ORIGINAL ARTICLE

Recombinant erythropoietin and blood transfusion in selected preterm infants

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Objectives: To comprehensively identify preterm infants likely to require blood transfusion and to investigate the effectiveness of recombinant erythropoietin in this high risk subgroup.
Design: Double blind randomised controlled trial.
Setting: Neonatal Intensive Care Unit, Middlemore Hospital, Auckland, New Zealand.
Patients: Preterm infants < 33 weeks gestation and < 1700 g birth weight meeting specific criteria indicating a high possibility of requiring blood transfusion.
Interventions: Predictors of blood transfusion were determined by analysis of preterm infants admitted to a neonatal intensive care unit over a two year period. Using the criteria developed, high risk infants entered the study and received erythropoietin or sham treatment until 34 weeks completed gestation. The sample size was calculated to detect a reduction of one blood transfusion per infant (significance level 5%, power 80%).
Results: The selection criteria had a positive predictive value for transfusion of 91% and a negative predictive value of 94%. Mean birth weights and gestational ages were similar in the two groups. Absolute reticulocyte counts and haemoglobin values were higher in the group receiving erythropoietin. There was no significant difference in the number of blood transfusions received in the treatment and control groups. However, comparing transfusions given to < 1000 g infants after 30 days of age, there were significantly fewer transfusions in the erythropoietin group (mean (SD) 0.5 (0.7) in those receiving erythropoietin and 1.6 (1.1) in the controls). No adverse effects were noted.
Conclusions: The selection criteria for the study were highly predictive of subsequent transfusion. In the group receiving erythropoietin, a reduction in transfusion requirements was apparent only in the < 1000 g birthweight group after 1 month of age.

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Declining haemoglobin levels in preterm infants often necessitate blood transfusion (BTF). Efforts to reduce the use of blood products and the complications arising from their use, such as transmission of infection, have included use of recombinant erythropoietin (Epo). Several double blind, randomised controlled studies have shown a decrease in the BTF requirements of preterm infants treated with Epo, as has a meta-analysis.[1–5] More recent papers have focused on < 1000 g infants.[6 7] Inclusion criteria for these studies have been almost entirely based on birth weight.

The rationale for the present study was to achieve a more comprehensive selection of all preterm infants—that is, < 37 weeks—in the nursery likely to require transfusion, to randomise them to either Epo or control and to monitor transfusion requirement throughout their hospital stay. This would allow Epo to be targeted to a specific high risk group of infants. The selection criteria for this study were based on a regression model of transfusions given in the nursery before use of Epo.

METHODS
Selection
A regression analysis of preterm infants admitted to the nursery at Middlemore Hospital over a two year period (1995–1996) and requiring blood transfusion was carried out. The features below were associated with BTF in infants whose gestation was < 33 weeks and birth weight < 1700 g. (Heavier, more mature infants only received blood for shock/acute blood loss, and transfusions for this indication were excluded from the analysis.)

(1) Packed cell volume at birth < 46% and a requirement for respiratory support (either continuous positive airway pres-

sure (CPAP) or intermittent positive pressure ventilation) for 48 hours or more.

(2) Blood loss > 9 ml in first 48 hours while requiring respiratory support (CPAP or intermittent positive pressure ventilation).

(3) BTF in first 48 hours.

(4) Birth weight < 1000 g.

(5) Mean weight gain < 7 g per day (measured from birth to packed cell volume of 38%). This criterion was also noted in a previous study to be useful in prediction of transfusion.[8]

The above were used as criteria for this study, and infants with one or more of these features were eligible. All except the weight gain criterion allowed selection by 48 hours of age. Exclusion criteria were the presence of major congenital abnormalities, clinically significant acquired or congenital infection at birth, and haemolysis.

Informed consent was obtained from the parents, and the study was approved by the regional ethics committee and the Middlemore Hospital Board.

