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Aher SM, Ohlsson A

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Late erythropoiesis-stimulating agents to prevent red blood cell transfusion in preterm or low birth weight infants.

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[Intervention Review]

Late erythropoiesis-stimulating agents to prevent red blood cell transfusion in preterm or low birth weight infants

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ABSTRACT

Background

Preterm infants have low plasma levels of erythropoietin (EPO), providing a rationale for the use of erythropoiesis-stimulating agents (ESAs) to prevent or treat anaemia. Darbepoetin (Darbe) and EPO are currently available ESAs.

Objectives

To assess the effectiveness and safety of late initiation of ESAs, between eight and 28 days after birth, in reducing the use of red blood cell (RBC) transfusions in preterm or low birth weight infants.

Search methods

We used the standard search strategy of Cochrane Neonatal to search the Cochrane Central Register of Controlled Trials (CENTRAL 2018, Issue 5), MEDLINE via PubMed (1966 to 5 June 2018), Embase (1980 to 5 June 2018), and CINAHL (1982 to 5 June 2018). We searched clinical trials databases, conference proceedings, and the reference lists of retrieved articles for randomised controlled trials and quasi-randomised trials.

Selection criteria

Randomised or quasi-randomised controlled trials of late initiation of EPO treatment (started at $\geq$ eight days of age) versus placebo or no intervention in preterm (< 37 weeks) or low birth weight (< 2500 grams) neonates.

Data collection and analysis

We performed data collection and analyses in accordance with the methods of the Cochrane Neonatal Review Group. We used the GRADE approach to assess the quality of the evidence.

Main results

We include 31 studies (32 comparisons) randomising 1651 preterm infants. Literature searches in 2018 identified one new study for inclusion. No new on-going trials were identified and no studies used darbepoetin.

Most included trials were of small sample size. The meta-analysis showed a significant effect on the use of one or more RBC transfusions (21 studies ($n = 1202$); typical risk ratio (RR) 0.72, 95% confidence interval (CI) 0.65 to 0.79; typical risk difference (RD) -0.17, 95%

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CI -0.22 to -0.12; typical number needed to treat for an additional beneficial outcome (NNTB) 6, 95% CI 5 to 8). There was moderate heterogeneity for this outcome (RR $I^2 = 66\%$; RD $I^2 = 58\%$). The quality of the evidence was very low. We obtained similar results in secondary analyses based on different combinations of high/low doses of EPO and iron supplementation. There was no significant reduction in the total volume (mL/kg) of blood transfused per infant (typical mean difference (MD) -1.6 mL/kg, 95% CI -5.8 to 2.6); 5 studies, 197 infants). There was high heterogeneity for this outcome ($I^2 = 92\%$). There was a significant reduction in the number of transfusions per infant (11 studies enrolling 817 infants; typical MD -0.22, 95% CI -0.38 to -0.06). There was high heterogeneity for this outcome ($I^2 = 94\%$).

Three studies including 404 infants reported on retinopathy of prematurity (ROP) (all stages or stage not reported), with a typical RR 1.27 (95% CI 0.99 to 1.64) and a typical RD of 0.09 (95% CI -0.00 to 0.18). There was high heterogeneity for this outcome for both RR ($I^2 = 83\%$) and RD ($I^2 = 82\%$). The quality of the evidence was very low. Three trials enrolling 442 infants reported on ROP (stage ≥ 3). The typical RR was 1.73 (95% CI 0.92 to 3.24) and the typical RD was 0.05 (95% CI -0.01 to 0.10). There was no heterogeneity for this outcome for RR ($I^2 = 18\%$) but high heterogeneity for RD ($I^2 = 79\%$). The quality of the evidence was very low. There were no significant differences in other clinical outcomes including mortality and necrotising enterocolitis. For the outcomes of mortality and necrotising enterocolitis, the quality of the evidence was moderate. Long-term neurodevelopmental outcomes were not reported.

Authors' conclusions

Late administration of EPO reduces the use of one or more RBC transfusions, the number of RBC transfusions per infant (< 1 transfusion per infant) but not the total volume (mL/kg) of RBCs transfused per infant. Any donor exposure is likely not avoided as most studies included infants who had received RBC transfusions prior to trial entry. Late EPO does not significantly reduce or increase any clinically important adverse outcomes except for a trend in increased risk for ROP. Further research of the use of late EPO treatment, to prevent donor exposure, is not indicated. Research efforts should focus on limiting donor exposure during the first few days of life in sick neonates, when RBC requirements are most likely to be required and cannot be prevented by late EPO treatment. The use of satellite packs (dividing one unit of donor blood into many smaller aliquots) may reduce donor exposure.

PLAIN LANGUAGE SUMMARY

Late erythropoiesis-stimulating agents to prevent red blood cell transfusion in preterm or low birth weight infants

Review question

We reviewed the evidence about the effectiveness and safety of late initiation of erythropoiesis-stimulating agents treatment between eight and 28 days after birth, for reducing the use of red blood cell (RBC) transfusions in preterm or low birth weight infants.

Background

The percentage of circulating red blood cells (haematocrit) falls after birth in all infants. This is particularly true in preterm infants due to their poor response to anaemia and to the amount of blood that is drawn for necessary testing. Low plasma levels of erythropoietin (EPO), a substance in the blood that stimulates red blood cell production in preterm infants, provide a rationale for the use of erythropoiesis-stimulating agents (EPO and darbepoetin) to prevent or treat anaemia.

Search date

The evidence is current to 5 June 2018.

Study characteristics

To date, 1651 infants (between eight and 28 days of age) born preterm have been enrolled in 31 studies of late administration of EPO to reduce the use of red blood cell transfusions and to prevent donor exposure.

There were no studies that used darbepoetin.

Study funding sources

We have not received any funding for this review and we have no conflicts of interest to declare.

Key results

The risk of receiving red blood transfusion is reduced following initiation of EPO treatment. However, the overall benefit of EPO is reduced as many of these infants had been exposed to donor blood prior to entry into the trials. Treatment with late EPO did not have any important effects on death or common complications of preterm birth, except for trends towards an increased risk for retinopathy of prematurity. Retinopathy of prematurity is a disease of the eye affecting infants born preterm. It is thought to be caused by disorganised growth of retinal blood vessels, which may result in scarring and retinal detachment. Retinopathy of prematurity can be mild and may resolve spontaneously, but it may lead to blindness in serious cases.

Quality of the evidence

The study quality varied and important information regarding the random sequence generation and whether the allocation was concealed or not was often missing. Sample sizes were small and long-term outcomes (18 to 24 months of corrected age) were not reported. The quality of the evidence was very low for the outcomes of “use of one or more red blood cell transfusions,” “retinopathy of prematurity (all stages or stage not reported)” and for “retinopathy of prematurity (stage ≥ 3)”. For the outcomes of “necrotising enterocolitis” and “mortality”, the quality of the evidence was moderate.

SUMMARY OF FINDINGS FOR THE MAIN COMPARISON [\[Explanation\]](#)

Late EPO compared with placebo or no intervention for complications of preterm birth - primary and secondary outcomes						
Patient or population: preterm infants with low birth weight Settings: NICU Intervention: Late EPO Comparison: Placebo or no intervention						
Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No of Participants (studies)	Quality of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				
	Placebo or no interven- tion	EPO				
Use of one or more red blood cell transfusions (low and high dose of EPO) during initial hos- pital stay	High risk population		RR 0.72 (0.65 to 0.79)	1202 (21)	⊕○○○ very low	Bias: We had concerns about random se- quence generation in 16 of the studies, about allocation concealment in 17 of the studies and about blinding (per- formance bias and de- tection bias) in 11 of the studies. We down- graded the quality of the evidence by one step. Heterogeneity/ Consistency: I ² for the typical RR was 66%and for the typical RD 58% (both moderate het-

						erogeneity). We downgraded the quality of the evidence by one step. Directness of evidence: Studies were conducted in the target population. Precision: Because of the large sample size (n = 1202), the point estimate was precise with a narrow 95% CI. Presence of publication bias: The funnel plot was asymmetrical - we downgraded the quality of the evidence by one step
		603 per 1000	434 per 1000 (392 to 477)			
Necrotising Enterocolitis $\geq$ Bell's stage 2 during initial hospital stay	High risk population			RR 0.88 (0.45 to 1.70)	656 (6)	⊕⊕⊕○ moderate Bias: We had concerns about random sequence generation in two of the studies, about allocation concealment in three of the studies and about blinding (performance bias and detection bias) in one of the studies. We downgraded the quality of the evidence by one step Heterogeneity/Consistency: I² for both the typical RR and for

					the typical RD was 0% (both no heterogeneity) . Directness of evidence: Studies were conducted in the target population. Precision: Because of the sample size (n = 656), the point estimate was quite precise with a narrow 95% CI. Presence of publication bias: As only six studies were included in the analyses we did not perform a funnel plot
	52 per 1000	46 per 1000 (23 to 88)			
Mortality during initial hospital stay (all causes)	High risk population		RR 0.82 (0.49 to 1.39)	767 (14)	⊕⊕⊕○ moderate Bias: We had concerns about random sequence generation in 11 of the studies, about allocation concealment in 7 of the studies and about blinding (performance bias and detection bias) in six of the studies. We downgraded the quality of the evidence by one step. Heterogeneity/Consistency: I² for the typical RR and for the RD was 0% (both

						no heterogeneity). Directness of evidence: Studies were conducted in the target population. Precision: Because of the large sample size (n = 1202), the point estimate was precise with a narrow 95% CI. Presence of publication bias: The funnel plot was symmetrical
		63 per 1000	52 per 1000 (31 to 88)			
Retinopathy of prematurity (all stages or stage not reported) during initial hospital stay	High risk population	RR 1.27 (0.99 to 1.64)		404 (3)	⊕○○○ very low	Bias: We had concerns about random sequence generation in two of the studies, about allocation concealment in none of the studies, and about blinding (performance bias and detection bias) in two of the studies. We downgraded the quality of the evidence by one step. Heterogeneity/Consistency: I ² for the typical RR was 83% and for the typical RD 82% (both high heterogeneity). We downgraded the quality of the evidence by one step.

					Directness of evidence: Studies were conducted in the target population. Precision: Because of the small sample size (n = 404), the point estimate was not precise. We downgraded the quality of the evidence by one step. Presence of publication bias: As there was only three studies included we did not perform a funnel plot
	328 per 1000	417 per 1000 (325 to 538)			
Retinopathy of prematurity (stage ≥ 3) during initial hospital stay	High risk population	RR 1.73 (0.92 to 3.24)	442 (3)	⊕○○○ very low	Bias: We had concerns about random sequence generation in two of the studies, about allocation concealment in none of the studies, and about blinding (performance bias and detection bias) in two of the studies. We downgraded the quality of the evidence by one step. Heterogeneity/Consistency: I^2 for the typical RR was 18% (no heterogeneity) and for the typical RD 79%

			(high heterogeneity). We downgraded the quality of the evidence by one step. Directness of evidence: Studies were conducted in the target population. Precision: Because of the small sample size (n = 442), the point estimate was not precise. We downgraded the quality of the evidence by one step. Presence of publication bias: As there was only three studies included we did not perform a funnel plot
	63 per 1000	109 per 1000 (58 to 203)	

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: Confidence interval; EPO: erythropoietin; I² = I-squared; NICU: Neonatal Intensive Care Unit; RR: Risk Ratio;

GRADE Working Group grades of evidence

High quality: Further research is very unlikely to change our confidence in the estimate of effect.

Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.

Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.

Very low quality: We are very uncertain about the estimate.

BACKGROUND

Description of the condition

After birth, the haemoglobin (a hemoprotein composed of globin and heme that gives red blood cells their characteristic colour; function primarily to transport oxygen from the lungs to the body tissues) concentration of newborn infants falls to minimal levels of 11 g/dL in term infants by eight to 12 weeks of age, and to 7.0 to 10.0 g/dL in preterm infants by six weeks of age (Stockman 1978). This process is called physiologic anaemia of infancy (Strauss 1986). In very low birth weight (VLBW) infants, the haematocrit (the ratio of the volume of red blood cells to the total volume of blood) falls to approximately 24% in infants weighing 1.0 to 1.5 kg and to 21% in infants weighing less than 1.0 kg at birth (Stockman 1986). In extremely low birth weight (ELBW) infants, this decline in haematocrit is not 'physiologic', as it may be associated with clinical findings that prompt red blood cell transfusions. To our knowledge, the diagnostic accuracy of different clinical signs and laboratory findings has not been studied (Cohen 1998). It is still unknown how low haematocrit levels can fall before clinical signs of anaemia occur and what the minimum haematocrit level is acceptable in infants requiring supplemental oxygen (Ohls 2002). Nevertheless, 'top-up' transfusions to treat low haemoglobin or low haematocrit levels are frequently used. As many as 80% of VLBW infants and 95% of ELBW infants receive blood transfusions during their hospitalisations (Widness 1996).

