Is Erythropoietin a Survival Factor for Red Blood Cells?[1]

Momir Polenakovic[2] and Aleksandar Sikole

ABSTRACT

Recombinant human erythropoietin (rhuEPO) therapy has been reported to maintain corrected hematocrit values by increasing the length of red blood cell (RBC) survival. This article presents a controlled study that assessed the RBC survival before, during, and after termination of prolonged rhuEPO treatment of chronic hemodialysis patients. Two groups of 20 patients were studied. The hematocrit value of each patient was below 28 vol%. One group (Group A) was treated with rhuEPO for 1 yr and then treatment was stopped because of the unavailability of the drug. The second group (Group B) was treated for 2 yr. Epoetin beta was administered subcutaneously. The initial dose was 20 U per kg body weight three times weekly. Upon reaching the target hematocrit value of 30 to 35 vol%, the dose was individualized for each patient, to maintain target range. RBC survival was determined by the chromium-51 technique. In Group A, RBC survival was determined: (1) before, (2) at 12 months, and (3) 1 yr after cessation of rhuEPO treatment. In Group B, RBC survival was determined: (1) at 24 months of therapy, and (2) 1 yr after cessation of rhuEPO treatment. RBC survival increased significantly in both patient groups under rhuEPO treatment. After cessation of therapy, the RBC survival decreased to pretreatment values. During the correction period, reticulocyte counts increased significantly in both groups. Over the maintenance period, they slightly decreased, and after termination of rhuEPO therapy, they decreased to the pretreatment values. The results of this study could suggest the possibility that RBC survival was prolonged by the action of EPO on the erythroid progenitors, resulting in the production of RBC with improved viability.

Key Words: Kidney failure (chronic), hemodialysis, renal anemia, erythropoietin, red blood cell survival

Anemia of chronic renal failure is a well-established entity, and depending on the primary renal disease, may vary in its severity (1). The various factors that may underlie the anemia of chronic renal failure include: reduced availability of erythropoietin (EPO), inhibitors of erythropoiesis, bone marrow fibrosis, and hemolysis as a result of extra corpuscular factors. Renal anemia is of hypoproliferative, normocytic, and normochromic type, but certain deficiency states, such as iron, folate, or vitamin B_{12} deficiency, may alter its nature (1,2).

One of the features of renal anemia is shortened red blood cell (RBC) survival. Most radioisotopic studies confirm the presence of mild hemolysis in renal anemia cases. Although the cause for the hemolysis is not yet fully understood, several factors in the uremic patient have been incriminated: parathyroid hormone, spermine, and ribonuclease (1–3).

Recombinant human erythropoietin (rhuEPO) treatment has been very effective in the correction of renal anemia, both by intravenous and subcutaneous administration. It has been reported that rhuEPO maintains corrected hematocrit (Hct) at a desired level by increasing the length of RBC survival, and not by a sustained elevated reticulocyte count, despite marked reduction of the rhuEPO dose required to maintain the Hct level (4).

The purpose of the study was to assess the RBC survival before and after prolonged subcutaneous rhuEPO treatment in chronic hemodialysis patients. Furthermore, because of unfortunate circumstances, we had the unusual possibility to observe the change of the RBC survival after cessation of rhuEPO therapy.

MATERIAL AND METHODS

Patients

In a prospective clinical study, two groups of patients were treated with rhuEPO by the same protocol, but for different time intervals (5). Forty patients were selected for the study on the basis of inclusion and exclusion criteria, and they signed informed consents to participate in the study. All patients were older than 18 yr of age and were anemic, with a Hct value below 28 vol%. They were treated by chronic hemodialysis at least 6 months before entering the study. The hemodialysis regime included 4 to 4.5 h of dialysis three times per week, with an acetate bath and cellulosic membrane of 1.1 to 1.3 m^2. The blood flow ranged between 250 and 280 mL/min. The vascular access for hemodialysis was from a Cimino-Brescia arteriovenous fistula. The dialysis dose used before the study for each patient remained constant throughout the entire study period. There have been no significant changes in weight or in the predialysis serum urea and creatinine level in the patients. The exclusion criteria were: iron, folic acid, and vitamin B_{12} deficiency; severe infection; malignant or systemic disease; acute liver infection; uncontrolled hypertension; epilepsy; pregnancy; hemolytic-uremic syndrome; and thrombocytosis (>500,000/ mm^3).