Randomisation
Stratified randomisation into three groups was carried out depending on when eligibility criteria were met. The groups were: < 1000 g; > 1000 g and up to 48 hours old; > 1000 g and > 48 hours of age. The reason for stratifying the > 1000 g infants into two groups was that approximately half were

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Abbreviations: BTF, blood transfusion; Epo, erythropoietin; CPAP, continuous positive airway pressure; CI, confidence interval

Table 1 Characteristics of the group of infants receiving erythropoietin (Epo) and the control group

	Control	Epo	p Value
Number of infants	21	22	
Birth weight (g)	972 (239)	956 (245)	0.92
Gestation (weeks)	27 (26–28)	26 (26–28)	0.59
Male: female	14/7	13/9	0.75
Prestudy blood loss (ml)	7.3 (5.8)	7.2 (3.8)	0.90
Birth haemoglobin (g/l)	157 (135–170)	145 (127–160)	0.79
Entry haemoglobin (g/l)	114 (108–141)	136 (109–145)	0.31
Entry reticulocytes ($\times10^9$/l)	141 (83–260)	162 (63–272)	0.89

Results shown are mean (SD) or median (interquartile range).

Table 2 Characteristics of <1000 g infants in control and erythropoietin (Epo) groups

	Control	Epo	p Value
Number	11	12	
Birth weight (g)	781 (131)	777 (98)	0.93
Gestation (weeks)	26 (24–27)	26 (24–26)	0.60
CPAP (days)	46.8 (31.6–59.9)	51.5 (20.9–63.3)	0.93
IPPV (days)	3.5 (0–10)	1 (0–4)	0.47
Days on oxygen	2.8 (1–8.8)	2.8 (0–4)	0.41
Blood sampling (ml)*	22.2 (9.8)	21.3 (6.0)	0.80

Results shown as mean (SD) or median (interquartile range).
*Blood sampling losses from admission to discharge.
CPAP, continuous postive airways pressure; IPPV, intermittent positive pressure ventilation.

likely to meet entry criteria after 2 weeks of age (based on the weight gain compared with packed cell volume criterion). We wanted to ensure that entry age would be comparable in the treatment and control arms. Infants were randomised by the hospital pharmacist to receive either Epo or no treatment (control group). The randomisation numbers were computer generated.

Management

Those in the Epo group received recombinant erythropoietin (Eprex; Janssen-Cilag, Auckland, New Zealand) at a dose of 1200 U/kg/week given subcutaneously in three divided doses until the age of 3 weeks when the dose was reduced to 600 U/kg/week. The higher initial Epo doses had been found to be effective in a previous study.[6] Treatment continued until 34 weeks completed gestation or for a minimum of three weeks (infants still on CPAP or respiratory support at 34 weeks continued treatment until 36 completed weeks). Although treatment ended at this age, monitoring of transfusion requirements continued until discharge or until a corrected gestation of 40 weeks was reached.

The infants randomised to the control group received sham treatment, to avoid subcutaneous placebo injections.[1 9] Treatment was administered by a designated study nurse not involved in clinical management decisions relating to the infants. On each of the treatment days, the nurse collected vials of Epo and saline, and 1 ml syringes were prepared in a side room. The syringes were labelled with the patient's name. A screen was placed around the bedside; those on Epo received a subcutaneous injection and an adhesive plaster was placed over the injection site. Those in the control group had a plaster applied to a similar site to those on Epo, the sites in both groups were then left covered until the next treatment day.

Ferrous gluconate (Fergon; Pacific Pharmaceuticals, Auckland, New Zealand) at a dose of 6 mg of elemental iron/kg/day was given to the Epo group once they had attained a postnatal age of 2 weeks provided that they were receiving at least 50% energy intake orally. Those in the control arm were given 2 mg/kg/day from the same age. The lower dose of oral iron in the controls was felt to be justified by the potential for oxidative stress and has been carried out in several other studies.[1 2 9] All iron supplements were provided by the pharmacy for named babies in dark brown bottles, and the infants in the control group received a more dilute preparation so that an equivalent volume was given, thus maintaining blinding. All infants received a multivitamin preparation (Vitadol C, Karicare; Nutricia Australasia, Central Park, Auckland, New Zealand) and vitamin E (25 IU per day).

For study purposes, blood samples were checked weekly (full blood count and reticulocytes) or two weekly (serum ferritin). Blood volumes removed for sampling were recorded.