Description of the intervention

Erythropoiesis-stimulating agents (ESAs) currently include erythropoietin (EPO) and darbepoetin (Darbe). Plasma erythropoietin levels in neonates are lower than those of older children and adults. Brown 1983 reported that, between two and 30 days of life, the mean EPO concentration was 10 mU/mL, as compared to 15 mU/mL in concurrently studied adults. A low plasma EPO level is a key reason that nadir haematocrit values of preterm infants are lower than those of term infants (Dallman 1981; Stockman 1986). Low plasma EPO levels provide a rationale for the use of EPO in the prevention or treatment of anaemia of prematurity. Studies in newborn monkeys and sheep have demonstrated that neonates have a larger volume of distribution and more rapid elimination of EPO, necessitating the use of higher doses than are required for adults (Ohls 2000). A systematic review of EPO administration noted a wide range of doses used, from 90 to 1400 IU/kg/week (Kotto-Kome 2004). Side effects reported in adults include hypertension, bone pain, rash, and rarely, seizures. Only transient neutropenia (low concentration of neutrophils, a type of white blood cell) has been reported in neonates (Ohls 2000).

How the intervention might work

The primary goal of ESAs therapy is to reduce transfusions. Most transfusions are given during the first three to four weeks of life. The larger or stable preterm infants who respond best to EPO receive few transfusions. ELBW infants, who are sick and have the greatest need for red blood cell (RBC) transfusions shortly after birth, have not consistently responded to EPO. This suggests that EPO is a more effective erythropoietic stimulator in more mature neonates. ELBW neonates are more likely to need transfusions even if their erythropoiesis is stimulated (Kotto-Kome 2004). In addition, ELBW neonates have a smaller blood volume and the relatively larger phlebotomy (phlebotomy - the drawing of blood) volumes that are required during hospital stay often necessitate 'early' transfusions. In contrast, 'late' transfusions are more often given because of anaemia of prematurity (Garcia 2002). Most preterm infants who require blood transfusions will receive their first transfusion in the first two weeks of life (Zipursky 2000). Reducing the number of RBC transfusions reduces the risk of transmission of viral infections and may reduce costs. Frequent RBC transfusions may be associated with retinopathy of prematurity (ROP) (abnormal blood vessel development in the retina of the eye) (Hesse 1997) and bronchopulmonary dysplasia (a chronic lung disease that affects newborns and infants) (BPD).

In preterm infants, iron is needed for erythropoiesis. As neonatal blood volume expands with rapid growth, infants produce large amounts of haemoglobin. Several studies have observed a decrease in serum ferritin concentration (an indication of iron deficiency (Finch 1982) during erythropoietin treatment). The use of higher, more effective doses of erythropoietin might be expected to increase iron demand and the risk of iron deficiency. Iron supplementation during erythropoietin treatment has been observed to reduce the risk of the development of iron deficiency (Shannon 1995). The range of iron doses used in EPO-treated infants is between 1 mg/kg/day to 10 mg/kg/day (Kotto-Kome 2004).

EPO has been found to have important non-haematopoietic (formation of blood cellular components) functions in the brain and other organs during development (Juul 2002). Administration of EPO could potentially have a neuro-protective effect in preterm infants, especially in perinatal asphyxia (Dame 2001; Juul 2002). This aspect of EPO use in neonates will be systematically reviewed separately (Yu 2010).

A slightly modified, long-acting version of EPO, darbepoetin alfa (Darbe), has been introduced (Egrie 2001). Darbepoetin was created by modifying five amino acids of the original EPO protein to generate two additional carbohydrate-binding sites, thereby significantly increasing circulating half-life and effectiveness. Compared with EPO, darbepoetin has an approximately three-fold longer serum half-life and greater in vivo potency. It can be administered less frequently to obtain the same biological response. A single subcutaneous dose of Darbe has been shown to accelerate erythropoiesis in preterm infants (Warwood 2005).

It is likely that additional studies of ESAs in preterm or LBW in-

infants have been published since the reviews noted above, which included reports up to October 2002. We have performed a series of Cochrane reviews on the use of EPO in preterm infants, including: *Early erythropoiesis-stimulating agents in preterm or low birth weight infants* (Ohlsson 2017), this review (late erythropoietin; Aher 2012a; Aher 2014), and *Early versus late erythropoietin to prevent red blood cell transfusion in preterm and/or low birth weight infants* (Aher 2012b). The cut-off of seven days of age or less for 'early' and more than seven days for 'late' treatment with EPO, although somewhat arbitrary, was chosen based on previously published meta-analyses (Garcia 2002; Kotto-Kome 2004), and allowed us to compare the results between our reviews and previously published reviews. It is of note that in the latest update of the *Early erythropoiesis-stimulating agents in preterm or low birth weight infants* (Ohlsson 2017) review, there was no significant difference in the incidence of ROP with the treatment with ESAs compared to controls.

Why it is important to do this review

This review concerns late administration of ESAs (starting in infants between eight and 28 days of age, after reaching 40 weeks' postmenstrual age (PMA)). The main rationale for such EPO therapy is to avoid exposure of neonates to multiple blood donors and the risks associated with this exposure. It is likely that many sick neonates would have received a transfusion prior to entry into trials enrolling infants more than seven days of age. We carried out a systematic review to evaluate all available studies where ESAs were begun after seven days of life, to assess the effect on the use of one or more red blood cell transfusions in preterm or very low birth weight infants.

OBJECTIVES

Primary objective

To assess the effectiveness and safety of late initiation of ESAs between eight and 28 days after birth in reducing red blood cell (RBC) transfusions in preterm or low birth weight infants.

Secondary objective

To distinguish between the effectiveness and safety of the intervention by subgroup analyses of low (≤ 500 IU/kg/week) and high (> 500 IU/kg/week) doses of EPO, and low (≤ 5 mg/kg/day) and high (> 5 mg/kg/day) doses of supplemental iron in reducing red blood cell transfusions in preterm or low birth weight infants.

METHODS

Criteria for considering studies for this review

Types of studies

Randomised or quasi-randomised controlled trials.

Types of participants

Preterm (< 37 weeks) and/or low birth weight (< 2500 grams) neonates between eight and 28 days of age.

Types of interventions

Late initiation of ESAs, at eight to 28 days of age, using any dose, route, or duration of treatment, versus placebo or no intervention. The use of any dose of supplemental iron.

Types of outcome measures

Primary outcomes

1. Use of one or more red blood cell transfusions.

Secondary outcomes

1. The total volume (mL/kg) of blood transfused per infant.
2. Number of transfusions per infant.
3. Number of donors to whom the infant was exposed.
4. Mortality during initial hospital stay (all causes of mortality).
5. Retinopathy of prematurity (ROP) (any stage and stage ≥ 3).
6. Proven sepsis (clinical symptoms and signs of sepsis and positive blood culture for bacteria or fungi).
7. Necrotising enterocolitis (NEC) (Bell's stage II or more).
8. Intraventricular haemorrhage (IVH); all grades and grades III and IV.
9. Periventricular leukomalacia (PVL); cystic changes in the periventricular areas.
10. Bronchopulmonary dysplasia (BPD) (supplementary oxygen at 28 days of age or at 36 weeks' postmenstrual age (PMA) and compatible X-ray).
11. Sudden infant death after discharge.
12. Long-term outcomes assessed at any age beyond one year of age by a validated cognitive, motor, language, or behavioural/school/social interaction/adaptation test.
13. Neutropenia.
14. Hypertension (as this outcome was frequently reported by trial authors, it has been added since the protocol was published).
15. Length of hospital stay (days).

16. Any side effects reported in the trials.

Search methods for identification of studies

We used the criteria and standard methods of Cochrane and Cochrane Neonatal (see the Cochrane Neonatal [search strategy](#) for specialised register). We searched for errata or retractions from included studies published in full-text on PubMed (www.ncbi.nlm.nih.gov/pubmed) on June 5th, 2018. We did not limit the search to any geographical region, language, or timing of publication.

Electronic searches

We conducted a comprehensive search including: Cochrane Central Register of Controlled Trials (CENTRAL 2018, Issue 5) in the Cochrane Library; MEDLINE via PubMed (1966 to 5 June 2018); Embase (1980 to 5 June 2018); and CINAHL (1982 to 5 June 2018) using the following search terms: (“Erythropoietin”[Mesh] OR Erythropoietin OR “recombinant human erythropoietin” OR rhuepo OR rhepo OR darbepoetin), plus database-specific limiters for RCTs and neonates (see [Appendix 1](#) for the full search strategies for each database; see [Appendix 2](#) for the search methods for previous versions of this review). We did not apply language restrictions.

We searched, on August 27th 2018, clinical trials registries for ongoing or recently completed trials (clinicaltrials.gov; the World Health Organization’s International [Trials Registry](#) and Platform, and the ISRCTN [Registry](#)).

Searching other resources

We also searched the reference lists of any articles selected for inclusion in this review in order to identify additional relevant articles.

Data collection and analysis

We used the standard methods of the Cochrane Neonatal Review Group and the *Cochrane Handbook for Systematic Reviews of Interventions* ([Higgins 2011](#)) for data collection and analysis.

Selection of studies

Two review authors (AO, SA) assessed all abstracts and published studies identified as potentially relevant by the literature search for inclusion in the review. Each review author extracted data separately onto a data abstraction form. They then compared the information and resolved differences by consensus. One review author (AO) entered the data into Review Manager 5 ([Review Manager 2014](#)) and the other (SA) cross-checked the printout against his own data abstraction forms and corrected errors.

For studies identified as abstracts only, we planned to contact the primary author to obtain further information. The two studies identified as abstracts did not provide enough information for us to be able to contact the authors ([Ahmadpour Kacho 2004](#); [Amin 2004](#)).

This update, in 2018, was conducted by both review authors (SA and AO).

Data extraction and management

The review authors separately extracted, assessed, and coded all data for each study using a form that was designed specifically for this review. We replaced any standard error of the mean by the corresponding standard deviation.

Assessment of risk of bias in included studies

Two review authors (AO, SA) independently assessed the risk of bias (low, high, or unclear) of all included trials using the Cochrane ‘Risk of bias’ tool ([Higgins 2017](#)) for the following domains:

- Sequence generation (selection bias);
- Allocation concealment (selection bias);
- Blinding of participants and personnel (performance bias);
- Blinding of outcome assessment (detection bias);
- Incomplete outcome data (attrition bias);
- Selective reporting (reporting bias);
- Any other bias.

Any disagreements were resolved by discussion or by a third assessor if necessary. See [Appendix 3](#) for a more detailed description of risk of bias for each domain.

For the original review, two review authors (SA, AO) who were not blinded to authors, institution or journal of publication, conducted independent quality assessments.

The 2012 update was conducted by one author (AO) and the updates in 2014 and 2018 by both authors (SA and AO).

Measures of treatment effect

The statistical methods include (typical, when applicable) risk ratio (RR), risk difference (RD), number needed to treat for an additional beneficial outcome (NNTB) or number needed to treat to treat for an additional harmful outcome (NNTH) for dichotomous outcomes, and mean difference (MD) for continuous outcomes, all reported with their 95% confidence intervals (CIs).

Unit of analysis issues

In all studies, the individual infant was the unit of analysis.

Dealing with missing data

We approached trial authors for additional data or for clarification of data.

Assessment of heterogeneity

We conducted heterogeneity tests including the I^2 statistic to assess the appropriateness of pooling the data (Higgins 2003). In this update of the review we used the following categories for heterogeneity: less than 25% no heterogeneity; 25% to 49% low heterogeneity; 50% to 74% moderate heterogeneity and 75% or greater high heterogeneity.

Assessment of reporting biases

We prepared a funnel for the primary outcome in Comparison 1 - use of one or more red blood cell transfusions (low and high dose of EPO).

Data synthesis

We performed meta-analysis using Review Manager 5 software (Review Manager 2014), supplied by Cochrane. For estimates of typical RR and RD, we used the Mantel-Haenszel method. For measured quantities, we used the inverse variance method. All meta-analyses were done using the fixed-effect model. We considered a P value of < 0.05 as statistically significant.

