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Efficacy and Safety of Recombinant Human Erythropoietin to Prevent the Anaemias of Prematurity

European Randomized Multicenter Trial

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Anaemias are a common threat to very low birth weight infants and a major problem in the intensive care of preterm infants. Contributing factors include anaemia of prematurity and blood losses. The major cause of anaemia of prematurity is an abrupt increase in oxygen availability after transition from placental to pulmonary oxygenation with a consequent decrease in erythropoietin (EPO) production. As anaemia develops there is a diminished EPO response to decreased oxygen availability. Additionally there is more rapid destruction of fetal cells. Due to rapid growth between 30 and 40 weeks' gestation there may also be haemodilution [1, 2]. Blood loss may be due to spontaneous perinatal haemorrhage, but the most significant cause of anaemia in the first weeks of life is diagnostic sampling. The smallest preterm infants requiring the highest level of intensive care may have their total blood volume removed for sampling within the first 28 days of life [3]. The view has become obsolete that anaemia is 'physiologic' in preterm infants [4]. It reduces the amount of available oxygen [5] and contributes to apnoeic spells, mesenteric hypoperfusion [6], necrotizing enterocolitis, and failure to thrive [7].

Infants of less than 1,000 g birth weight requiring artificial ventilation will need frequent red cell transfusions. Therefore, they may be confronted with more than 8 donors within 28 days and thus the risk of acquiring viral infections is significant. 'Walking' donor systems, using a constant donor – preferably the infant's father – [8], do not exclude the risk of transfusion-acquired viral infections. Infants who are allowed to become anaemic may be symptomatic [9]. The best descriptors of this anaemia are red cell volume and haematocrit [10]. Infants with higher red cell volumes at birth require a shorter period of intensive care and have reduced morbidity and mortality.

Red cell volume can be manipulated by controlled placento-fetal transfusion at birth [11] or transfusion of adult red cells. Successful stimulation of the infant's own erythropoiesis will maintain red cell volume, i.e. the total amount of red cells in the circulation, thus reducing the need for red cell transfusion in the period of intensive care and prevent the anaemia of prematurity.

Recent studies have suggested that erythroid progenitors from preterm infants differentiate in response to recombinant human EPO (rhEPO) [12, 13]. rhEPO has been used successfully to stimulate erythropoiesis in more than 2,000 adults and children with anaemia due to renal failure [14, 15]. The aim of this study was to investigate whether treatment with rhEPO reduces the anaemias of prematurity and thus the need for transfusion by one third in preterm infants.

Patients, Materials and Methods

Ethical approval and informed consent was obtained by each participating centre. 171 preterm infants with gestational age of 28–32 completed weeks were admitted to the five study centres from April 1989 to February 1990. 47 infants were not eligible for study due to participation in other therapeutic trials (n = 28) or lack of parental consent (n = 19). 31 preterm infants were excluded prior to randomization for the following reasons: polycythaemia (venous haematocrit 55% or higher on 3rd day of life, regardless of cause), n = 10; congenital malformation, n = 8; haemolytic disease of the newborn, n = 3; exchange transfusion, n = 2; renal failure (urine output <30 ml/kg/24 h and/or serum creatinine >180 μmol/l on 3rd day of life), n = 2; other reasons (e.g. investigator absent), n = 6.

A total of 93 preterm infants were entered in the study. Details are shown in table 1. The study was of randomized open-controlled parallel-group design. Stratification was performed according to the need for ventilatory support on the 3rd day of life. Randomization was performed by prenumbered sealed envelopes.

EPO Treatment. rhEPO was supplied from Boehringer Mannheim GmbH in vials containing 500 U/ml in liquid preparation. A volume of 0.1 ml of the preparation was diluted with 0.9 ml 0.15 *M* NaCl and a dose of 30 U/kg was given by subcutaneous injection into the

Table 1. Randomized patients according to stratification, birth weight, and gestational age

	rhEPO n = 43	Control n = 50
Male/female, n	27/16	29/21
Birth weight, g (mean ± SD)	1,380 ± 324	1,295 ± 323
500– 999 g (n, vent/spont)	7/2	5/4
1,000–1,499 g (n, vent/spont)	9/11	10/17
≥1,500 g (n, vent/spont)	6/8	8/6
Gestational age, weeks (mean ± SD)	30 ± 1	30 ± 1
28 weeks, n	3	3
29 weeks, n	5	11
30 weeks, n	16	13
31 weeks, n	19	22
32 weeks, n	0	1

rhEPO = rhEPO-treated group; Control = control group; vent = artificial ventilation; spont = spontaneous breathing.

thigh every 3rd day from the 4th to the 25th day of life. Control infants were not given subcutaneous injections of placebo, but were otherwise managed identically. Iron treatment was started on day 14 with 2 mg Fe^{2+}/day orally, if there were no feeding or intestinal problems, and was given in the form of Fe(II)-chloride, Fe(II)-ascorbate or polysaccharide-iron complex.