Blood transfusions

These were given according to a set protocol. Indications were:

- packed cell volume 36–40% and critically ill with:
  - (a) requirement for oxygen > 45% via CPAP;
  - (b) ventilation (mean airway pressure > 10 cm water);
  - (c) severe sepsis;
  - (d) active bleeding;
- packed cell volume 31–35% and:
  - (a) requirement for oxygen (up to 45%) via CPAP;
  - (b) ventilation (mean airway pressure 7–10 cm water);
- packed cell volume 21–30% and:
  - (a) gain less than 10 g/day averaged over one week;
  - (b) experience either at least 10–12 apnoeic or bradycardic episodes in 12 hours or two or more such episodes requiring bag and mask ventilation within a 24 hour period, not due to other causes and not responsive to methylxanthine treatment;
  - (c) have a sustained tachycardia (> 170 beats/min) or tachypnoea (> 70/min) per 24 hours and not attributable to other causes;
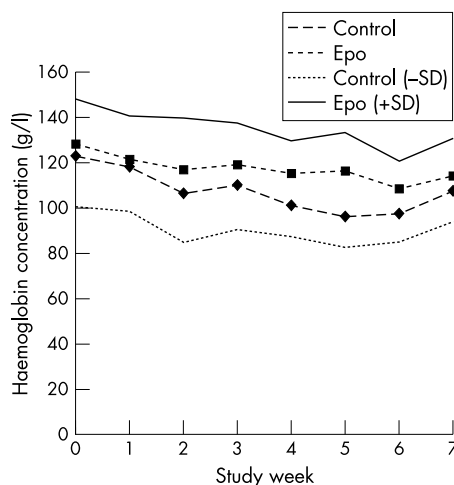
Figure 1 Haemoglobin concentrations in control and erythropoietin (Epo) groups. Study entry is week 0.
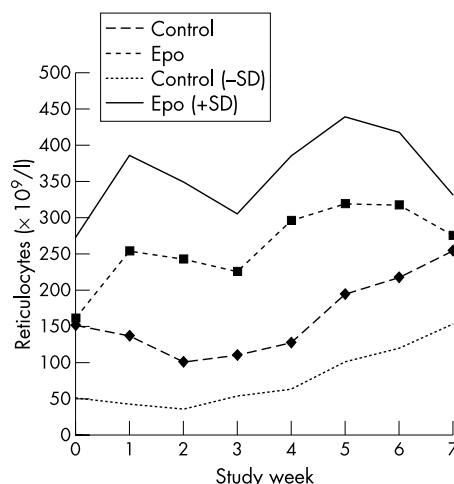

Figure 2 Reticulocyte counts ($\times 10^9$/l) in control and erythropoietin (Epo) groups. Study entry is week 0.

– (d) develop cardiac decompensation secondary to a clinically apparent patent ductus arteriosus;
– (e) positive pressure ventilation on low settings (mean airway pressure < 7 cm water) or nasal CPAP;
– (f) those requiring surgery
• packed cell volume ≤ 20% and reticulocyte count < 100 × 10^9/l.

Statistical analysis

The primary outcome measure was the number of transfusions in each group from birth to discharge or 40 weeks corrected gestation. Previous experience from the neonatal unit indicated that an average of 1.25 transfusions/patient would need to be avoided to prevent one donor exposure (mean transfusions before the study was five per patient; unpublished data). Infection risk is theoretically linked to the number of donor exposures. In practice, a reduction of one transfusion is the least amount of blood that could lead to one less donor exposure. Therefore, a reduction of 1 BTF was deemed to be a conservative estimate of what may be clinically relevant. To detect an overall reduction of 1 BTF with a significance level of 5% and a power of 80%, 21 infants were needed per group. Based on pre-study estimates, this sample size would give the study 97% power to detect a 50% reduction in transfusions in the Epo group.
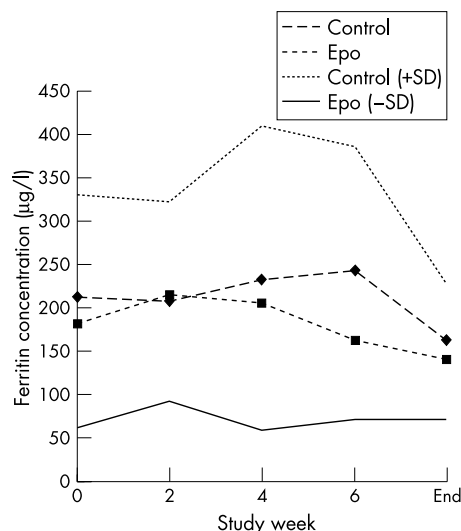
Figure 3 Ferritin concentrations in control and erythropoietin (Epo) groups. Study entry is week 0 and the final ferritin concentration at the end of the study is denoted as "End".