Quality of evidence

- We used the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach, as outlined in the [GRADE Handbook](#) (Schünemann 2013), to assess the quality of evidence for the following (clinically relevant) outcomes in infants exposed to late EPO versus placebo or sham injection. To make the outcomes of this review comparable to the “Early erythropoiesis-stimulating agents in preterm or low birth weight infants review” (Ohlsson 2017), we chose to use the same primary outcome and the same secondary outcomes, when available, and in which two or more studies were included in the analyses for this review. For this review, no long-term outcomes were reported. No studies using Darbe were identified.

Primary outcomes:

- Use of one or more red blood cell transfusions (low and high doses of EPO).

Secondary outcomes:

- NEC ($\geq$ Bell's stage 2);
- Mortality during initial hospital stay (all causes of mortality);
- Retinopathy of prematurity (ROP) (any stage);
- Retinopathy of prematurity (ROP) (stage ≥ 3).

IVH is probably not a relevant outcome in this review as most haemorrhages occur during the first few days of life and infants were enrolled later in these studies. We did not include this outcome in the 'Summary of findings' table.

In this review, no long-term follow-up results were reported.

Two authors (AO, SA) independently assessed the quality of the evidence for each of the outcomes above. We considered evidence from randomised controlled trials as high quality but downgraded the evidence one level for serious (or two levels for very serious) limitations based upon the following: design (risk of bias), consistency across studies, directness of the evidence, precision of estimates, and presence of publication bias. We used the [GRADEpro GDT](#) Guideline Development Tool to create a 'Summary of findings' table to report the quality of the evidence.

The GRADE approach results in an assessment of the quality of a body of evidence in one of four grades:

1. High: We are very confident that the true effect lies close to that of the estimate of the effect.
2. Moderate: We are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
3. Low: Our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.
4. Very low: We have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

Subgroup analysis and investigation of heterogeneity

We performed subgroup analyses for low (≤ 500 IU/kg/week) and high (> 500 IU/kg/week) doses of EPO, and in addition within those subgroups for no iron, low (≤ 5 mg/kg/day) and high (> 5 mg/kg/day) doses of supplemental iron (co-intervention).

Sensitivity analysis

In an attempt to further explore the heterogeneity observed in the primary outcome and subgroup analyses, we performed a post hoc analysis comparing the results of studies that we judged as high quality with those that we identified as of lower quality or could not precisely define their quality because of lack of information. We compared the results of studies that used well defined criteria for red blood cell transfusions to those that used no criteria or less well defined criteria.

RESULTS

Description of studies

For the previous feedback-initiated update, the study by Romagnoli (Romagnoli 2000) was moved from the early EPO review (Ohlsson 2012) to this late EPO review as the infants were of a mean (SD) age of 10 (± 1) days at the initiation of EPO treatment. The study enrolled 230 infants. The 2012 update identified one

new study for inclusion as did this update in 2018. As a result, 31 studies (32 comparisons) including 1651 preterm or low birth weight infants met the inclusion criteria. These studies were performed in 22 countries (Argentina, Australia, Austria, Belgium, Brazil, Canada, Finland, France, Germany, Greece, Iran, Israel, Italy, Japan, Norway, South Africa, Spain, Switzerland, Taiwan (Republic of China), the UK, Turkey, the USA). We decided to include studies that enrolled some infants who were less than eight days at study entry if most of the infants enrolled were older at entry. We made one further deviation from our protocol, as we included studies that enrolled infants beyond 28 days of age. Most of these studies enrolled some infants who were less than 28 days

old but the inclusion criteria did not have 28 days of postnatal age as an upper limit. We could not separate out data for infants that were under 28 days at enrolment. The inclusion of these studies makes our review more comprehensive. The age at enrolment is stated for each study in the table [Characteristics of included studies](#).

Results of the search

The study flow diagram shows the results of the searches ([Figure 1](#)). Only one new study ([Basiri 2015](#)) was identified for this update. No new ongoing trial was identified. No study investigated Darbe.

Figure 1. Study flow diagram: review update

Included studies

We included 31 studies (32 comparisons) in this review. We treated the report by Giannakopoulou as two separate studies; under [Giannakopoulou 1998a](#), we reported on the 32 infants weighing < 1000 grams and under [Giannakopoulou 1998b](#), we reported on the 36 infants weighing 1000 to 1300 grams. The studies are detailed in the table [Characteristics of included studies](#) and are briefly discussed below.

The detailed guidelines, used for transfusions, are outlined in the Additional Table ([Table 1: Transfusion Guidelines](#)).

[Akisu 2001](#) was a single centre study performed at University of Ege, Izmir, Turkey.

- Objective: To evaluate the effect of EPO on lipid peroxidation and the activities of erythrocyte antioxidant enzymes in very low birth weight (VLBW) infants.
- Population: Appropriately grown preterm infants with post menstrual age (PMA) < 33 weeks' gestational age and birth weight < 1500 grams.
- Intervention: The EPO group received high doses of EPO from day 10 of life, totaling 750 IU/kg/week (high dose). Infants in the control group received no placebo. All infants received 3 mg/kg/day (low dose) of elemental iron.
- Outcomes assessed: Use of one or more red blood cell transfusions.

[Al-Kharfy 1996](#) was a single centre study performed in Canada.

- Objective: To determine whether treatment with EPO reduces transfusion requirements in preterm neonates at risk of having bronchopulmonary dysplasia (BDP) and requiring multiple transfusions.
- Population: Appropriately grown preterm infants with birth weight (BW) < 1250 grams and having a 75% probability of having BPD determined on day 10 of life and postnatal age 10 to 17 days.
- Intervention: The EPO group received EPO 200 IU/kg body weight, by subcutaneous (sc) injection, on Monday, Wednesday and Friday for six weeks (600 IU/kg/week; high dose). The control group received sham injections. Oral ferrous sulphate solution was administered to the EPO group at 6 mg of elemental iron/kg/day (high dose) and the control group received 2 mg of elemental iron/kg/day (low dose).
- Outcomes assessed: Number of transfusions per infant, mortality, sepsis, retinopathy of prematurity (ROP) (stage ≥ 3), hypertension, BPD at 28 days of age.

[Atasay 2002](#) was a single centre study performed in Turkey.

- Objective: To investigate the effect of early EPO treatment on induction of erythropoiesis and the need for transfusion in VLBW infants with acute neonatal problems.

- Population: Infants with BW < 1500 grams and PMA < 32 weeks' gestational age.

- Intervention: The EPO group received EPO 600 IU/kg/week (high dose) sc, at seven to ten days and continued over seven to eight weeks. The control group received no EPO, placebo, or iron. The EPO group was supplemented with oral iron (ferro glycine sulphate) at the dose of 3 mg/kg/day (low dose).

- Outcomes assessed: Use of one or more red blood cell transfusion(s).

[Bader 1996](#) was a two-centre study performed in Israel.

- Objective: To assess whether an iron dose of 6 mg/kg/day is sufficient to maintain serum ferritin at adequate levels (as per authors).

- Population: Preterm infants with PMA < 34 weeks' gestational age and BW < 1750 grams and postnatal age three to five weeks.

- Intervention: The EPO group received EPO 300 IU/kg/day sc three times a week (900 IU/kg/week; high dose), for a total duration of four weeks. The control group received no placebo or other intervention. Two weeks into the study, elemental iron supplementation was begun in both groups at a dose of 6 mg/kg/day (high dose).

- Outcomes assessed: Use of one or more red blood cell transfusions, side effects, sudden infant death syndrome (SIDS).

[Basiri 2015](#) was a single centre study performed in Iran.

- Objective: To evaluate the effectiveness of EPO treatment in preventing anaemia of prematurity.

- Population: Preterm infants of 24 to 31 weeks' postmenstrual age.

- Intervention: The study group 250 U rhEpo/kg BW (0.1 ml solution/kg BW) given sc 3 days a week every other day on Monday, Wednesday and Friday. (= 3 x 250 U/week = 750 IU/week; high EPO). The control group received equal volume normal saline 0.1 ml solution/kg BW. Infants in both groups were given oral ferrous sulphate, 4 mg/kg/day during the entire treatment period (4 mg/kg/day; low iron (< 5 mg/kg/day)).

- Outcomes assessed: Use of one or more red blood cell transfusion(s), unspecified side effects.

[Bechensteen 1993](#) was a four-centre study performed in Norway.

- Objective: To determine whether VLBW infants respond to EPO with increased erythropoiesis.

- Population: Preterm infants with BW 900 grams to 1400 grams at three weeks of age.

- Intervention: The EPO group received EPO 100 IU/kg three times a week (300 IU/kg/week; low dose) sc from three to seven weeks of age. The control group received neither EPO nor placebo. Oral iron 18 mg/day (high dose) regardless of weight,

began at the start of the study (three weeks). If serum iron concentration fell below 16 micromol/L, the dose was increased to 36 mg/day.

- Outcomes assessed: Use of one or more red blood cell transfusions, mortality, hypertension, neutropenia, side effects.

Bierer 2009 was a single centre study performed in the USA.

- Objective: To determine if EPO administration to neonates requiring surgery would stimulate erythropoiesis.
- Population: Neonates requiring surgery. Post-natal age < 28 days. The majority of infants were preterm and/or LBW.
- Intervention: The EPO group received EPO 200 IU/kg per day or 400 IU/kg sc three times weekly (high dose). The placebo group received an equal volume of normal saline if the infant was on total parenteral nutrition and, if not, a sham dose was given sc and a BandAid was placed over the sham injection site. Infants received oral iron supplementation when enteral feeds reached 60 mL/kg/day.
- Outcomes assessed: Volume transfused during hospitalisation (mL/kg), number of donors the infants was exposed to.

Chen 1995 was a single centre study performed in Taiwan, Republic of China.

- Objective: To evaluate the safety and efficacy of EPO for the treatment of anaemia of prematurity.
- Population: Preterm infants with PMA $\leq$ 33 weeks' gestational age and BW $\leq$ 1750 grams, haemoglobin (Hb) < 10 g/dL and haematocrit (Hct) < 30%.
- Intervention: The EPO group (A) received 150 IU/kg iv twice a week (300 IU/kg/week; low dose); Group B received packed washed erythrocyte transfusion, when their Hb levels were < 10 g/dL with signs and symptoms attributed to anaemia or who had a Hb level < 8 g/dL even if asymptomatic; group C did not receive EPO or erythrocyte transfusions (three infants excluded from total 19, as they received erythrocyte transfusion later because of frequent episodes of apnoea). All infants received oral elemental iron 3 mg/kg/day (low dose). Group A and C were included in this review.
- Outcomes assessed: Mortality, adverse effects.

Corona 1998 was a single centre study performed in Italy.

- Objective: To evaluate the efficacy of EPO, establish the optimal dose, the age at which to start, the duration of the treatment, any adverse effects, and the reduction in red blood cell transfusions.
- Population: Preterm infants (BW < 1500 grams and < 33 weeks' postmenstrual age).
- Intervention: EPO group A received EPO 150 IU/kg/week sc low dose; EPO group B received 300 IU/kg/week sc low dose; the control group (group C) received no treatment. All groups received oral iron 4 mg/kg/day (low dose).

- Outcomes assessed: Use of one or more red blood cell transfusions, total volume (mL/kg) of blood transfused per infant (means but no standard deviation (SD) provided), side effects.

Donato 1996 was a single centre study performed in Argentina.

- Objective: To assess the efficacy of three different doses of recombinant human EPO (rHuEPO) to reduce the need for transfusion in preterm infants with BW < 1500 grams.
- Population: Preterm infants with PMA < 34 weeks' gestational age and BW < 1500 grams.
- Intervention: The placebo group (A) received human seroalbumin. The three EPO groups: Group B received EPO 50 IU/kg (150 IU/kg/week; low dose), Group C received EPO 100 IU/kg (300 IU/kg/week; low dose) and Group D received EPO 250 IU/kg (750 IU/kg/week; high dose) sc during eight consecutive weeks. All participants were given oral iron 6 mg/kg/day (high dose) and folic acid (2 mg/day) supplements, starting on day 15 of age and continuing during whole treatment period.
- Outcomes assessed: Use of one or more red blood cell transfusions, average number of transfusions per infant during treatment, mortality, mean length of hospitalisation time, side effects, hypertension, SIDS.

Emmerson 1993 was a single centre study performed in the UK.

- Objective: To investigate the safety and efficacy of EPO for the prevention of anaemia of prematurity.
- Population: Infants between 27 and 33 weeks' postmenstrual age.
- Intervention: The EPO group received low dose EPO (between 50 and 150 IU) twice a week from seven days of age and the placebo group received 4% albumin from seven days of age until discharge home. All infants received iron (6.25 mg) in the form of ferrous glycine sulphate from four weeks of age (high dose).
- Outcomes assessed: Use of one or more red blood cell transfusions, volume transfused (mL/kg), mortality, hospital stay, SIDS, neutropenia.

