplasia was associated with a slower decline of serum Epo levels induced by hypoxia.[40] However, the rate of Epo disappearance from the plasma of dogs with normal, hypoplastic or hyperplastic marrow, was later shown to be similar, whether the experiment was performed in nephrectomized[41] or unmanipulated[42] animals. Epo accumulation in the kidney and bone marrow of rats was minimal after intravenous injection of a tracer dose of rHuEpo.[43] Furthermore, erythropoietin life span was similar in normal rats and in rats with bone marrow suppressed by cyclophosphamide or hypertransfusion or stimulated by hemolysis or bleeding.[44] The pharmacokinetics of rHuEpo in hemodialysis patients was not different before and after 6 weeks of treatment with rHuEpo.[45] Therefore, variations observed in serum Epo levels after intensive chemotherapy cannot simply be explained by changes in Epo consumption by the bone marrow. Indeed, experiments on hypobaric hypoxemia in mice previously treated or untreated with rHuEpo suggested that variations in plasma Epo levels during periods of rapidly expanding erythropoiesis are the reflection of a decrease in the rate of production rather than in the rate of utilization by proliferating erythroid precursors.[46] Therefore, whereas it is indisputable that marrow erythropoietic activity independently influences serum Epo levels, it is possible that this happens through some other (yet to be elucidated) mechanism by which marrow erythropoiesis influences the rate of Epo production by the kidney. Some other factors linking the erythron to Epo production may also exist. For instance, products resulting from red cell hemolysis may indirectly stimulate marrow erythropoietic activity as well as renal Epo production.[47,48]

We conclude that serum Epo levels are not only influenced by tissue oxygenation but also depend on the mass of erythroid precursors in the bone marrow. This is particularly true in the course of ASCT, but should also be taken into account when interpreting the adequacy of a serum Epo value in other situations. It remains to be determined whether the erythroid precursor mass acts directly by utilizing circulating Epo or indirectly by influencing the rate of Epo production.

Contributions and Acknowledgments

YB was responsible for the conception of the study and the writing of the paper. FB was responsible for data handling. GF was responsible for the conception of the study and revision of the paper.

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Disclosures

Conflict of interest: none.

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