Serial immunoreactive erythropoietin levels in autologous blood donors

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The variations in plasma erythropoietin (EPO) concentration during preoperative deposit of autologous blood were studied in 12 patients (8 men, 4 women). Four donations were scheduled at weekly intervals. A predonation hemoglobin concentration of 11 g per dL (110 g/L) was required. Hemoglobin concentration decreased from 14.3 ± 1.1 g per dL (143 ± 11 g/L) (mean ± SD) before the first donation to 11.7 ± 0.7 g per dL (117 ± 7 g/L) on Day 22 (p<0.0001). Reticulocyte counts increased from a median of 31,800 (range, 4900-95,000) per μL (median, 32 × 10⁹/L [range, 5-95 × 10⁹/L]) to 93,800 (16,800-194,900) per μL (median, 94 × 10⁹/L [range, 17-195 × 10⁹/L]) on Day 28 (p<0.01). Plasma EPO concentration was 17.8 ± 5.1 mU per mL prior to the first donation and displayed a small and transient peak after each donation. A sustained elevation followed each peak. Although plasma EPO concentration differed significantly from the baseline value after the first donation, only the peak concentrations after the second (35.5 ± 15.5 mU/mL), third (38.0 ± 14.5 mU/mL), and fourth (36.1 ± 11.0 mU/mL) donations exceeded the normal range. The moderate, biphasic increase in plasma EPO concentration and the moderate increase in erythropoiesis suggest two strategies in autologous blood donation that should be investigated with respect to efficiency and safety: 1) more aggressive donation schemes, which reduce donation intervals and/or the minimum hemoglobin concentration and 2) the administration of recombinant human EPO.

**Materials and Methods**

**Patients**

With their informed consent, we studied 12 consecutive patients scheduled for hip arthroplasty or revision hip arthroplasty and participating in the preoperative autologous blood deposit program. Anemia, coronary heart disease, congestive heart failure, uncontrolled hypertension, severe obstructive or restrictive pulmonary disease, infectious disease, cerebral sclerosis, and syncope were considered exclusion criteria for predonation hematocrits) to enhance endogenous EPO formation. Alternatively, recombinant human EPO may be administered, a treatment that is effective in the management of the anemia that occurs with renal failure and has recently been shown to allow the withdrawal of larger amounts of blood from animals who are phlebotomized repeatedly and from autologous blood donors.

To obtain a rational basis for such preoperative donation strategies, knowledge of the pattern of variations in endogenous EPO concentration following repeated phlebotomy would be helpful. A recent study suggested only slight increases during preoperative deposit. However, in that study, EPO values were determined only before each donation, and thus transient increases may have been missed. In our investigation, we measured plasma EPO concentration both before and after repeated phlebotomy for autologous blood donation.

**Abbreviations:** EPO = erythropoietin.
preoperative blood deposit. In addition, we excluded from the study patients with malignant disease and those with a serum creatinine level >1.3 mg per dL (100 μmol/L).

Of the patients studied, 8 were men, and 4 were women. Their mean age was 58.0 ± 10.5 (± SD), and the range was 39 to 75 years. Their mean height was 167.3 ± 9.6 cm (± SD), with a range of 150 to 190 cm. Their mean weight was 70.1 ± 15.4 kg (± SD), with a range of 46 to 110 kg.

Blood donation

The aim of the program was to obtain four donations of 450 mL of blood at weekly intervals. A patient was deferred for 1 week if the hemoglobin concentration prior to donation fell below 11 g per dL (110 g/L). Fe(-II)-aspartate tetrahydrate (Spartocine, UBC Chemie, Kerpen, Germany), 350 mg, was prescribed three times daily, a dose that corresponds to three doses of 50 mg of Fe2+. Blood samples for blood cell counts, hemoglobin, and EPO determination were obtained before each donation and on the first, third, and fifth days thereafter. Serum iron and serum ferritin concentrations were determined at weekly intervals. The blood samples taken totalled 25 mL during the week after each donation appointment.