Giannakopoulou 1998a and **Giannakopoulou 1998b** was a single centre study performed in Greece.

- Objective: To stimulate erythrocyte production by the use of EPO and thereby decrease the requirement for red blood cell transfusions.
- Population: Preterm infants with BWs $\leq$ 1300 grams, postnatal age > 20 days.
- Intervention: The EPO group received EPO 300 IU/kg body weight, three times a week (900 IU/kg/week; high dose) from 20 days of age for six to eight weeks. The control group did not receive any placebo. All infants received oral elemental iron 10 mg/kg/day (high dose).
- Outcomes assessed: Mortality, side effects, hypertension, neutropenia.
- We treated this report as two separate studies; Under **Giannakopoulou 1998a** we reported on the 32 infants weighing

< 1000 g and under [Giannakopoulou 1998b](#) we reported on the 36 infants weighing 1000 to 1300 grams.

[Griffiths 1997](#) was a study conducted in four neonatal intensive care units (NICU) in Yorkshire, England.

- Objective: To evaluate the role of EPO in reducing iron supplementation, which may exacerbate free radical change, leading to lung disease.
- Population: Preterm infants with postmenstrual age ≤ 32 weeks and/or BW ≤ 1500 grams, requirement for mechanical ventilation and/or supplemental oxygen at birth. Postnatal age seven to 14 days.
- Intervention: The EPO group received 480 IU/kg/week (low dose) and the control group received placebo (4% human albumin) starting at seven to 14 days of age. All infants received oral iron (3.0 mg/kg/day) (low dose) from four weeks after birth.
- Outcomes assessed: Mortality, BPD (at 36 weeks' postmenstrual age), number of blood transfusions per infant (medians provided), SIDS.

[Javier Manchon 1997](#) was a multi-centre study involving three centres in Barcelona, Spain.

- Objective: To test the therapeutic effect of EPO on anaemia of prematurity.
- Population: Preterm infants < 34 weeks' postmenstrual age, who at 28 days after birth had Hb levels < 10.5 g/dL.
- Intervention: The EPO group received high dose EPO (200 IU/kg/day) three days a week for four weeks and ferrous sulphate 4 mg/kg/day (low dose). The control infants did not receive placebo, EPO, or iron.
- Outcomes assessed: Use of one or more red blood cell transfusions between 30 and 60 days of age.

[Juul 2003](#) was a single centre study performed in the USA.

- Objective: To determine whether enterally dosed EPO stimulates erythropoiesis in preterm infants.
- Population: Preterm infants with BW between 700 to 1500 grams and receiving at least 30 mL/kg per day of enteral feeding.
- Intervention: The EPO group received 1000 IU/kg (enterally) per day divided into two daily feedings (7000 IU/kg/week; high dose), for 14 days. The placebo group received 5% dextrose in water for 14 days. All participants received supplemental iron (iron dextran, 1.0 mg/kg/day, or enteral ferrous sulphate 6 mg/kg/day (high dose)).
- Outcomes assessed: Phlebotomy loss (mL) and packed red blood cell transfusion volume (mL). Evidence of feeding intolerance and other adverse effects.

[Kivivuori 1999](#) was a four-centre study performed in Helsinki and Espoo, Finland.

- Objective: To compare oral and intramuscular routes of administration of iron in EPO treated VLBW infants.
- Population: VLBW infants (birth weight ranged from 625 to 1470 grams).

- Intervention: One EPO group received EPO 300 IU/kg sc three times/week, 900 IU/kg/week (high dose) sc and oral iron 6 mg/kg/day (high dose). Another EPO group received EPO 900 IU/kg/week and weekly intramuscular (im) iron 12 mg/kg (high dose). The control group received im iron 12 mg/kg/week but no EPO.

- Outcomes assessed: Use of one or more red blood cell transfusions, adverse effects.

[Kumar 1998](#) was a single centre study performed in the USA.

- Objective: To evaluate the efficacy and safety of EPO in VLBW infants with anaemia of prematurity.
- Population: Preterm infants (PMA < 32 weeks, BW < 1250 grams) with anaemia of prematurity.
- Intervention: The EPO group received 300 IU/kg/dose of EPO sc twice a week (600 IU/kg/week; high dose) for six weeks. The control group received an identical volume of placebo suspension (normal saline). All infants received elemental iron 6 mg/kg/day (high dose).
- Outcomes assessed: Use of one or more red blood cell transfusions, number of erythrocyte transfusions (per infant), entry to discharge duration (days), side effects.

[Maier 2002](#) was a multicentre study performed in 14 centres in four European countries (Belgium, France, Germany, Switzerland).

- Objective: To investigate whether EPO reduces the need for transfusions in extremely low birth weight (ELBW) infants and to determine the optimal dose of treatment.
- Population: Preterm infants with BW 500 to 999 grams.
- Intervention: The EPO group received EPO 200 IU/kg/dose three times a week sc (600 IU/kg/week, high dose). The volume was increased by the equivalent of 50 IU/kg per dose if the haematocrit declined by 6% during any two-week period during the trial, but was withheld if the haematocrit was > 45%. The control group received an identical volume of placebo. Enteral iron 3 mg/kg/day (low dose) was given to all infants from days three to five and was increased at days 12 to 14 to 6 mg/kg/day (high dose) and to 9 mg/kg/day (high dose) at days 24 to 26 of life.
- Outcomes assessed: Use of one or more red blood cell transfusions, donor exposure, mortality during hospital stay, necrotising enterocolitis (NEC), IVH, periventricular leukomalacia (PVL), ROP, days in oxygen, days in NICU, days in hospital.

[Meyer 1994](#) was a single centre study performed in South Africa.

- Objective: To assess the efficacy of EPO in the treatment of anaemia of prematurity.
- Population: Preterm infants (≤ 32 weeks' postmenstrual age, weight ≤ 1500 grams, postnatal age two to eight weeks, central haematocrit Hct $\leq 35\%$).
- Intervention: The EPO group received high dose EPO (200 IU/kg three times a week; 600 IU/kg/week). The control group

received an identical volume of placebo. All infants received 3 mg/kg/day of iron (low dose).

- Outcomes assessed: Use of one or more red blood cell transfusions, sepsis, NEC, SIDS.

Pollak 2001 was a single centre study conducted in Vienna, Austria.

- Objective: To test the efficacy and safety of combining intravenous iron in amounts approximately the in-utero accretion rate and the postnatal iron loss with EPO in VLBW infants.
- Population: Preterm infants < 31 weeks' postmenstrual age and < 1300 grams birth weight not treated with red blood cell transfusions during the study period.
- Intervention: During a three-day run-in baseline period, 9 mg/kg/day of iron poly maltose complex (high dose) was administered to all participants in all groups. This was followed by a treatment period during which participants received: 1) the same oral iron supplementation dose alone (oral iron group - control group); 2) 300 IU/kg/day of EPO iv at three-day intervals (600 IU/kg/week, high dose) along with the same oral iron supplement as the oral iron group (EPO + oral iron group); or 3) 2 mg of intravenous iron sucrose/kg/day + EPO as in group two (iv iron + EPO group). To maintain comparability of iron intake among the three groups, this last group also received EPO and oral iron in an identical manner to the EPO + oral iron group.
- Outcomes assessed: Mortality, sepsis, ROP, BPD (oxygen dependency at 36 weeks' postmenstrual age).

Reiter 2005 was a single centre study performed in the USA.

- Objective: To determine the effectiveness of a 10-day EPO course in preterm infants.
- Population: Preterm infants < 32 weeks' postmenstrual age, haematocrit $\leq$ 28%, post-conceptual age of < 48 weeks or five months chronological age.
- Intervention: The EPO group received 300 IU/kg/day (high dose) and 6 mg/kg/day of enteral iron (high dose) versus iron only. Both groups received the intervention for 10 days.
- Outcomes assessed: Use of one or more red blood cell transfusions, volume of red blood cells required (mL/kg).

Rocha 2001 was a single centre study performed in Brazil.

- Objective: To assess the efficacy of EPO for the prevention and treatment of anaemia of prematurity.
- Population: Preterm infants with PMA up to 33 weeks, BW up to 1550 grams and postnatal age between 10 and 35 days.
- Intervention: The two EPO groups received either daily doses of 100 IU/kg of EPO or twice weekly doses of 350 IU/kg (700 IU/kg/week, high dose). The EPO groups were given iron (ferrous sulphate) 3 mg/kg/day enterally, and increased to 6 mg/kg/day in the second week of treatment. In the control group, iron supplementation was initiated around the 30th day of life. The control group did not receive any placebo.

- Outcomes assessed: Mean number of blood transfusions per participant (no SD provided), two or more blood transfusions per participant.

Romagnoli 2000 was a single centre study performed in Italy.

- Objective: To assess the effectiveness of rhEPO.
- Population: Infants with PMA $\leq$ 30 weeks and those infants of 31 to 34 weeks' PMA suffering from respiratory distress syndrome and requiring mechanical ventilation.
- Intervention: Treated infants received rhEPO doses of 300 IU/kg body weight sc on each Monday, Wednesday and Friday from the 2nd to the 7th week of life (high dose). Iron supplementation (Intrafer-Geymonat) was given at a dose of 1 mg/kg per day iv from the 2nd to the 4th week (low dose); thereafter, 12 mg/kg/day orally until the 7th week of life.
- Outcomes assessed: Retinopathy of prematurity (stage 1 to 2 and stage 3 to 4), IVH (> grade 2), sepsis, NEC, number of infants transfused, number of transfusions per infant.

Ronnestad 1995 was a single centre study performed in Norway.

- Objective: To investigate whether EPO given to infants < 32 weeks' gestational age, fed with their own mother's milk supplemented with a bovine milk protein and electrolyte fortifier together with moderate iron supplementation, would ameliorate the anaemia and thus reduce the need for blood transfusions after the second week of life.
- Population: Preterm infants, PMA < 32 weeks. Age 14 to 22 days.
- Intervention: The EPO group received EPO 150 IU/kg three times per week (450 IU/kg/week; low dose) or placebo. All infants received 4 mg/kg/day of iron (low dose) as ferrous fumarate.
- Outcomes assessed: Neutropenia.

Samanci 1996 was a single centre study performed in Turkey.

- Objective: To determine whether EPO would prevent anaemia of prematurity and reduce the need for transfusion in infants with VLBW.
- Population: Preterm infants with PMA $\leq$ 32 weeks, BW of $\leq$ 1250 grams. Postnatal age at the first dose was two to four weeks.
- Intervention: The EPO group received 200 IU/kg sc three times weekly (600 IU/kg/week, high dose), for four weeks. The control group received an equivalent volume of placebo sc, three times weekly, for four weeks. All infants received oral supplements of elemental iron (3 mg/kg/day) (low dose) during the study period.
- Outcomes assessed: Use of one or more red blood cell transfusions, number of blood transfusions per infant, NEC, IVH, major adverse events.

Shannon 1991 was a three-centre study performed in the USA.

- Objective: Not stated.
- Population: Preterm infants with BWs $\leq$ 1250 grams.

- Intervention: The EPO group received EPO 100 IU/kg, twice each week (200 IU/kg/week; low dose) for six weeks. The control group received intravenous injections of an identical volume of placebo twice each week for six weeks. All infants received 3 mg/kg/day of oral iron (low dose) and continued at the discretion of the attending physician.

- Outcomes assessed: Use of one or more red blood cell transfusions, mortality, NEC, hypertension, neutropenia, side effects.

[Shannon 1992](#) was a single centre study performed in the USA.

- Objective: Not stated.
- Population: Preterm infants with PMA < 33 weeks and BW < 1250 grams.
- Intervention: The EPO group received EPO 100 IU/kg, 5 times a week (500 IU/kg/week; high dose). The control group received an identical volume of placebo suspension, five times a week. Oral iron was started in all infants at 3 mg/kg/day (low dose), divided into three doses and given between feedings. The iron dose was increased to 6 mg/kg/day (high dose) for infants who were tolerating full caloric feedings.
- Outcomes assessed: Use of one or more red blood cell transfusions, major adverse events.

[Shannon 1995](#) was a multi-centre study at 11 centres in the USA.