Patient monitoring: Infants' heart rate, respiratory rate and blood pressure were recorded throughout the study. Monitoring for liver parenchymal damage and renal failure was performed on days 3, 13, and 25. Ultrasound brain scan for the detection of intraventricular haemorrhage and ophthalmoscopy for retinopathy of prematurity was carried out. During the study period, rhEPO treatment was withheld if the haematocrit rose to >50% or the haemoglobin to >17 g/dl. If these values were achieved by transfusions, and were maintained for at least 3 days after the last transfusion, rhEPO administration was discontinued until haemoglobin concentration had fallen below a value which was 3 g/dl above the level recommended as indication for transfusion (fig. 1). Moreover, the treatment was stopped if the platelet count was $>600 \times 10^9$/l, and if renal failure, hypertension or severe local reactions occurred. Patients were withdrawn from the study if they required an exchange transfusion, or medication with possible toxicity to bone marrow. Proven vertical infection and loss of parental consent were also indications for withdrawal.

Transfusion was strictly regulated as shown in figure 1. Volume of transfused red cells was determined by multiplying the total transfused volume by the haematocrit of the donor pack. Where the haematocrit of the transfused blood was unavailable the red cell volume transfused was calculated using a donor haematocrit of 70%. Transfused volume was divided

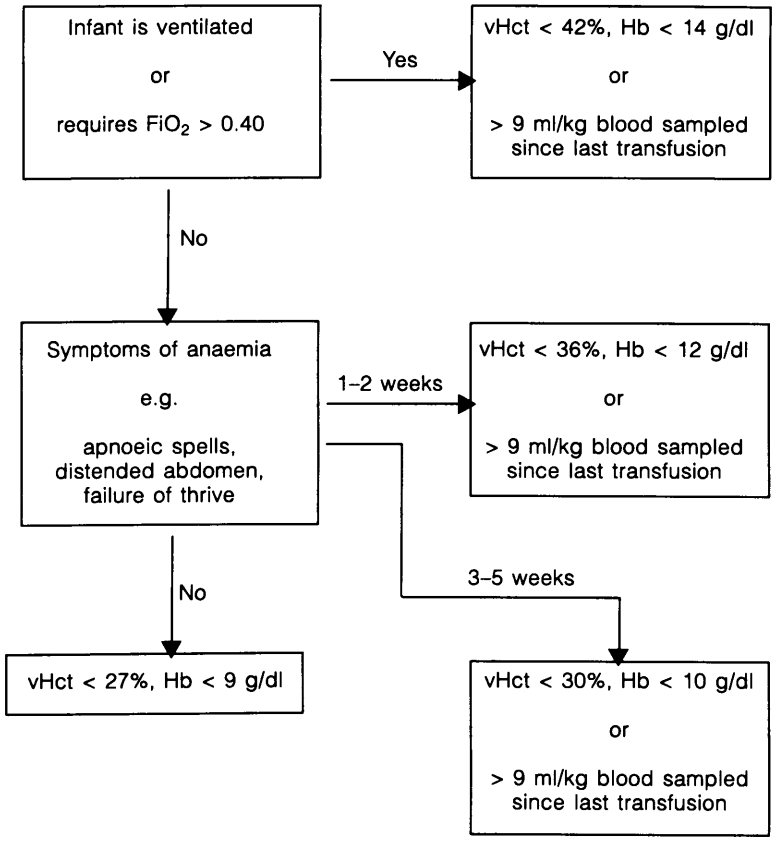

Fig. 1. Indication for transfusion of packed red cells.

by birth weight. The total volume of blood removed for diagnostic purposes during the study period was carefully recorded.

Haematological Studies. Venous haematocrit, haemoglobin and platelet counts were performed on days 3, 7, 10, 13, 19, and 25 prior to the administration of rhEPO. Reticulocyte count, serum ferritin, serum protein and serum EPO were checked on days 3, 13, and 25. The total volume of blood required for the study was 2,700 µl.

Statistical Methods. Mean values between groups were tested with the Mann-Whitney test. For categorical data the χ^2 test or Fisher's exact test were used respectively.