The patients were randomized into two groups of 20 patients each, their basic clinical and demographic data were recorded (Table 1), and they were then enrolled in the prospective study.

Treatment Protocol

Each group of patients was treated by the following protocol. The initial dose of epoetin beta (Recormon; Boehringer-Mannheim, Mannheim, Germany) was 20 U per kilogram body weight (U/kg body wt), administered subcutaneously three times weekly, 10 min before each dialysis session. The dose could be increased by 20 U/kg body wt per single dose every 4 wk, if Hct rise was too slow or absent. This period was called the correction period. After reaching the target Hct of 30 to 35 vol%, the last administered dose was reduced by half and adjusted thereafter individually, to keep the Hct value in the range of 30 to 35 vol%. It was the maintenance period of the study. During the rhuEPO therapy, body iron stores were regularly monitored by determination of serum ferritin, transferrin, and total iron binding capacity. The patients were treated with oral iron therapy in the form of ferrous sulfate if serum ferritin level fell below 200 ng/mL or if transferrin saturation index fell below 20%.

One group of patients (Group A) was treated for 1 yr and treatment was then discontinued because of unavailability of the drug. The second group (Group B) was treated for 2 yr and the treatment was also then discontinued for the same reason.

Determination of RBC Survival

The following procedure was used: at the onset of a dialysis session, 10 mL of blood were withdrawn from the patient into a sterile 50-mL glass tube containing 2 to 3 mL acid-citrate-dextrose (Grade I NIH). One hundred mCi radioactive sodium chromate (Cr51) with high specific activity was then added to the mixture. After 30 to 40 min of incubation at room temperature with intermittent gentle agitation, the tube was centrifuged (1000 × g for 12 min at 25°C). The supernatant

TABLE 1. Demographic and basic clinical data of patients

Patients	Group A	Group B
Age (yr)	48.3 (range, 23 to 69)	47.6 (range, 25 to 70)
Sex		
Male	12	11
Female	8	9
Hemodialysis Duration (months)	47.6 (range, 21 to 111)	60.5 (range, 20 to 133)
Primary Renal Disease		
Chronic Glomerulonephritis (N)	6	5
Chronic Interstitial Pyelonephritis (N)	2	4
Calculous Pyelonephritis (N)		3
Balkan Nephropathy (N)	2	1
Hereditary Nephropathy (N)	2	2
Nephrosclerosis (N)	3	1
Uric Nephropathy (N)	2	
Undifferentiated Nephropathy (N)	3	4

was discarded using a sterile needle connected to a vacuum. Labeling efficiency was over 80%/dose measured in a dose calibrator. Approximately four-fifths of the Cr51-labeled RBC was injected intravenously into the patient at the end of the same dialysis session, and the needle was flushed by sterile saline. A heparinized blood sample of 5 mL was withdrawn after 24 h and was used as the "Day 0" sample. Subsequently, blood samples were drawn three times weekly at appropriate intervals for the next 3 wk or until the radioisotope concentration in the blood had decreased to one-half its original (Day 0) level. The data were plotted on semilog graphs and RBC survival was determined by regression analysis (6,7).

RBC survival was determined in both patient groups (8,9). In Group A, it was determined before the onset of rhuEPO therapy in 18 patients. Two patients were unavailable for determination. All 18 patients were tested again at 12 months of rhuEPO therapy. The third determination of RBC survival was performed 1 yr after cessation of rhuEPO treatment in 12 patients of this group.

In Group B, RBC survival was determined in 18 patients after 24 months of rhuEPO therapy. The second determination was made 1 yr after termination of therapy in 11 patients from this group.

Hematocrit (vol%) level was determined weekly, and uncorrected reticulocyte count (% peripheral RBC) was determined monthly over the entire study period, using standard laboratory methods. Throughout the study, all laboratory parameters were determined from blood taken before hemodialysis sessions.

Results were expressed as mean (M) ± SD. The t test was applied to observe the difference between different time intervals of the study.

RESULTS

Group A

During the correction period of 16 wk, Hct value had risen to the target range of 30 to 35 vol% in all patients, and was thereafter maintained within the target limits by low subcutaneous doses of rhuEPO. The highest mean rhuEPO dose during the correction period for the whole group reached 35 U/kg body wt per single dose. The mean maintenance dose varied between 20 and 30 U/kg body wt applied three times weekly.