- Objective: To assess whether treatment with EPO would stimulate erythropoiesis and thereby reduce the need for erythrocyte transfusions in preterm infants.
- Population: Preterm infants with PMA < 31 weeks with BW of $\leq$ 1250 grams.
- Intervention: The EPO group received 100 IU/kg/day (from Monday through Friday (500 IU/kg/week; high dose)) for six weeks or until the infants were ready to be discharged home. Doses of EPO (or placebo) were adjusted weekly according to changes in body weight. The control group received an identical volume of placebo suspension. Participants received oral iron supplements at study entry to achieve a total enteral intake of 3 mg/kg/day of elemental iron (low dose). Total iron intake was increased to 6 mg/kg/day (high dose) when the infants tolerated full caloric feeding enterally.
- Outcomes assessed: Use of one or more red blood cell transfusions, mean number of erythrocyte transfusions per infant, mortality, sepsis, NEC, ROP, hypertension, SIDS, side effects.

[Whitehall 1999](#) was a single centre study conducted in Australia.

- Objective: To evaluate safety and efficacy of EPO in reducing the need for red cell transfusions in anaemia of prematurity.
- Population: Infants with PMA $\leq$ 32 weeks.
- Intervention: Infants in the EPO group received 400 IU/kg every second day x 10 doses (high dose). Infants in the control group received no placebo. Both groups received 3 mg/kg/day of

iron (low dose) increased to 6 mg/kg/day (high dose), as tolerated.

- Outcomes assessed: Total volume (mL/kg) of blood transfused, number of transfusions per infant, mortality during hospital stay.

[Yamada 1999a](#) was a single centre study conducted in Japan.

- Objective: To assess the efficacy of EPO in the treatment of anaemia of prematurity.
- Population: Infants with BW 1000 to 1499 grams, PMA < 33 weeks, haemoglobin < 12 g/dL and oral milk intake > 50 mL/kg/day.
- Intervention: The EPO group received EPO (200 IU/kg twice a week, low dose) for eight weeks and the control group received no study drug or placebo. All infants received 3 mg/kg/day of oral iron (low dose).
- Outcomes assessed: Use of one or more red blood transfusions, total volume of blood transfused (mL/infant), number of transfusions per infant, side effects.

[Yamada 1999b](#) was a single centre study conducted in Japan.

- Objective: To assess the efficacy of EPO in the treatment of anaemia of prematurity.
- Population: Infants with BW 500 to 999 grams, PMA < 33 weeks, haemoglobin < 13 g/dL and oral milk intake > 50 mL/kg/day.
- Intervention: The EPO group received low dose EPO (200 IU/kg twice a week) for eight weeks and the control group received no study drug or placebo. All infants received 3 mg/kg/day of oral iron (low dose).
- Outcomes assessed: Use of one or more red blood transfusions, total volume of blood transfused (mL/infant), number of transfusions per infant, side effects.

Three different routes of administration were used; subcutaneous (sc) ([Shannon 1992](#); [Bechensteen 1993](#); [Emmerson 1993](#); [Meyer 1994](#); [Yamada 1999a](#); [Yamada 1999b](#); [Ronnstad 1995](#); [Shannon 1995](#); [Al-Kharfy 1996](#); [Bader 1996](#); [Donato 1996](#); [Samanci 1996](#); [Griffiths 1997](#); [Javier Manchon 1997](#); [Corona 1998](#); [Giannakopoulou 1998a](#); [Giannakopoulou 1998b](#); [Kumar 1998](#); [Kivivouri 1999](#); [Whitehall 1999](#); [Romagnoli 2000](#); [Akisu 2001](#); [Rocha 2001](#); [Atasay 2002](#); [Reiter 2005](#); [Bierer 2009](#); [Basiri 2015](#)), intravenous (iv) ([Pollak 2001](#); [Shannon 1991](#); [Chen 1995](#)), oral ([Juul 2003](#)), or iv or sc ([Maier 2002](#)). The dose of EPO varied from 150 IU/kg/week ([Donato 1996](#); [Corona 1998](#)) to 2100 IU/kg/week ([Reiter 2005](#)) when given subcutaneously. When given intravenously, the dose varied from 200 IU/kg/week ([Shannon 1991](#)) to 300 IU/kg/week ([Chen 1995](#)). [Juul 2003](#) provided 7000 IU/kg/week enterally.

Different EPO preparations were used; Recormon (Boehringer-Mannheim, Germany); Eprex (provided by Cilag Zug, Switzerland, Ortho Pharmaceutical Canada Ltd., Janssen-Cilag or Guler Pharmaceutical Corp, Istanbul, Turkey) ([Bechensteen 1993](#); [Emmerson 1993](#); [Meyer 1994](#); [Chen 1995](#);

Ronnestad 1995; Al-Kharfy 1996; Bader 1996; Samanci 1996; Griffiths 1997; Giannakopoulou 1998a; Giannakopoulou 1998b; Kivivuori 1999; Whitehall 1999; Atasay 2002); Amgen (Shannon 1991), Eogen alpha (Amgen, Inc. Thousand Oaks, CA, USA) (Reiter 2005); unnamed products (Shannon 1992; Shannon 1995; Javier Manchon 1997; Corona 1998; Kumar 1998; Yamada 1999a; Yamada 1999b; Romagnoli 2000; Rocha 2001; Juul 2003; Bierer 2009), NeoRecormon (F. Hofman-La Roche, Basel, Switzerland) (Maier 2002), Erypo (Janssen-Cilag Pharma, Vienna, Austria) (Pollak 2001), Hemax (Bio Sidus, S. A.) (Donato 1996) and PDpoietin (Pooyesh Darou Inc, Iran) (Basiri 2015).

Three studies did not state that guidelines for red blood cell transfusions were in place (Shannon 1991; Chen 1995; Akisu 2001). In only one study was it explicit that infants who had received erythrocyte transfusions prior to study entry were excluded (Samanci 1996). For transfusion guidelines, see Additional Table (Table 1: *Transfusion Guidelines*).

Excluded studies

One study (Ohls 1991) was excluded as it compared an EPO-treated group with a group receiving blood transfusions. Two studies (Messer 1993; Testa 1998) were excluded as they were not randomised controlled trials. Two studies reported only as abstracts were excluded as one study from Saudi Arabia lacked information to ascertain whether the study was a randomised controlled trial or not (Amin 2004) and the other study conducted in Iran did not provide the age of the infants at the time of enrolment (Ahmadpour Kacho 2004). We were unable to contact the authors

for additional information. Warwood 2005 was a dose-finding study of darbepoetin (longer-acting and more potent than EPO). Infants were randomised to receive either one or four microgram/kg of a single dose of darbepoetin, without an untreated control group. Meyer 1996, Bechensteen 1997 and Pathak 2003 lacked an untreated control group. There were no outcomes of interest in the study by Widness 2006. In the searches in February 2010, we identified two additional studies (Badiie 2006; Pasha 2008). However, as they did not report on any of our prespecified outcomes we excluded them. In the search conducted in 2012, we identified and excluded three additional studies. In Mohammadzadeh 2005, both groups received EPO. Warwood 2011 reported that “only one of the 20 study subjects qualified for and received a subsequent rbc transfusion during the hospitalisation”. The authors did not state if the infant who received the transfusion belonged to the darbepoetin or the control group. Ohls 2012 compared two dosing schedules; once a week versus three times a week of EPO in infants aged seven days or more.

Risk of bias in included studies

The assessment of individual studies is presented in the table ‘Characteristics of included studies’ and summarised in the ‘Risk of Bias’ graph (Figure 2) and in the ‘Risk of bias’ summary (Figure 3). All studies were reported as randomised controlled trials. Information on which to base our judgements on whether a study used concealed allocation or not was often not clearly reported. In general, the studies were of small sample size, ranging from eight (Shannon 1992) to 230 infants (Romagnoli 2000). The studies often lacked a sample size calculation.

Figure 2. Risk of bias graph: review authors’ judgements about each risk of bias item presented as percentages across all included studies.

Figure 3. Risk of bias summary: review authors' judgements about each risk of bias item for each included study.

	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding (performance bias and detection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)	Other bias
Akisu 2001	?	?	+	+	?	+
Al-Kharfy 1996	?	+	+	+	?	+
Atasay 2002	?	?	?	+	?	+
Bader 1996	?	?	+	+	?	+
Basiri 2015	+	?	+	+	?	+
Bechensteen 1993	?	+	+	+	?	+
Bierer 2009	+	+	+	+	?	+
Chen 1995	?	?	+	+	?	+
Corona 1998	?	?	+	+	?	+
Donato 1996	?	?	+	+	?	+
Emmerson 1993	?	?	+	+	?	+
Giannakopoulou 1998a	?	?	+	+	?	+
Giannakopoulou 1998b	?	?	+	+	?	+
Griffiths 1997	?	+	+	+	?	+
Javier Manchon 1997	?	?	+	+	?	+
Juul 2003	?	?	+	+	?	+
Kivivuori 1999	?	?	+	+	?	+
Kumar 1998	?	?	+	+	?	+
Maier 2002	?	+	+	+	?	+
Meyer 1994	+	?	+	+	?	+
Pollak 2001	?	+	+	+	?	+
Reiter 2005	?	?	+	+	?	+
Rocha 2001	?	?	+	+	?	+
Romagnoli 2000	+	+	+	+	?	?
Rønnestad 1995	?	?	+	+	?	+
Samanci 1996	+	?	+	+	?	+
Shannon 1991	?	?	+	+	?	+
Shannon 1992	?	?	+	+	?	+
Shannon 1995	+	+	+	+	?	+
Whitehall 1999	+	+	+	+	?	+
Yamada 1999a	?	?	+	+	?	+
Yamada 1999b	?	?	+	+	?	+

We performed two post hoc secondary analyses for the primary outcome 'Use of one or more red blood cell transfusions'. In the first, we compared those studies that used concealed allocation (a placebo or sham-injection to blind the intervention) and in which there was blinding of outcome measure assessment to those studies in which this was not evident from the published report. In the second post hoc analysis, we compared the studies that used well defined criteria for red blood cell transfusions with those that used no or less well defined criteria.

Random sequence generation

There was low risk of bias in seven studies (22% of the studies; Meyer 1994; Shannon 1995; Samanci 1996; Whitehall 1999; Romagnoli 2000; Bierer 2009; Basiri 2015). In the remaining 78% of the studies, there was an unclear risk of bias.

Allocation

We considered the concealment of allocation to be appropriate in nine studies (28% of the studies; Bechensteen 1993; Shannon 1995; Al-Kharfy 1996; Griffiths 1997; Whitehall 1999; Romagnoli 2000; Pollak 2001; Maier 2002; Bierer 2009). In the remaining 23 studies (72% of the studies), there was an unclear risk of bias.

Blinding

We considered there to be a low risk of performance bias and detection bias in 15 studies (47% of the studies; Shannon 1991; Shannon 1992; Emmerson 1993; Meyer 1994; Ronnestad 1995; Shannon 1995; Al-Kharfy 1996; Donato 1996; Samanci 1996; Griffiths 1997; Kumar 1998; Maier 2002; Juul 2003; Bierer 2009; Basiri 2015). Many studies did not use a placebo or sham injection, precluding blinding of the intervention and the outcome measure assessment. There was a high risk of bias in 16 studies (50% of the studies; Bechensteen 1993; Chen 1995; Bader 1996; Javier Manchon 1997; Corona 1998; Giannakopoulou 1998a; Giannakopoulou 1998b; Kivivuori 1999; Whitehall 1999; Yamada 1999a; Yamada 1999b; Romagnoli 2000 Akisu 2001; Pollak 2001; Rocha 2001; Reiter 2005). In one study (3% of the studies; Atasay 2002), there was an unclear risk of bias.

Incomplete outcome data

There was a low risk of bias for 30 studies (94% of the studies) and a high risk of bias in two studies (6% of the studies; Juul 2003; Kivivuori 1999).

Selective reporting

The risk of bias was unclear to us for all the studies (100%) as the study protocols were not available for us to read.

Other potential sources of bias

We had serious concerns about possible 'other bias' in one study (Rocha 2001). The study Romagnoli 2000 was reported in a 'Research letter' format allowing only few details. We obtained information from the Editorial office of the European Journal of Pediatrics, that 'Research letters' are peer-reviewed. For details regarding these two studies, please see the notes under [Characteristics of included studies](#).

Effects of interventions

See: [Summary of findings for the main comparison](#)

For this update, one new study was identified (Basiri 2015). This added 60 infants to the review. The review now includes 31 studies (32 comparisons) that randomised 1651 infants. For details of results, see [Data and analyses](#).

All studies compared late initiation of erythropoietin (EPO) (8 - 28 days) versus placebo or no intervention.