Table 2. Cumulated packed red cell volume transfused until day 25

	Red cells transfused, ml/kg				Number of infants with/ without transfusion	
	rhEPO		control		rhEPO	control
	mean	SD	mean	SD		
Spontaneous breathing	6.6	9.1	10.9	14.0	8/11	15/10
Artificial ventilation	21.2	21.1	22.3	25.8	15/4	14/6
All	14.1	17.8	16.5	20.8	23/15	29/16

rhEPO = rhEPO-treated group; control = control group.

Table 3. Haematologic values on day 25

	Spontaneous breathing				Artificial ventilation			
	rhEPO, n = 19		control, n = 25		rhEPO, n = 19		control, n = 20	
	mean	SD	mean	SD	mean	SD	mean	SD
Hct, %	36	8	38	5	41	6	39	7
Hb, g/dl	11.8	2.1	12.3	1.5	13.6	2.0	12.7	2.2
WBC, $\times 10^9$/l	10.2	2.0	10.3	3.3	10.6	3.8	11.2	3.1
Platelets, $\times 10^9$/l	390	149	381	165	318	179	336	162
Reticulocytes, %	5.0	6.2	3.7	4.8	7.7	14.9	2.5	2.1
Ferritin, ng/ml	163	74	170	78	252	171	201	90

rhEPO = rhEPO-treated group; control = control group.

Results

Efficacy

The cumulated volume of red cells transfused within the first 25 days is shown in table2. A total of 31 infants (15 rhEPO, 16 control infants) did not require any transfusions. There was no significant difference between the two groups of infants. The effect of rhEPO treatment on haematological values is

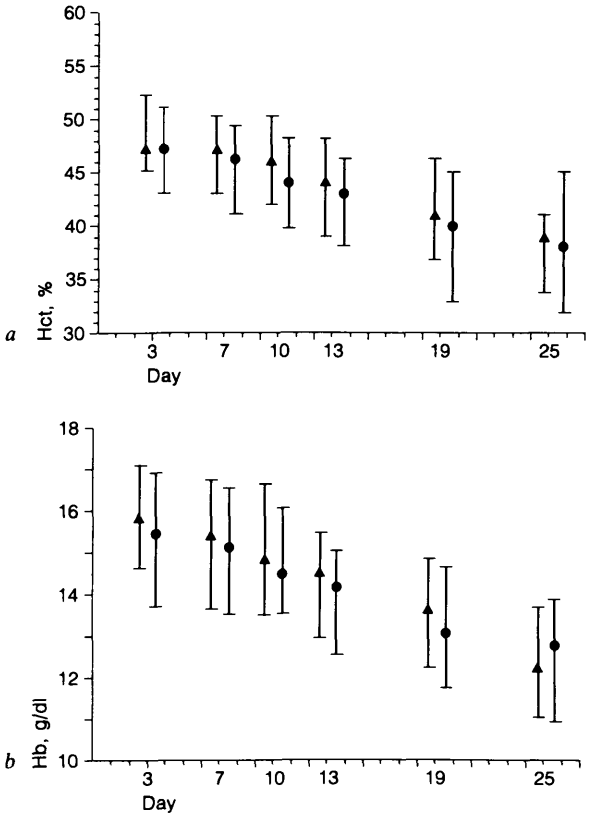

Fig. 2. Development of anaemia in preterm infants during the first 25 days of life. *a* Venous haematocrit. *b* Haemoglobin. Median ± quartiles. No difference can be observed between rhEPO-treated (●) and control infants (▲).

shown in table 3 and figures 2–4. There was no significant difference in any of the parameters among treated and nontreated, ventilated and nonventilated infants. Cumulated iron dose up to day 25 is shown in table 4. Although it was different in each centre, there were no signs of iron deficiency. Median ferritin levels on day 13 (before iron treatment was initiated) were 186 ng/ml in the rhEPO group and 189 ng/ml in the control group (NS). Ferritin levels of less than 100 ng/ml were never observed during the study period. The serum ferritin concentrations remained unchanged throughout the study (fig. 3). Total blood volume sampled for diagnostic purpose within 25 days is shown in