The mean reticulocyte value before the study was 1.21 ± 0.55%. During the correction period, it rose to the maximum level of 2.22 ± 0.73% (P < 0.001). In the maintenance phase of the study, the mean reticulocyte count ranged between 1.32 ± 0.64% and 1.9 ± 0.86%, averaging 1.79 ± 0.16% (P < 0.001). After termination of rhuEPO therapy, the mean reticulocyte value was 1.26 ± 0.84% (P = not significant [NS]). The difference between the maximum level of 2.22 ± 0.73% and the average level of the maintenance period (1.79 ± 0.16%) was also significant (P < 0.05).

The RBC survival at different time intervals of the study for this group of patients are presented in Figure 1. Before the onset of epoetin treatment, RBC survival, i.e., mean Cr51 half-life (t½) was 23.3 ± 2.6 days, at a mean Hct value of 23.8 ± 2.1 vol%. At 12 months of treatment and mean Hct level of 33.2 ± 1.8 vol%, the
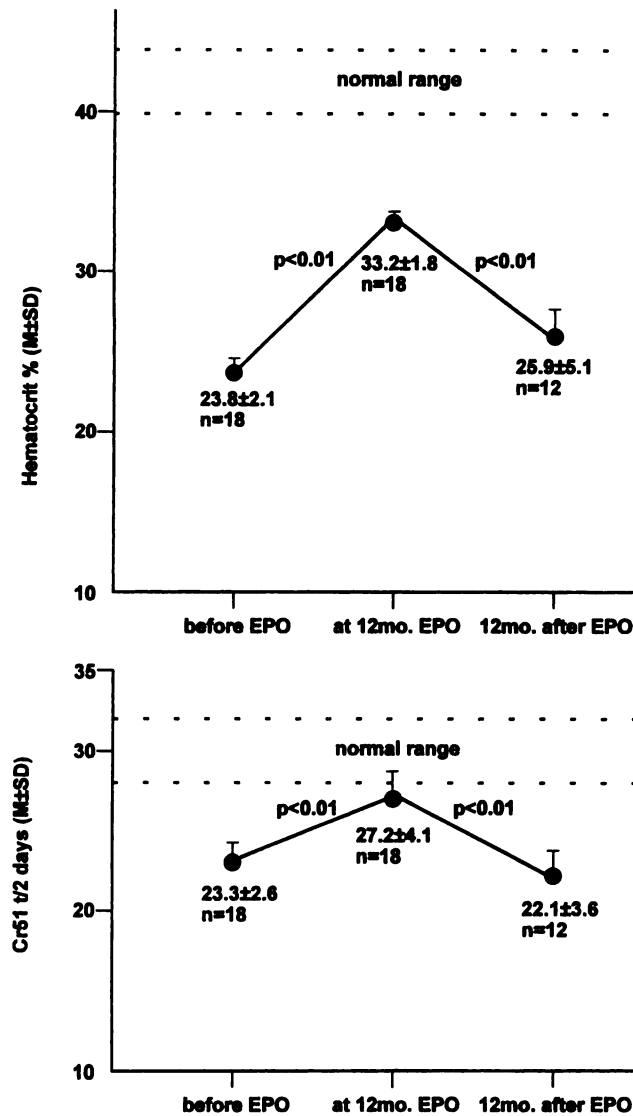
Figure 1. The hematocrit data of Group A patients at the time of determination of RBC survival are presented in the upper graph. The data of the Cr51 half-life of Group A patients are shown on the lower graph, determined at three intervals of the study: (1) before the start of erythropoietin treatment, (2) at 12 months of rhuEPO therapy, and (3) 12 months after cessation of rhuEPO therapy. The values are expressed as mean ± SD. Paired t test was used for statistical analysis.

mean Cr51 t½ rose to 27.2 ± 4.1 days. One year after termination of rhuEPO therapy, Cr51 T ½ decreased to 22.1 ± 3.6 days. The Hct level at that time had also dropped to 25.9 ± 5.1 vol%. These changes were significant.

The mean body weight of this group before the start of therapy was 66.5 ± 10.42 kg. At 12 months of therapy, it was 66.8 ± 11.04 kg (P = NS). After 1 yr of termination of rhuEPO treatment, it was 61.33 ± 8.98 kg in the remaining 12 patients (P = NS). The differ-

ences between the three intervals were all nonsignificant. The predialysis serum urea level was 33 ± 5.17 mmol/L before rhuEPO therapy, 35.58 ± 4.52 mmol/L at 12 months of treatment, and 33.67 ± 6.29 mmol/L 1 yr after cessation of rhuEPO treatment. These differences were also nonsignificant. These parameters support the fact that the dialysis dose did not change during the study.