Determination of erythropoietin

We determined the EPO concentration in EDTA-plasma samples by radioimmunoassay as described previously, using a rabbit antiserum derived against recombinant human EPO, iodinated recombinant human EPO (Amersham Laboratories, Amsberg, UK) as a tracer and using the 2nd International Reference Preparation for EPO (WHO/International Laboratory for Biological Standards, London, UK) as a standard. The interassay coefficient of variation is 7 percent. The geometric mean EPO level for healthy adults is 17.9 mU per mL, and the 5 to 95 percentile is 11 to 31 mU per mL (n = 84).

Blood cell count

Hemoglobin concentration, red cell count, mean corpuscular volume, and white cell and platelet counts were determined by automated routine laboratory technique. Reticulocytes were counted manually.

Determination of serum ferritin and serum iron

Serum iron concentration was determined photometrically. Serum ferritin concentration was determined by immunofluorescence assay (normal range: men, 35-490 fig/L; women, 25-150 fig/L).

Calculated values

Blood volume was estimated according to equations (1a) and (1b).18

\[
\text{PBV} = 0.0236 \times Hb^{0.725} \times W^{0.425} - 1.229 \quad (\text{men}) \tag{1a}
\]

\[
\text{PBV} = 0.0248 \times Hb^{0.725} \times W^{0.425} - 0.425 \quad (\text{women}) \tag{1b}
\]

where PBV = predicted blood volume, H = height (cm), and W = weight (kg).

Red cell volume was estimated according to equation (2).19

\[
\text{EV} = \text{PBV} \times \text{Hct} \times 0.896 \tag{2}
\]

where EV = red cell volume and Hct = hematocrit (venous).

The recovery from red cell loss between donations, given that the hematocrit of the donated blood corresponded to that before donation and that the blood volume of each patient prior to donation did not change in the course of preoperative deposit,20 was estimated according to equation (3).

\[
\text{ER}_{n+1} = E_{n} - (\text{EV}_{n} + \text{DV}_{n} \times \text{Hct}_{n} \times 0.985) \tag{3}
\]

where ER = recovery from red cell loss between the nth and (n+1)th donations, EVn and EV = red cell volume of the nth and (n+1)th donations, DVn = the volume donated at the nth donation, and Hctn = (venous) Hct before the nth donation.

Statistics

The data were analyzed via statistical software.21 Sample distribution was tested for normality as described by Shapiro-Wilk.22 Samples were compared by the paired t test or the Wilcoxon signed-rank test, as appropriate. The significance level was set at 5 percent.

Results

The mean calculated blood volume in these 12 patients was 4550 ± 904 mL (± SD), and the range was 2819 to 6580 mL. The mean red cell volume before the first donation was 1646 ± 446 mL (± SD), and the range was 809 to 2519 mL. At the first and second appointments, all 12 patients donated 1 unit of blood; at the third appointment, 10 patients donated blood, and at the fourth appointment, 8 patients did so. A total of 4 units was preoperatively deposited by each of 8 patients, as was a total of 3 units by each of 2 patients and 2 units by each of another 2 patients.

The pattern of variation in the hemoglobin concentration is shown in Fig. 1. Repeated phlebotomies led to a gradual decrease in hemoglobin concentration from 14.3 g per dL (143 g/L) (mean) to 13.2, 12.6, 11.9, and 11.7 g per dL (132, 126, 119, and 117 g/L) (mean). Recovery from red cell loss by increased erythropoiesis can be estimated as 70 mL of red cells during the week after the first donation and 98 mL during the week following the fourth donation appointment. Reticulocyte count increased from a median of 31,800 (median, 32 × 10⁹/
L (range, 4900-95,000) per μL (range, 5.95 × 10^6/L) before the first donation to a median of 93,800 (94 × 10^6/L) (range, 16,800-194,900) per μL (17-195 × 10^6/L) on Day 28 (p < 0.01). Platelet and white cell counts did not change significantly.