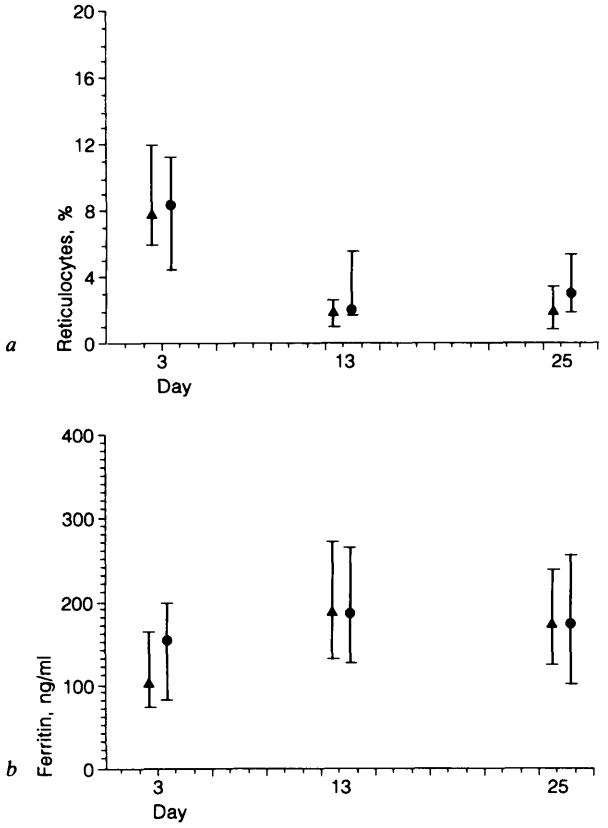

Fig. 3. Reticulocyte concentration *(a)* and serum ferritin levels *(b)* in preterm infants during the first 25 days of life. Median ± quartiles. No difference can be observed between rhEPO-treated (●) and control infants (▲).

figure 5. It is highest in the smallest infants and in each birth weight group is higher in infants requiring ventilatory support.

Safety

The study was discontinued in 10 infants as shown in table 5. No infant developed arterial hypertension (i.e. systolic blood pressure above 90 mm Hg) or suffered severe local reaction at the site of injection. The incidence of complications reported during the study is shown in table 6. No infant treated with rhEPO died. There were two deaths in the control group (one of a 610 g

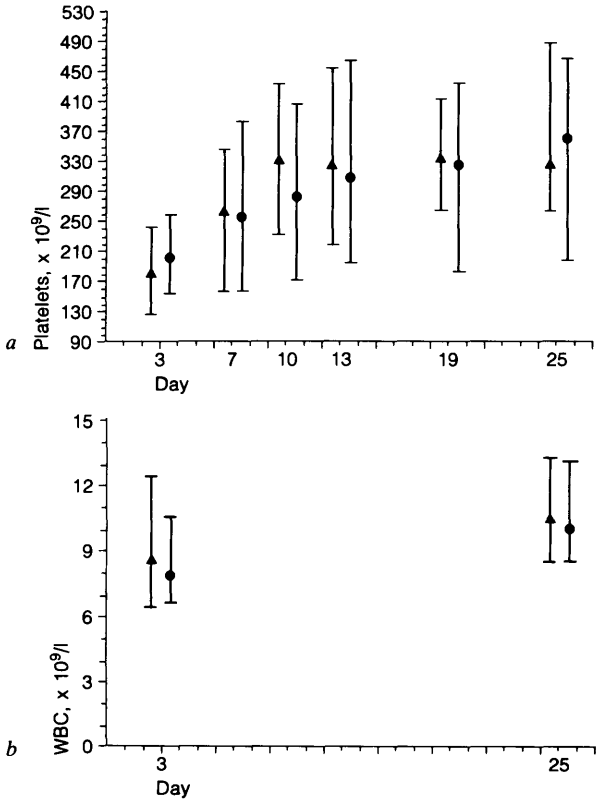

Fig. 4. a Typical rise of platelet count in preterm infants during the first 25 days of life. *b* White blood cell count on days 3 and 25 in preterm infants. Median ± quartiles. No difference can be observed between rhEPO-treated (●) and control infants (▲).

infant from necrotizing enterocolitis and one of a 1,380 g infant from hydrocephalus and brain atrophy).

Discussion

This study has shown that rhEPO at a subcutaneous dose of 70 U/kg/week is safe in preterm infants. Treatment with this regimen did not affect haematocrit, haemoglobin or volume of red cells transfused per kilogram birth

Table 4. Iron administration up to day 25

	Infants without any iron, n	Infants treated with iron, n	Mean cumulated iron dose (mg) in iron-treated infants
Münster	1	18	25
Heidelberg	6	10	17
Glasgow	1	17	53
Berlin-Charité	4	6	31
Berlin-West	2	18	30
All	14	69	32