Group B

This group was treated with rhuEPO for 2 yr and the Hct value was maintained at 30 to 35 vol%. The maximum rhuEPO dose for the group during the correction period reached 49 U/kg body wt, whereas it ranged between 17 and 27 U/kg body wt during the maintenance period. Figure 2 presents data on the Hct value and the RBC survival at the time of determination. The mean Cr51 t½ at 24 months of rhuEPO therapy was 27.3 ± 3.7 days, at a mean Hct level of 33.5 ± 2.6 vol%. One year after termination of therapy and a reduced Hct level of 24 ± 2.9 vol%, Cr51 t½ was significantly lower at 22.6 ± 2.3 days.

During the study, reticulocytes varied similarly to those of Group A. Mean reticulocyte percentage before the onset of therapy was 1.2 ± 0.43%. It rose to a maximum of 2.58 ± 1.16% (P < 0.001) during the correction period and ranged between 1.39 ± 0.72% and 1.84 ± 0.84% during the maintenance period, with a mean average of 1.71 ± 0.26% (P < 0.001). After termination of rhuEPO therapy, it decreased to 1.3 ± 0.53% (P = NS). The difference between the maximum level (2.58 ± 1.16%) and the average maintenance level (1.71 ± 0.26%) was also significant (P < 0.01).

Mean body weight of this group at 24 months of rhuEPO therapy was 62.73 ± 9.51 kg. In the 11 patients of this group after a year of termination of rhuEPO, it was 57.3 ± 9.08 kg. This change was not significant. Serum predialysis urea was 30.08 ± 3.99 mmol/L at 24 months of therapy, and 31.1 ± 3.25 mmol/L 1 yr after termination of rhuEPO. The changes of these parameters were nonsignificant, indicating that the dialysis dose was not changed during the study period.

DISCUSSION

Our results suggest that subcutaneous rhuEPO treatment effectively corrects anemia in uremic dialysis patients, and that corrected Hct value is maintained with relatively low doses of rhuEPO, provided that the iron stores in the body are well preserved with adequate supplementation.

In both patient groups, RBC survival, as expressed by the Cr51 technique, was significantly increased during treatment, with rhuEPO approaching normal range. The RBC survival of Group A at 12 months of treatment was comparable to that of Group B at 24 months of treatment, but both values were significantly increased when compared with the pretreatment value of Group A.
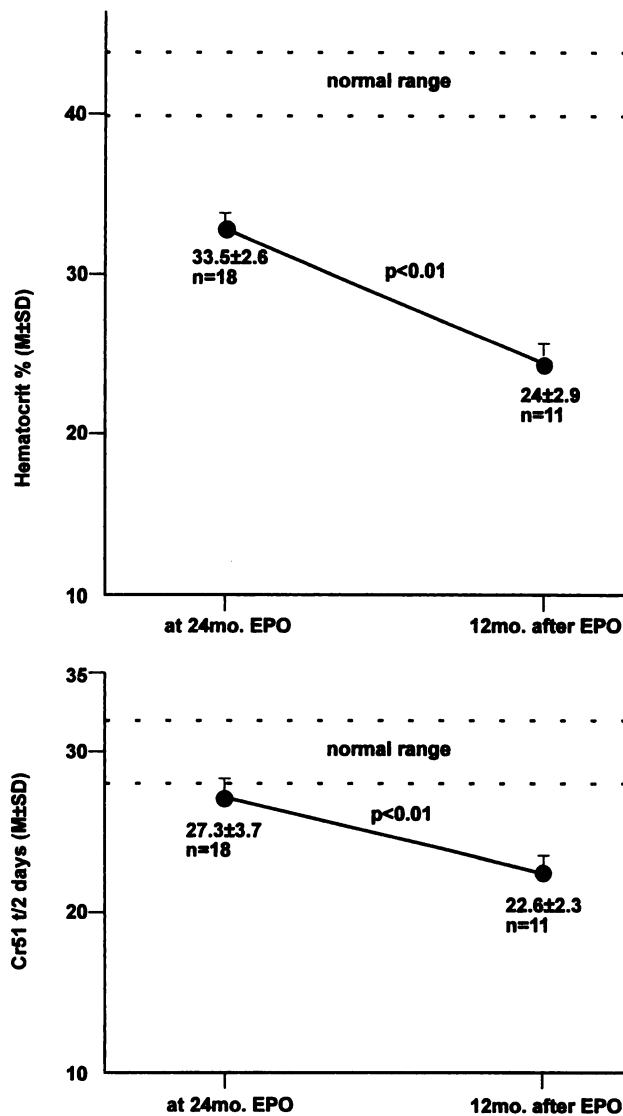
Figure 2. The hematocrit values of Group B patients at the times of determination of Cr51 half-life of the red blood cells are presented in the upper graph. The RBC survival of Group B patients, determined at two intervals, is presented in the lower graph: (1) at 24 months of rhuEPO therapy and (2) 12 months after cessation of rhuEPO therapy. The values are expressed as mean ± SD. Paired t test was used for statistical analysis.