Primary Outcome:

The use of one or more red blood cell transfusions (Outcome 1.1)

See [Figure 4](#).

Figure 4. Forest plot of comparison: I Late initiation of EPO (8 - 28 days) vs placebo or no intervention, outcome: 1.1 Use of one or more red blood cell transfusions (low and high dose of EPO).

A total of 21 studies including 1202 infants reported on the use of one or more red blood cell transfusions following the use of either low or high dose of EPO. There was a significant reduction in the use of one or more red blood cell transfusions (typical risk ratio (RR) 0.72, 95% confidence interval (CI) 0.65 to 0.79; typical risk difference (RD); -0.17, 95% CI -0.22 to -0.12; number needed to treat for an additional beneficial outcome (NNTB); 6, 95% CI 5 to 8). There was moderate heterogeneity for this outcome (for RR; I² = 66.0%; for RD; I² = 58.0%). The quality of the evidence was very low.

Subgroup analyses:

We conducted further analyses including studies that used a **high dose of EPO** (> 500 IU/kg/week) or a **low dose of EPO** (≤ 500 IU/kg/week).

High dose of EPO (Outcome 1.2)

The summary estimates for 15 studies including 972 infants testing a **high dose of EPO (Outcome 1.2)** were statistically significant with a typical RR of 0.77 (95% CI 0.68 to 0.86), a typical RD of -0.14 (95% CI -0.19 to -0.08) and a NNTB of 7 (95% CI 5 to 13). There was moderate heterogeneity for this outcome for RR (I² = 63%) and for RD (I² = 62%).

We conducted a subgroup analysis for **high dose of EPO in combination with high dose of iron (Outcome 1.2.1)**. Six studies

(n = 318) showed a typical RR of 0.74 (95% CI 0.62 to 0.88), a typical RD of -0.16 (95% CI -0.24 to -0.08) and NNTB of 6 (95% CI 4 to 13). There was high heterogeneity for both RR (I² = 79%) and for RD (I² = 78%).

Nine studies of **high EPO and low dose of iron (Outcome 1.2.2)** (n = 654) showed a typical RR of 0.78 (95% CI 0.67 to 0.90), a typical RD of -0.13 (95% CI -0.20 to -0.06) and NNTB of 8 (95% CI 5 to 20). There was moderate heterogeneity for RR (I² = 52%) and low heterogeneity for RD (I² = 29%).

Low dose of EPO (Outcome 1.3)

The summary estimates for seven studies including 239 infants testing a **low dose of EPO (Outcome 1.3)** were statistically significant with a typical RR of 0.53 (95% CI 0.42 to 0.67), a typical RD of -0.34 (95% CI -0.45 to -0.23) and a NNTB of 3 (95% CI 2 to 4). There was moderate heterogeneity for RR (I² = 59%) and no heterogeneity for RD (I² = 14%).

We conducted a subgroup analysis for **low dose of EPO in combination with high dose of iron (Outcome 1.3.1)**. Three studies (n = 77) showed a typical RR of 0.50 (95% CI 0.31 to 0.79), a typical RD of -0.31 (95% CI -0.49 to -0.13) and a NNTB of 3 (95% CI 2 to 8). There was no significant heterogeneity for this outcome for RR (I² = 0%) and RD (I² = 0%).

Four studies (n = 162) evaluated the effectiveness of **low dose of EPO in combination with low dose of iron (Outcome 1.3.2)**.

The typical RR was 0.54 (95% CI 0.41 to 0.71), the typical RD was -0.36 (95% CI -0.49 to -0.22) and the NNTB was 3 (95% CI 2 to 5). There was high heterogeneity ($I^2 = 76\%$) for RR and moderate heterogeneity for RD ($I^2 = 53\%$).

Secondary outcomes:

The total volume (mL/kg) of blood transfused per infant (Outcome 1.4)

Five studies including 197 infants reported on the total volume of blood transfused per infant. The typical mean difference (MD) between the groups was not statistically significant, with a typical MD of -1.61 mL/kg (95% CI -5.78 to 2.57) transfused per infant. There was high heterogeneity ($I^2 = 92\%$). The very high heterogeneity may indicate that the data are skewed and it brings uncertainty to the estimate. [Corona 1998](#) ($n = 60$) reported on this outcome but provided only the means with no standard deviation (SD). In the two EPO groups combined, the mean was 20 mL/kg and in the control group it was 32 mL/kg ($P < 0.01$, according to the authors).

Number of red blood cell transfusions per infant (Outcome 1.5)

The number of red blood cell transfusions per infant was reported in 11 studies enrolling 817 infants. The significant typical MD was -0.22 (95% CI -0.38 to -0.06) favouring the EPO group. There was high heterogeneity ($I^2 = 94\%$). In [Griffiths 1997](#) ($n = 42$), the median number of blood transfusions was lower for the infants in the EPO group (difference in medians -2 (95% CI -4 to 0)).

Number of donors to whom the infant was exposed (Outcome 1.6)

Two studies reported on donor exposure in 165 enrolled infants. The significant MD was 0.45 (95% CI 0.20 to 0.69) indicating a higher donor exposure number in the EPO group. There was high heterogeneity for this outcome ($I^2 = 93\%$).

Mortality during initial hospital stay (all causes of mortality) (Outcome 1.7)

Thirteen studies (14 comparisons) including 767 infants reported on mortality during initial hospital stay. The non-significant typical RR was 0.82 (95% CI 0.49 to 1.39) and the typical RD was -0.01 (95% CI -0.05 to 0.02). There was no statistically significant heterogeneity for this outcome for either RR ($I^2 = 0\%$) or RD ($I^2 = 0\%$). The quality of the evidence was moderate.

Retinopathy of prematurity (ROP) (all stages or stage not reported) (Outcome 1.8)

Three studies including 404 infants reported on ROP (all stages), with a non-significant typical RR 1.27 (95% CI 0.99 to 1.64) and a typical RD of 0.09 (95% CI -0.00 to 0.18). There was high heterogeneity for this outcome for both RR ($I^2 = 83\%$) and RD ($I^2 = 82\%$). The quality of the evidence was very low.

Retinopathy of prematurity (ROP) stage ≥ 3 (Outcome 1.9)

Three trials enrolling 442 infants reported on severe ROP (stage ≥ 3). The typical RR was 1.73 (95% CI 0.92 to 3.24) and the typical RD was 0.05 (95% CI -0.01 to 0.10); neither was statistically significant. There was unimportant heterogeneity for this outcome for RR ($I^2 = 18\%$) but high heterogeneity for RD ($I^2 = 79\%$). The quality of the evidence was very low.

Proven sepsis (clinical symptoms and signs of sepsis and positive blood culture) (Outcome 1.10)

Five studies including 551 infants reported on this outcome. The typical RR was 0.75 (95% CI 0.52 to 1.09) and the typical RD was -0.05 (95% CI -0.11 to 0.01), neither statistically significant. There was no heterogeneity for this outcome for either RR ($I^2 = 0\%$) or RD ($I^2 = 0\%$).

Necrotising enterocolitis (NEC) (Bell's stage II or higher) (Outcome 1.11)

Six studies including 656 infants reported on NEC. In some studies, the stage was not reported but the results were included in the meta-analyses. The typical RR was 0.88 (95% CI 0.45 to 1.70) and the typical RD -0.01 (95% CI -0.04 to 0.02). Neither estimate was statistically significant. There was no heterogeneity for this outcome for either RR ($I^2 = 0\%$) or RD ($I^2 = 0\%$). The quality of the evidence was moderate.

Intraventricular haemorrhage (IVH); all grades (Outcome 1.12)

Four studies including 454 infants reported on intraventricular haemorrhage (all grades). In [Samanci 1996](#), there were no events in either group and that study was therefore disregarded for the RR. The non-significant typical RR was 0.87 (95% CI 0.53 to 1.42) and typical RD was -0.02 (95% CI -0.07 to 0.04). There was no heterogeneity for either RR ($I^2 = 0\%$) or RD ($I^2 = 0\%$). IVH was probably not a relevant outcome in this review as most haemorrhages occur during the first few days of life and infants were enrolled later in these studies.

Periventricular leukomalacia (PVL); cystic changes in the periventricular areas (Outcome 1.13)

One study enrolling 145 infants reported on PVL. The non-significant RR was 4.80 (95% CI 0.57 to 40.05) and the RD was 0.05 (95% CI -0.01 to 0.12). The test for heterogeneity was not applicable.

Bronchopulmonary dysplasia (BPD) (supplemental oxygen at 28 days of age) (Outcome 1.14)

Two studies ($n = 285$) reported on BPD at 28 days. In [Al-Kharfy 1996](#), all infants in both groups had BPD. The typical RR was 1.25 (95% CI; 1.00 to 1.55) and the RD was 0.10 (95% CI 0.00 to 0.20). There was very high heterogeneity for both RR ($I^2 = 97\%$) and RD ($I^2 = 88\%$). Both results were of borderline statistical significance (test for overall effect: $Z = 1.99$ ($P = 0.05$)). The extremely high heterogeneity for this analysis may relate to the fact that in one study all the infants had BPD at 28 days of age. The results should be interpreted with caution.

Bronchopulmonary dysplasia (BPD) (supplemental oxygen at 36 weeks' postmenstrual age (PMA)) (Outcome 1.15)

Three studies enrolling 216 infants reported on the use of supplemental oxygen at 36 weeks' PMA. The typical RR was 0.89 (95% CI 0.59 to 1.35) and the typical RD was -0.03 (95% CI -0.15 to 0.08); neither was statistically significant. There was moderate heterogeneity for RR ($I^2 = 56\%$) and for RD ($I^2 = 59\%$).

Sudden infant death (SIDS) after discharge (Outcome 1.16)

Six studies including 363 infants reported on SIDS. The typical RR was 1.06 (95% CI 0.25 to 4.52) and the typical RD was 0.00 (95% CI -0.03 to 0.04). Neither was statistically significant. There was no heterogeneity for either RR ($I^2 = 0\%$) or RD ($I^2 = 0\%$).

Neutropenia (Outcome 1.17)

Five studies (six comparisons) enrolling 164 infants reported on neutropenia. The typical RR was 0.28 (95% CI 0.05 to 1.54) (in only two studies did the outcome of interest occur), and the typical RD was -0.04 (-0.11 to 0.03); neither was statistically significant. There was no heterogeneity for RR ($I^2 = 0\%$) and for RD ($I^2 = 0\%$).

Hypertension (Outcome 1.18)

Seven studies including 361 infants reported on hypertension. The RR was 1.20 (95% CI 0.46 to 3.14) and the RD 0.01 (95% CI -0.04 to 0.05); neither was statistically significant. There was no statistically significant heterogeneity for either RR ($I^2 = 21\%$) or RD ($I^2 = 0\%$).

Length of hospital stay (days) (Outcome 1.19)

Length of hospital stay was reported in two studies enrolling 55 infants. There was no significant difference between the groups with a typical MD of -0.35 days (95% CI -12.83 to 12.13). There was no heterogeneity ($I^2 = 0\%$).

Long-term outcomes assessed at any age beyond one year of age by a validated cognitive, motor, language, or behavioural/school/social interaction/adaptation test

Long-term neurodevelopmental outcomes were not reported in any study.

Any side effects reported in the trials

There were no serious side effects reported in most of the trials that specifically reported on adverse events ([Shannon 1991](#); [Shannon 1992](#); [Bechensteen 1993](#); [Yamada 1999a](#); [Yamada 1999b](#); [Chen 1995](#); [Shannon 1995](#); [Bader 1996](#); [Donato 1996](#); [Samanci 1996](#); [Corona 1998](#); [Giannakopoulou 1998a](#); [Giannakopoulou 1998b](#); [Kumar 1998](#); [Kivivuori 1999](#); [Rocha 2001](#); [Juul 2003](#)). [Griffiths 1997](#) reported a total of 41 different types of adverse events, with infection (positive blood cultures), pneumonia, and patent ductus arteriosus being the most common.

Secondary (post hoc) analyses:

In an attempt to further explore the heterogeneity observed in the primary outcome and subgroup analyses, we performed a post hoc analysis comparing the results of studies that we judged as high quality with those that we identified as of lower quality or could not precisely define their quality because of lack of information. We also compared the results of studies that used strict criteria for red blood cell transfusions to those that used no criteria or less strict criteria.