Plasma EPO concentration before the first blood donation amounted to 17.8 ± 5.1 mU per mL (mean ± SD). As shown in Fig. 2, plasma EPO concentration displayed a small, transient peak after the first donation, a more pronounced peak after the second donation, and a longer-lasting peak after the third and fourth donations. A sustained elevation followed each peak. Although plasma EPO concentration was significantly elevated after the first donation, only those peak concentrations following the second, third, and fourth donations exceeded the normal range.

Of the variables associated with iron metabolism, serum iron concentration decreased significantly from a median of 170 (range, 40-400) μg per L to 50 (range, 20-210) μg per L (p < 0.01). Mean corpuscular volume (88 ± 4 μm^3 [88 ± 4 fl] prior to donation vs. 89 ± 5 μm^3 [89 ± 5 fl] on Day 28), and mean corpuscular hemoglobin (31 ± 2 pg vs. 32 ± 2 pg) did not change significantly during the time of investigation.

**Discussion**

After blood donation, blood volume is restored within 48 to 72 hours. The gradual fall of the hemoglobin concentration after each donation in our study complies with these results. Recovery from red cell loss, however, is slow in patients who exhibit the range of hemoglobin <10 g per dL (<100 g/L), would increase EPO levels by 100 mU per mL and more. Thus, the sensitivity of EPO secretion to changes in the hemo-
globin concentration appears rather low, in the range of low-normal to slightly subnormal hemoglobin values.

The rate of erythropoiesis can be increased to twice normal or more if the hematocrit is lowered to 32 to 37 percent (0.32-0.37). A total of 500 mL of blood can be donated in weekly intervals if iron is given orally in high dosage. After repeated donations, the hematocrit stabilizes near 30 percent (0.30). In our patients, we found a drop in the hemoglobin concentration of 15 percent 1 week after the fourth donation. The decline in hemoglobin concentration was associated with an increased erythropoiesis, as reflected by the elevation in reticulocyte counts and the estimated recovery from red cell loss. Whereas reticulocytosis represents both increased erythropoiesis and premature release of red cells from the bone marrow, the estimated recovery of red cell volume indicates a stimulation of erythropoiesis to twice the normal rate.

The oral iron supplementation prescribed constitutes a compromise between desirable dosage and low patient compliance resulting from gastrointestinal side effects. The drop in serum iron and serum ferritin concentrations shows that iron absorption cannot cope with increased demand. The serum ferritin concentrations measured, however, are usually not associated with depleted iron stores. It has also been demonstrated that, within the range of hemoglobin concentrations attained in the present study, sufficient iron for erythropoiesis can be supplied either from full iron stores or—in patients with little or no storage iron—from high-dose oral iron medication alone. Thus, iron availability was probably not the factor that limited erythropoiesis.

In the absence of iron deficiency, erythropoiesis is mainly determined by the availability of EPO. As the increase in EPO concentration and the stimulation of erythropoiesis are only moderate following repeated phlebotomy, different strategies may be adopted to increase EPO levels and erythropoiesis.

1. The application of exogenous recombinant human EPO has been shown to accelerate the recovery of red cell volume in phlebotomized animals as well as in autologous blood donors. Its use in healthy persons, however, is still hampered by the facts that not all side effects of the recombinant hormone may yet be known and that the reported side effects are not completely understood in regard to their causal mechanisms. If these concerns are overcome with increased experience in the treatment of chronic anemia with recombinant human EPO, its use may acquire a place in preoperative autologous blood donation.

2. It is tempting to speculate that modified donation schemes may result in more efficient stimulation of erythropoiesis. Reducing the time between donations, to 3 days, for example, may lead to the occurrence of EPO peak concentrations at shorter intervals. In view of the exponential inverse relationship between EPO levels and hematocrit, a reduction in the minimum predonation hematocrit could lead to a more significant increase in EPO concentrations. Whether such donation schemes do indeed increase EPO levels and erythropoiesis during autologous blood donation and decrease perioperative homologous blood requirements, and whether a lower minimum predonation hematocrit is acceptable in certain groups of patients remains to be investigated.

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