One year after cessation of therapy, RBC survival was significantly reduced and comparable in both Group A and Group B. Furthermore, mean Cr51 half-life values were comparable to the pretreatment value of Group A. Thus, EPO treatment significantly increased RBC survival of uremic dialysis patients and this effect was continuously maintained under treatment but diminished after cessation of therapy, indicating that EPO directly influenced the longevity of RBC.

Our results are in agreement with those of Schwartz et al. (4,10), Hughes et al. (11), and Eschbach (1). Some other authors have reported that RBC survival is not affected during rhuEPO treatment, and that the correction of anemia depends solely on increased RBC production (12–14). The basic objection to the latter studies would be that they were too short in follow-up time because the effect of rhuEPO on RBC survival might be related to the duration of treatment.

The reticulocyte counts varied similarly in both patient groups. They rose during the correction period to a maximum level. Then, as the rhuEPO dose was individualized and tapered down to keep the Hct value within the limits of 30 to 35 vol% over the maintenance period, the reticulocyte counts decreased and leveled off. The difference between pretreatment counts and maintenance counts could indicate the presence of early reticulocyte release and minute prolongation of RBC survival. After cessation of therapy, reticulocytes further decreased just slightly. It is important to note that the increase in reticulocyte counts in our study was considerably smaller than, for example, that in the study of Schwartz et al. (4). This difference could be explained by the different rhuEPO doses and routes of administration used in the studies. One of the aims of our study was the slow correction of the Hct value, to avoid occurrence of serious adverse events. Therefore, the doses used in the correction period were smaller and the target Hct value was also lower (38 vol% in the study of Schwartz et al. [4] versus 30 to 35 vol% in ours). For these reasons, the correction period in our study lasted longer. However, in principle, the dynamics of reticulocytosis in our study entirely resembled the pattern of the study of Schwartz et al.

It is not yet fully elucidated how rhuEPO influences normalization of erythropoiesis. Koury and Bondurant (15) and Spivak et al. (16) have shown that EPO acts as both a mitogen and a survival factor. They proposed that the possible mechanism of action of EPO on the colony-forming units–erythroids was stimulation of DNA synthesis, retardation of DNA breakdown, and prevention of apoptosis (programmed cell death). Based on in vitro experiments, Bondurant et al. (17) have postulated that "three possibly interrelated processes occur simultaneously during colony development: cell growth and replication, extensive differentiation and maintenance of all functions necessary for survival. The last area has only recently been identified as a mechanism of hormonal regulation." Their "model of erythropoiesis" proposes that individual progenitors within the EPO-dependent population exhibit a range of sensitivities to EPO such that there is an extended dose range of EPO over which individual progenitors may survive and continue proliferation and differentiation. Thus, the level of EPO ultimately controls RBC production by regulating the number of dependent progenitors that survive or die. So, at a given level of EPO, some cells die

whereas others survive and progress through differentiation.

Our results, as well as those of Schwartz et al. (4,10), suggest that the viability of the RBC increases with EPO therapy. EPO may exert a number of molecular effects on erythroid cells whose ultimate effect would be the increased viability of RBC (17). These processes may stabilize the erythrocyte membrane and make the RBC more resistant to the aggressive uremic environment. Lerche et al. (18) have provided evidence for this effect of EPO. They found significant improvement of the whole RBC deformability after 30 wk of rhuEPO treatment of 20 adults and ten children with ESRD. They concluded that the disturbed erythropoiesis in uremic patients also caused impaired mechanical membrane properties that could not be attributed to the direct influence of "uremic toxins" on RBC alone, and furthermore, that the performed experiments did not exclude the direct action of rhuEPO on the mechanics of the membrane.