The Mann-Whitney U test was used for non-parametric variables, and the *t* test for normally distributed data. In assessing the mean difference in transfusions between the two groups, non-equal variance was assumed. Repeated measures were analysed using analysis of variance.

RESULTS

Selection criteria

A total of 107 infants < 33 weeks gestation and < 1700 g birth weight were admitted to the nursery during the study period (1997–1999), and 55 met eligibility criteria (none met the criterion of blood loss of 9 ml or more in the first 48 hours). There were 12 study refusals; of the remaining 43, 22 were randomised to Epo and 21 to the control group. Of the 12 study refusals, 11 received at least 1 BTF and a further three of 52 not meeting the entry criteria were transfused. Combining the results of those who declined study entry and the 21 in the control group, there were 33 infants of whom 30 received at least 1 BTF. This indicates that the selection criteria had a positive predictive value for transfusion of 30/33 (91%) and a negative predictive value of 49/52 (94%).

Comparison of control and Epo groups

Characteristics on study entry were similar in the two groups (tables 1 and 2). The median entry haemoglobin levels appeared lower in the control group (table 1), although this was not significant. The number of cases in each group with low haemoglobin on study entry (defined as < 110 g/l, the 25th centile for the study group as a whole) were equal (five per group; results not shown). Figures 1–3 show the results for haemoglobin concentration, reticulocyte count, and serum ferritin respectively. Analysis of variance for repeated measures indicated significantly higher haemoglobin (p = 0.04) and reticulocyte counts (p = 0.003) in the Epo group. There were no significant differences in ferritin levels during the study (p = 0.13) or at its completion (p = 0.3).

Blood transfusions

There were no significant differences in the mean number of transfusions given to infants in the two groups when all the patients were considered together (table 3). The mean difference in transfusions was 0.49 lower in the Epo group with a 95% confidence interval (CI) of −1.1 to 2.3 (assuming non-equal variance). A similar non-significant difference was

Table 3 Comparison of blood transfusions (BTFs) in the control and erythropoietin (Epo) groups

	Control	Epo	p Value
Mean total BTFs	3.2 (3.4)	2.5 (2.3)	0.41
BTFs before entry	0.64 (1.0)	0.71 (1.2)	0.95
Number of cases transfused	19/21	15/22	0.28
Number receiving 1 BTF	4/21	8/22	0.3
Donor exposure	2.95 (3.8)	1.86 (1.5)	0.23
BTFs to >1000 g	0.7 (1.0)	1.5 (1.5)	0.19
BTFs to <1000 g	5.2 (3.0)	3.3 (2.6)	0.14
Mean BTFs to <1000 g >30 days	1.6 (1.1)	0.5 (0.7)	0.01
Number of BTFs to <1000 g >30 days	18/57	6/40	0.025

Results shown are mean (SD).

found when those < 1000 g were analysed as a subgroup (table 3). Here, the mean transfusion difference was 1.84 (95% CI −0.6 to 4.3).

Infants in the < 1000 g subgroup who received Epo showed a significant reduction in BTFs given after the age of 1 month (30 days; p = 0.01). The mean difference was 1.13 (95% CI 0.34 to 1.92). From table 3, it is apparent that over one third of transfusions given to the < 1000 g control group were given after 1 month of age. No adverse effects attributable to Epo were noted.