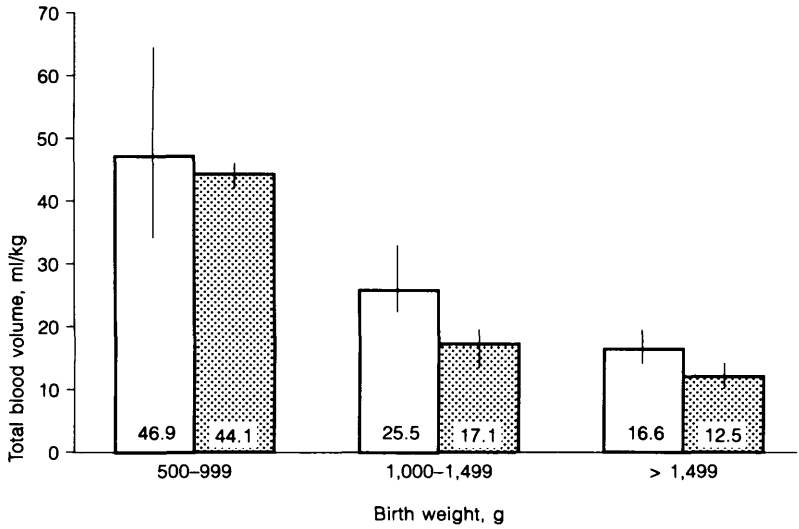

Fig. 5. Total blood volume sampled up to day 25 (ml/kg birth weight; median ± quartiles), according to birth weight and stratification. □ = Artificial ventilation; □ = spontaneous breathing.

weight. Although reticulocyte counts in infants receiving rhEPO were slightly higher than in the control infants at days 13 and 25, this failed to reach statistical significance. The expected fall in serum ferritin concentration did not occur, confirming that rhEPO given to preterm infants at this dose was not effective. Shannon et al. [16] in a double-blind placebo-controlled study administering 100 U/kg twice weekly to treat the anaemia of prematurity also

Table 5. Major reasons for discontinuation of the study in 10 infants, explaining the total number of 83 infants in tables 2–4 (only one reason is indicated per infant)

Stop rule	rhEPO	Control
Death	0	1
Hct >50%, Hb >17 g/dl	3	0
Platelets $>600 \times 10^3$/l	1	0
Renal failure	0	1
Exchange transfusion	0	1
Consent withdrawal	1	1
Others (transfer)	0	1
All	5	5

rhEPO = rhEPO-treated group; control = control group.

Table 6. Incidence of complicating disorders of prematurity, independent of discontinuation of the trial

Complications	rhEPO (n = 43)	Control (n = 50)
Necrotizing enterocolitis	1	3
Patent ductus arteriosus	1	3
Intraventricular haemorrhage	5	5
Bronchopulmonary dysplasia	6	5
All complicating disorders	13	16

rhEPO = rhEPO-treated group; control = control group.

failed to show a response. This failure to demonstrate a haemopoietic response may be due to inadequate dosage, suboptimal nutrition including iron deficiency, inflammation, infection, or the requirement for other growth factors.

Premature infants may generally require larger doses of rhEPO than older children and adults: in an animal study using rhesus monkeys, George et

al. [17] found that rhEPO doses of 100 or 250 U/kg, applied twice weekly, produced significant increases in haemoglobin in adults, but no change in newborn animals. Oster et al. [18] used doses of 300–600 U/kg/week to prevent chemotherapy-induced anaemia, Niemeyer [19] has used doses of up to 2,000 U/kg daily in an attempt to treat Blackfan-Diamond disease. EPO has been used in a dose of 1,200 U/kg/week to increase preoperative collection of autologous blood [20]. Halpérin et al. [21] in a recent uncontrolled pilot study reported an increase in reticulocytes and stabilization of haematocrit in preterm infants treated with higher doses (up to 300 U/kg/week) of EPO. However, since the EPO treatment was started after the 3rd week of life, the infants were slightly more mature; therefore, Halpérin's study was different from ours not only in terms of EPO dosage. It cannot be excluded that the positive response was due to a normal development of reticulocytes and haematocrit at this age.

Ohls et al. [22] studied the erythroid 'burst-promoting' activity in the serum of patients with the anaemia of prematurity. Burst-promoting activity was similar to that found in adult blood and cord sera. Their findings indicate that the anaemia of prematurity like the anaemia of end-stage renal disease is associated with a specific deficiency of EPO.

The erythropoietic potential of a fetus gaining weight from 1 to 3 kg is 90 ml red cells. If this potential is to be realized, maintenance of nutritional status, especially with regard to haematopoiesis, will be important. Achievement of optimal erythropoiesis should reduce the requirement for donor transfusion whilst maintaining blood volume for optimal oxygen delivery and tissue perfusion. Maintenance of this physiological state of the blood is also likely to minimize the complications of preterm delivery, especially those related to inadequate oxygenation and perfusion by the blood such as prolonged dependence on respiratory support [7]. For these reasons we believe that controlled studies with higher doses of rhEPO are now indicated in preterm infants.

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