Our results, as well as the work of other authors, suggest that RBC survival may be prolonged because of the action of EPO on the erythroid progenitors. The consequence of the molecular effects of EPO on the erythroid cells could result in increased viability of the peripheral red blood cells.

ACKNOWLEDGMENTS

REFERENCES

1. **Eschbach JW:** The anemia of chronic renal failure: Pathophysiology and the effects of recombinant erythropoietin. Kidney Int 1989;35:134-148.
2. **Massry SG:** Pathogenesis of the anemia of uremia: Role of secondary hyperparathyroidism. Kidney Int 1983; 16[Suppl 24]:204-207.
3. **Bogin E, Massry SG, Levi J, Djaldelli M, Bristol G, Smith J:** Effect of parathyroid hormone on osmotic fragility of human erythrocytes. J Clin Invest 1982;69:1017-1025.
4. **Schwartz AB, Kelch B, Terzian L, et al.:** One year of rhuEPO therapy prolongs RBC survival and may stabilize RBC membranes despite natural progression of chronic renal failure to uremia and need for dialysis. ASAIO (Am Soc Artif Intern Organs) Trans 1992;M692.
5. **Polenakovic M, Sikole A, Masin G, et al.:** Clinical use of human recombinant erythropoietin (rhuEPO) for treatment of the anemia of end-stage renal disease-one year experience with Recormon®. Maked Med Pregled 1993; [Suppl 12]:65-74.
6. **ICSH-International Committee of Standardization in Haematology:** Recommended methods for red blood cell survival studies. Br J Haematol 1980;45:655-660.
7. **Bogdanova V, Georgievska S, Tadzer IS:** A rapid method of estimation red blood cell survival time. Macedonian Academy of Sciences and Arts, Section of Biological and Medical Sciences, Contributions XI, 1-2, 1990.
8. **Sikole A, Polenakovic M, Spasovski G, Masin G:** Red-cell survival in chronic haemodialysis patients treated with rhuEPO [Abstract]. Nephrol Dial Transplant 1993; 8:964A.
9. **Polenakovic M, Sikole A:** Is erythropoietin a survival factor for the red blood cells [Abstract]. Int J Artif Organs 1995;18[Suppl 8]:428A.
10. **Schwartz AB, Kahn SB, Kelch B, Kim KE, Pequignot E:** RBC improved survival due to recombinant human erythropoietin explains effectiveness of less frequent, low dose subcutaneous therapy. Clin Nephrol 1992; 38[Suppl 5]:283-289.
11. **Hughes RT, Cotes PM, Pippard MJ, et al.:** Subcutaneous administration of recombinant human erythropoietin to subjects on continuous ambulatory peritoneal dialysis: An erythrokinetic assessment. Br J Haematol 1990;75: 268-273.
12. **Najean Y, Moynot A, Deschryver F, et al.:** Kinetics of erythropoiesis in dialysis patients receiving recombinant erythropoietin treatment. Nephrol Dial Transplant 1989; 4:350-355.
13. **Zehnder C, Blumberg A:** Human recombinant erythropoietin treatment in transfusion dependent anemic patients on maintenance hemodialysis. Clin Nephrol 1989; 31:55-59.
14. **Cotes PM, Pippard MJ, Reid CDL, Winearls CG, Oliver DO, Royston PJ:** Characterization of the anaemia of chronic renal failure and the mode of its correction by a preparation of human erythropoietin (r-HuEPO): An investigation of the pharmacokinetics of intravenous erythropoietin and its effects on erythrokinetics. Q J Med 1989;70[Suppl N262]:113-137.
15. **Koury MJ, Bondurant MC:** Erythropoietin retards DNA breakdown and prevents programmed death in erythroid cells. Science (Washington DC) 1990;248:378.
16. **Spivak JL, Pham T, Isaacs M, Hankins WD:** Erythropoietin is both a mitogen and a survival factor. Blood 1991;77:1228.
17. **Bondurant M, Kelley L, Koury MJ.** Is erythropoietin a differentiation factor, a mitotic factor, or a survival factor? In: Bauer C, Koch KM, Scigalla P, Wieczorek L, Eds. Erythropoietin: Molecular Physiology and Clinical Applications. New York: Marcel Dekker Inc.; 1993:241-251.
18. **Lerche D, Schmidt R, Zoellner K, et al.:** Rheology in whole blood and in red blood cells under recombinant human erythropoietin therapy. Contrib Nephrol 1989; 76:299-305.