Use of one or more blood transfusions (secondary analysis based on quality) (Outcome 1.20)

For six high-quality studies enrolling 417 infants, the typical RR was 0.83 (95% CI 0.73 to 0.95); the typical RD was -0.12 (95% CI -0.21 to -0.04). For 15 studies of uncertain quality enrolling 785 infants, the typical RR was 0.63 (95% CI 0.54 to 0.73) and the typical RD was -0.20 (-0.26 to -0.14). The summary effect size was larger in the studies of poor quality. There was low heterogeneity for the high-quality studies ($I^2 = 49\%$ for RR and 47% for RD), as well as for the studies of uncertain quality which had moderate heterogeneity ($I^2 = 71\%$ for RR and 61% for RD).

Use of one or more blood transfusions (secondary analysis based on criteria for red blood cell transfusions) (Outcome 1.21; Outcome 1.21.1; Outcome 1.21.2)

We considered 16 studies enrolling 1023 infants to have used strict (although variable) guidelines for red blood cell transfusions, and three studies enrolling 97 infants to have used no criteria or less strict criteria. We excluded two studies for which we were unable to translate the text regarding possible transfusion guidelines (Yamada 1999a; Yamada 1999b). For the 16 studies using strict red blood cell transfusion guidelines, the typical RR was 0.76 (95% CI 0.69 to 0.85) and the typical RD was -0.15 (95% CI -0.20 to -0.09); NNTB 7 (95% CI 5 to 11). There was moderate heterogeneity for RR ($I^2 = 61\%$) and RD ($I^2 = 53\%$). For the

studies using no criteria or less strict criteria, the typical RR was 0.25 (95% CI 0.08 to 0.77) and the typical RD was -0.21 (95% CI -0.36 to -0.07); NNTB 5 (95% CI 3 to 14). There was no heterogeneity for the studies using no criteria or less strict criteria for RR ($I^2 = 0\%$) and RD ($I^2 = 0\%$). The summary effect size was larger for the studies that did not use strict guidelines for red blood cell transfusions compared to those that did.

Funnel plot

A funnel plot for the primary outcome 'Use of one or more red blood cell transfusions' was asymmetric, with a relative absence of smaller studies not having a protective effect (see Figure 5).

Figure 5. Funnel plot of comparison: I Late initiation of EPO (8 - 28 days) vs placebo or no intervention, outcome: I.1 Use of one or more red blood cell transfusions (low and high dose of EPO).

Enterally-dosed EPO

One study (Juul 2003) using enterally-dosed EPO found that the intervention did not significantly influence erythropoiesis or iron utilisation when given for a two-week period, nor did it elevate the

serum EPO concentration in preterm or term infants. The authors concluded that enterally-dosed EPO is not an effective substitute for parenteral administration (Juul 2003).

DISCUSSION

Summary of main results

The current review included 31 studies (32 comparisons) meeting our inclusion criteria. These studies included a total of 1651 preterm or low birth weight infants and reported on at least one of the outcomes of interest for this systematic review.

The results showed that late administration of erythropoietin reduces the use of one or more blood transfusions following study entry. These results were quite consistent (overlapping confidence intervals) when including studies that used both low and high doses of EPO in combination with low and high doses of iron.

With the inclusion of [Romagnoli 2000](#), in which there was a statistically significant increase in retinopathy of prematurity (ROP) at all stages and at stage 3 or higher, the overall estimates in the meta-analyses now showed a trend towards increase for both these outcomes. Three studies including 404 infants reported on ROP (all stages), with a typical RR 1.27 (95% CI 0.99 to 1.64) and a typical RD of 0.09 (95% CI -0.00 to 0.18). This outcome was not statistically significantly different between the groups. There was high heterogeneity for this outcome for both RR ($I^2 = 83\%$) and RD ($I^2 = 82\%$). Three trials enrolling 442 infants reported on severe ROP (stage 3 or higher). The typical RR was 1.73 (95% CI 0.92 to 3.24) and the typical RD was 0.05 (95% CI -0.01 to 0.10); neither was statistically significant. There was minimal heterogeneity for this outcome for RR ($I^2 = 18\%$) but high heterogeneity for RD ($I^2 = 79\%$). As the cut-off for early EPO treatment versus late EPO treatment was set arbitrarily at eight completed days of age of initiation of treatment, we thought it justified to include all studies that have reported on ROP in secondary analyses. Details of these analyses were presented in the 2014 update of the early EPO review ([Ohlsson 2012](#)). We combined all studies that reported on ROP at stage 3 or higher regardless of at what age the EPO treatment was initiated. We included seven studies from the early EPO review and three studies from the late EPO review that reported on this outcome. A total of 10 studies enrolling 1303 infants reported on this outcome. There was a significantly increased risk of ROP stage 3 or higher (typical RR 1.48 (95% CI 1.02 to 2.13); typical RD 0.03 (95% CI 0.00 to 0.06) ($P = 0.04$ for RR and 0.03 for RD); number needed to treat for an additional harmful outcome (NNTH) 33 (95% CI 17 to infinity)). There was no heterogeneity for RR ($I^2 = 0\%$) and moderate heterogeneity ($I^2 = 50\%$) for RD.

Overall completeness and applicability of evidence

The number needed to treat for an additional beneficial outcome (NNTB) to avoid one red blood cell transfusion was low (range 3 to 8, for different combinations of EPO and iron). The clinical importance of this finding is lessened by the fact that any donor

exposure was not avoided, as many infants required red blood cell transfusions prior to study entry. Only two studies reported on donor exposure. In the study by [Bierer 2009](#), there was a statistically significant increase in the number of donors the infants were exposed to but not in the study by [Maier 2002](#). In the meta-analysis of the two studies, a significant difference in the typical mean difference for number of transfusions was noted indicating a higher donor exposure number in the EPO group. There was very high heterogeneity for this outcome ($I^2 = 93\%$), reducing the importance of the finding. In addition, in the 2012 and 2013 updates there was no statistically significant reduction in the total volume (mL/kg) of blood transfused per infant. There was a small reduction in the mean number of transfusions (0.2) per infant. The need for intravenous, intramuscular, or subcutaneous injections with EPO/iron treatment in the neonatal period is associated with repeated painful stimuli and could potentially have adverse long-term effects. This has not been addressed in any study.

Quality of the evidence

The study quality varied and important information regarding the random sequence generation and whether the allocation was concealed or not was often missing. No study was reported according to the 'Consort' statement ([CONSORT 2012](#)). Sample sizes were small and long-term outcomes (18 to 24 months corrected age) were not reported. In only one study ([Samanci 1996](#)), did the authors state that infants were not eligible to enter the study if they had previously received a red blood cell transfusion. Most studies followed guidelines for red blood cell transfusions, although these varied between the studies.

For the primary outcome of 'use of one or more blood transfusions', the typical risk ratio (RR) for six high-quality studies was 0.83 (95% CI 0.73 to 0.95). For 15 studies of uncertain quality, the typical RR was 0.63 (95% CI 0.54 to 0.73). The CIs for these analyses were not overlapping, indicating that there were statistically significant differences in the effect sizes between studies that could be ascertained as being of high quality and studies of uncertain quality. There was a reduction in heterogeneity when the high-quality studies were analysed separately. Studies of higher quality often show lower effect sizes ([Schulz 1995](#)). Judging the quality of a study depends to a large extent on the information published, and obtaining additional information from the authors may change the evaluations. The typical effect size for studies that used strict red blood cell transfusion guidelines was smaller (RR 0.76) than for studies that used no or less strict criteria (RR 0.25). Of concern is the finding of moderate heterogeneity for the primary outcome, including all combinations of low and high EPO and low and high iron treatment. The heterogeneity remained for individual combinations of EPO and iron. The heterogeneity could possibly be explained by the fact that the studies were conducted in 22 countries, with presumably different care practices. Of note, the control rates for red blood cell transfusions varied

markedly between studies. As noted in the Additional Table 1, there was large variation in the guidelines for red blood cell transfusions. The results of these post hoc analyses should therefore be interpreted with caution.

Potential biases in the review process

As outlined in Figure 5 (Funnel plot of comparison: 1 Late initiation of EPO (8 - 28 days) versus placebo or no intervention, outcome: 1.1 Use of one or more red blood cell transfusions (low and high dose of EPO)), there was a paucity of smaller studies not having a protective effect. It is possible that such studies have been conducted and that the results have not been published. Several studies were supported by pharmaceutical companies and some authors were employed by such companies. We are not aware of any potential biases in our own review process.

Agreements and disagreements with other studies or reviews

Systematic reviews of the efficacy of EPO in anaemia of prematurity have been published (Vamvakas 2001; Garcia 2002; Kotto-Kome 2004). Vamvakas 2001 concluded that there is extreme variation in the results, and, until this variation is better understood, it is too early to recommend EPO as standard treatment for the anaemia of prematurity. Garcia 2002 concluded that administering EPO to VLBW neonates can result in a modest reduction in late erythrocyte transfusions and that this effect is dependent on the dose of EPO used. Kotto-Kome 2004 concluded that if EPO is begun in the first week of life, a moderate reduction can be expected in the proportion of VLBW neonates transfused. The reduction was less significant for early transfusion than for late transfusion. Direct comparisons regarding the results of this systematic overview and previous reviews (Vamvakas 2001; Garcia 2002; Kotto-Kome 2004) are not appropriate as our review included a much larger sample of studies. In 2015, the French Society of Neonatology published "Recombinant human erythropoietin in neonates: guidelines for clinical practice". The publication is labelled as a systematic evidence review. They reported that "Early EPO reduced the risk of RBC transfusions, donor exposure, and the number of transfusions in very preterm infants (LE2) (LE = level of evidence). Late EPO reduced the risk of RBC transfusions and the number of transfusions in very preterm infants (LE2). There is no difference between the effectiveness of early and late EPO (LE2). There is no difference between high-dose and low-dose EPO (LE2). The level of evidence is too low to recommend the intravenous route. EPO has no impact on the rate of bronchopulmonary dysplasia, necrotising enterocolitis (LE3), and retinopathy of prematurity (LE2). The level of evidence is too low to recommend EPO for neuro protection in very preterm or term infants". They concluded; "EPO to reduce RBC transfusion

in very preterm infants is recommended (Level A). The optimal time to start therapy is unknown (Level B). The recommended dose is 750 IU/kg/week via three subcutaneous injections for 6 weeks (Level B)". To our knowledge, there is no other recent systematic review for late EPO (Lopez 2015).

AUTHORS' CONCLUSIONS

Implications for practice

Late EPO administration results in a reduction in the use of one or more red blood cell transfusions following initiation of therapy. It minimally reduces the number of red blood cell transfusions per infant. It is not associated with reductions in mortality or other neonatal morbidities. The use of late EPO is not associated with any short-term serious side effects except for a possible association with retinopathy of prematurity (ROP) stage 3 or higher. A large proportion of extremely low BW/preterm neonates require red blood cell transfusions during the first few days of life, when neither early nor late EPO administration could possibly have an impact. The decision to use late EPO will depend on the baseline rate of red blood cell transfusions in this population in a specific neonatal intensive care unit, the costs, the associated pain, and the values assigned to the clinical outcomes. Other means of reducing the need for red blood cell transfusions should be considered, including reduced blood sampling, and the use of 'satellite packs' from directed or universal donors.

Implications for research

There is no need for further research to assess the effectiveness of the late use of EPO in reducing red blood cell transfusions. Its effectiveness has been established in populations that were exposed to donor blood prior to study entry, minimising the clinical importance of this effect. Future research should focus on strategies to minimise red blood cell donor exposure (using multiple aliquots from a properly tested single donor) during the first week of life, when the likelihood of need for red blood cell transfusions is at its peak. Such strategies, in combination with late EPO treatment, may reduce further donor exposure in early infancy. All ongoing and planned studies should monitor the incidence of retinopathy of prematurity.

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Ms. Yolanda R Brosseau conducted the literature searches in 2012. Ms. Colleen M Ovelman, Managing Editor, Cochrane Neonatal, conducted the literature searches in 2013 and 2018.

The methods section of this review is based on a standard template used by Cochrane Neonatal.