Blood donors

There were no significant differences in the number of donors to which infants were exposed. There was, however, a trend towards lower donor exposure in the infants < 1000 g when the transfusion needs over 1 month of age were considered (p = 0.08). When cases in the study exposed to more than one donor were compared, the relative risk of greater donor exposure in the Epo group was 0.78 (95% CI 0.41 to 1.49), which was not significant.

DISCUSSION

The aim of the study was to reduce the use of BTF in the whole nursery preterm population. This meant that selection criteria for the study were important to identify a high risk population to target for Epo treatment. The selection criteria used were predictive of the need for BTF with a high positive and negative predictive value; indeed only three (6%) preterm infants (< 37 weeks gestation) not meeting selection criteria were given BTF during the study period. The selection criterion based on initial phlebotomy loss was not reached in any of the cases, and this probably reflects current nursery practice to minimise sampling losses and improved microassays for laboratory testing. The transfusion criterion based on weight gain may have a physiological explanation in that improved weight gain (either from better nutrition or on the basis of lesser illness severity) is associated with enhanced erythropoiesis.[10][11] Therefore, it could be expected that low weight gain and relatively low packed cell volume would indicate higher likelihood of transfusion.

The results indicated Epo responsiveness in terms of increased haemoglobin concentration and reticulocyte counts in the treatment group, but this resulted in significantly reduced transfusion requirements only in the < 1000 g group after 1 month (30 days) of age. Based on our pre-study estimates, the study had an 80% power to detect a difference of 1 BTF between groups. However, it has been argued that to change clinical practice, a 50% reduction in transfusion rates in the Epo group would be needed.[12] Applying this to the present study—that is, a 50% reduction from a pre-study estimate of five transfusions/patient—the power of our study was 97%. Therefore, it seems very unlikely that we missed a clinically important effect of Epo on transfusions. The study size

does not allow us to rule out rare adverse effects of Epo. However, this was not the aim, and meta-analysis of pooled studies comprising several hundred patients has not shown up such effects.[5]

Most Epo studies starting soon after birth have also shown a lack of useful effect in the early weeks of life. A multicentre European study entering very low birthweight infants showed no benefit in the first two weeks after birth.[1] Likewise, Donalto et al[13] studying infants < 1250 g birth weight found no transfusion reduction from Epo treatment before 2 weeks of age (all infants received Epo after this age). A recent American study enrolling infants < 1250 g at birth showed no benefit in reducing transfusions.[12] In contrast with these results, two smaller studies (one terminated early after 20 infants were randomised) noted a significant decrease in BTF in the first two weeks.[4][6] The finding that Epo is generally less effective at reducing transfusion in the early postnatal period may be related to the effects of illness and transfusions on erythropoiesis, protein deficiency, and high phlebotomy losses.[14]

The finding of reduced BTF after 1 month of age in < 1000 g in this study should be interpreted with some caution because of small numbers. The observation of decreased transfusions in this subgroup receives some support from previous literature, although comparison with earlier studies is somewhat limited because of the stricter entry criteria in this study. The first American multicentre study, which showed a relatively small transfusion reduction after Epo treatment, had an average entry age of about 3 weeks and about two thirds of the infants were < 1000 g.[3] The South African study enrolled infants at an average postnatal age of nearly 4 weeks with a significant reduction in transfusions.[2] The most recent American multicentre study showed fewer transfusions to infants < 1000 g beginning after about 4 weeks of age and reaching statistical significance between weeks 7 and 10.[12] These results suggest that a clinically useful Epo effect is more likely to be obtained when the infants have reached a stable growing phase of their postnatal period. The < 1000 g infants in this study still received one third of their transfusions after 1 month of age, but none received transfusions after 34 weeks. Whether Epo would have the same effect if started at 1 month of age requires further study.

The controls received a lower dose of oral iron than the treatment group; however, ferritin levels were not significantly different in the two groups either during or at the end of the study.

In conclusion, the entry criteria for the study enabled selection of 94% of the entire cohort of all preterm infants transfused in the nursery over a two year period. However, a benefit from the use of Epo was only noted in infants < 1000 g after 1 month of age.

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