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* Indicates the major publication for the study

CHARACTERISTICS OF STUDIES

Characteristics of included studies [ordered by study ID]

Akisu 2001

Methods	Randomised controlled study, single centre, University of Ege, Izmir, Turkey Study period: Not stated Funding sources: Not stated Declarations of interest: None reported	
Participants	40 AGA preterm infants with PMA < 33 weeks and BW < 1500 grams were enrolled on day 10 of life	
Interventions	Infants assigned to the treatment group (n = 20) received r-HuEPO (Recormon, Boehringer-Mannheim, Germany) sc 3 times per week, totaling 750 IU/kg/week (high dose). 20 infants were assigned to the control group with no placebo given. All infants received 3 mg/kg/day (low dose) of elemental iron. All infants received poly-vitamin supplements, vitamin D and folate	
Outcomes	Exposure of a proportion of infants to one or more red blood cell transfusions	
Notes	Unknown whether infants who had received blood transfusions prior to study entry were included or not. This study focused on lipid peroxidation and antioxidant enzyme activities in preterm infants. Guidelines for transfusion were not stated.	
<i>Risk of bias</i>		
Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	No information provided
Allocation concealment (selection bias)	Unclear risk	No information provided
Blinding (performance bias and detection bias) All outcomes	High risk	The control group did not receive a placebo.
Incomplete outcome data (attrition bias) All outcomes	Low risk	Outcomes reported for all randomised infants
Selective reporting (reporting bias)	Unclear risk	The protocol for the study was not available to us and therefore we could not ascertain if there were deviations from the protocol

Other bias	Low risk	Appeared free of other biases

Al-Kharfy 1996

Methods	A double-blind (sham-injection in the placebo group), randomised, controlled trial, single centre, Canada Study period: March 1992 to May 1994 Funding sources: Supported by a grant from the Vancouver Foundation Declarations of interest: None reported
Participants	55 preterm infants with BW < 1250 grams, appropriate for PMA, postnatal age 10 to 17 days, Hct less than 45% and a > 75% probability of having BPD, determined at 10 days of age by the predictive score of Sinkin et al. were included. Infants were randomly assigned, within 250 g BW strata and between 10 and 17 days of age. 41 infants received the 6-week treatment schedule.
Interventions	Infants assigned to the treatment group (n = 27) received r-HuEPO (Eprex, Ortho Pharmaceutical, Canada Ltd), in a dosage of 200 IU/kg body weight, by sc injection, on Monday, Wednesday and Friday for 6 weeks (600 IU/kg/week; high dose). In control infants (n = 28), because of a desire to avoid repeated sc placebo injections, sham injections were given and an adhesive dressing was applied to the sham injection site. The care of the infant was then handed back to nursery personnel unaware of treatment assignment. Vitamin E (25 IU/day) and folic acid (0.05 mg/day) began when enteral feeding reached 50% of total fluid intake. Oral ferrous sulphate solution was administered to the treatment group at 6 mg of elemental iron/kg/day (high dose) and the control group received 2 mg of elemental iron/kg/day
Outcomes	Number of transfusions per infant Mortality Sepsis ROP (stage = 3) Hypertension BPD (at 28 days)
Notes	Unclear whether infants who had received blood transfusions prior to study entry were included or not. This study exclusively enrolled those infants, who were predicted to have > 75% probability of having BPD and requiring multiple transfusions. All enrolled infants were included in the data analyses. Sham injections were given in the control group. Erythrocyte transfusions were in accordance with the guidelines developed by the Canadian Paediatric Society Fetus and Newborn Committee

Risk of bias

Al-Kharfy 1996 (Continued)

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	No information provided
Allocation concealment (selection bias)	Low risk	Blinding of randomisation - yes (numbered sealed envelopes)
Blinding (performance bias and detection bias) All outcomes	Low risk	Sham-injection was given in the placebo group Blinding of outcome measure assessment - yes
Incomplete outcome data (attrition bias) All outcomes	Low risk	Outcomes reported on all randomised infants
Selective reporting (reporting bias)	Unclear risk	The protocol for the study was not available to us so we could not judge if there were deviations from the protocol
Other bias	Low risk	Appeared free of other bias

Atasay 2002

Methods	Prospective, randomised, placebo-controlled trial, single centre, Turkey Study period: October 1997 to February 1999 Funding sources: None stated Declarations of interest: None reported
Participants	27 VLBW infants, with BW < 1500 grams, PMA < 32 weeks and postnatal age 7 to 10 days
Interventions	14 infants received 600 IU/kg/week (high dose) rHuEPO (Cilag AG Schaffhausen, Switzerland), by sc route, at 7 - 10 days and continued over 7 to 8 weeks. 13 infants were assigned to control group (no EPO or placebo). Infants in the study group were supplemented with oral iron (ferro glycine sulphate) at the dose of 3 mg/kg/day (low dose)
Outcomes	Exposure of a proportion of infants to one or more red blood cell transfusions
Notes	Infants were enrolled at age 7 to 10 days. We decided to include this study in the late EPO systematic review, although some infants may have been < 8 days old at enrolment. Unknown if infants who had received blood transfusions prior to study entry were included or not. Transfusion guidelines were followed. The authors did not provide information on the mean age of the infants at enrolment. Strict transfusion criteria were used in this trial.

<i>Risk of bias</i>		
Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	No information provided
Allocation concealment (selection bias)	Unclear risk	No information provided
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Blinding of intervention not stated Blinding of outcome measure assessment not stated
Incomplete outcome data (attrition bias) All outcomes	Low risk	Outcomes reported for all randomised infants
Selective reporting (reporting bias)	Unclear risk	The protocol for the study was not available to us so we could not judge if there were deviations from the protocol
Other bias	Low risk	Appeared free of other bias

Bader 1996

Methods	Randomised, controlled clinical trial, 2 centres, Israel, Study period: January 1, 1992 to December 31, 1992 Funding sources: None stated Declarations of interest: CILAG Int. supplied rHuEPO
Participants	29 preterm infants (BW < 1750 grams, PMA < 34 weeks and postnatal age of 3 to 5 weeks, mean postnatal age 34 ± 14 days)
Interventions	15 infants received 300 IU/kg/day of rHuEPO (Eprex, Cilag Int.) sc to lateral aspect of right arm 3 times a week (900 IU/kg/week; high dose), for a total duration of 4 weeks 14 infants received no placebo or other intervention. All infants received enteral vitamin E (25 IU/day), vitamin A and D (400 and 1500 IU/day) and folic acid. Two weeks into the study, elemental iron supplementation was begun in both groups at a dose of 6 mg/kg/day (high dose)
Outcomes	Exposure of a proportion of infants to one or more red blood cell transfusions SIDS Side effects
Notes	Unclear whether infants who had received blood transfusions prior to study entry were included or not. Criteria for blood transfusion were in place.

<i>Risk of bias</i>		
Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	No information provided
Allocation concealment (selection bias)	Unclear risk	No information provided - infants were randomly assigned
Blinding (performance bias and detection bias) All outcomes	High risk	Blinding of intervention - no; no placebo was used Blinding of outcome measure assessment - no
Incomplete outcome data (attrition bias) All outcomes	Low risk	Outcomes reported on all randomised infants
Selective reporting (reporting bias)	Unclear risk	The protocol for the study was not available to us so we could not judge if there were deviations from the protocol
Other bias	Low risk	Appeared free of other bias

Basiri 2015

Methods	Randomised, double-blinded, placebo-controlled study in a single centre in Hamadan, Iran	
Participants	60 preterm infants with BW < 1500 grams, PMA 24 to 31 weeks. Age at enrolment: EPO group 13.40 ± 5.71 days. Control group 10.96 ± 5.5 days	
Interventions	30 infants received rHuEpo 250 IU/kg BW (0.1 ml solution/kg BW given sc 3 days a week every other day on Monday, Wednesday and Friday. (= 3 x 250 IU/week = 750 IU/week = high dose) The control group received equal volume normal saline 0.1 ml solution/kg BW All infants in both groups were given oral ferrous sulphate, 4 mg/kg/day during the entire treatment period (4 mg/kg/day = low dose iron)	
Outcomes	Need for blood transfusion	
Notes	The authors reported no side effects.	
Risk of bias		
Bias	Authors' judgement	Support for judgement

Basiri 2015 (Continued)

Random sequence generation (selection bias)	Low risk	Computer-based random-number generated sequence
Allocation concealment (selection bias)	Unclear risk	The study medication was randomly assigned to each participant. No further information provided
Blinding (performance bias and detection bias) All outcomes	Low risk	The rHuEPO and placebo drug solutions were indistinguishable
Incomplete outcome data (attrition bias) All outcomes	Low risk	Outcomes reported on all infants enrolled
Selective reporting (reporting bias)	Unclear risk	The study protocol was not available to us, so we could not judge if there were any deviations from the protocol or not
Other bias	Low risk	Appeared free of other bias

Bechensteen 1993

Methods	Randomised, open, controlled study, 4 participating centres Study period: Dates not reported Funding sources: Cilag provided financial support and provision of Eprex Declarations of interest: One of the authors (AGB) was a recipient of a research fellowship from the Norwegian Cancer Society. The financial support of Semper AB, Sweden was acknowledged for the preparation of freeze-dried human milk protein
Participants	29 preterm infants (BW 900 to 1400 grams, AGA) at 3 weeks of age
Interventions	14 infants received rHuEPO (Eprex, Cilag), 100 IU/kg 3 times a week (300 IU/kg/week; low dose) sc from 3 to 7 weeks of age. 15 infants received neither EPO nor placebo. Oral iron 18 mg/day (high dose) regardless of weight, began at the start of the study (3 weeks). If serum iron concentration fell below 16 micromol/L, the dose was increased to 36 mg/day
Outcomes	Exposure of a proportion of infants to one or more red blood cell transfusions Mortality Hypertension Neutropenia Side effects
Notes	Infants receiving blood transfusions < 96 hours before start of study were excluded. Indications for blood transfusions were 1) Hb < 80 g/L, or 2) at the discretion of the clinician caring for the infant according to symptoms and signs. This study concentrated more on different haematological parameters rather than the

Bechensteen 1993 (Continued)

	need for RBC transfusions	
<i>Risk of bias</i>		
Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	No information provided
Allocation concealment (selection bias)	Low risk	Prenumbered sealed envelopes were used.
Blinding (performance bias and detection bias) All outcomes	High risk	Blinding of intervention - no Blinding of outcome measure assessment - no
Incomplete outcome data (attrition bias) All outcomes	Low risk	Outcomes reported on all randomised infants
Selective reporting (reporting bias)	Unclear risk	The protocol for the study was not available to us so we could not judge if there were deviations from the protocol
Other bias	Low risk	Appeared free of other bias

Bierer 2009

Methods	Randomised, placebo-controlled study, 1 centre, the USA Study period: August 2001 to May 2005 Funding sources: Supported by grants from National Institutes of Health HD00988 and M01 RR 00997 Declarations of interest: None reported
Participants	20 infants; 10 infants in the EPO group (BW (SEM) 2034 ± 308 g, 8 ± 2 days old); 10 infants in the placebo group (BW (SEM) 2400 ± 184 G, 7 ± 2 days old)
Interventions	The EPO group received EPO 200 IU/kg per day or 400 IU/kg sc 3 times weekly (high dose). The placebo group received an equal volume of normal saline if the infant was on total parenteral nutrition and, if not, a sham dose was given sc and a BandAid was placed over the sham injection site. Infants received oral iron supplementation (dose not specified) when enteral feeds reached 60 mL/kg/day. Dosing continued until the infant reached study day 14 unless the infant was discharged from the hospital or died before the end of the study period
Outcomes	Volume transfused during hospitalisation (mL/kg) Number of transfusion per infant Number of donors the infants was exposed to

Bierer 2009 (Continued)

Notes	We included this study in our late EPO review as most infants would have been > 7 days and would have weighed < 2500g on study entry	
<i>Risk of bias</i>		
Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	A random number list was used.
Allocation concealment (selection bias)	Low risk	EPO or placebo was dispensed from the pharmacy in total parenteral nutrition iv administration or in syringes for sc dosing. If iv access was not present, EPO was administered sc. For infants randomised to receive placebo, iv administration consisted of TPN with placebo (an equal volume of normal saline) added, whereas sc administration consisted of sham dosing, followed by placement of a BandAid over the sham injection site
Blinding (performance bias and detection bias) All outcomes	Low risk	Blinding of intervention - yes Blinding of outcome measure assessment - yes
Incomplete outcome data (attrition bias) All outcomes	Low risk	Outcome data reported for all randomised infants
Selective reporting (reporting bias)	Unclear risk	The study was not entered into a trials registry and we could not tell if there were any deviations from the study protocol
Other bias	Low risk	Appeared free of other bias

Chen 1995

Methods	Randomised, controlled clinical trial, single centre, Taiwan, Republic of China Study period: June 1992 to May 1994 Funding sources: None reported Declarations of interest: None reported
Participants	67 preterm infants (BW $\leq$ 1750 grams and PMA $\leq$ 33 weeks) Mean age at entry: Group A 22.3 $\pm$ 6.6 days; Group C 22.3 $\pm$ 6.6. days
Interventions	26 infants (Group A) received rHuEPO (Eprex, Cilag Zug, Switzerland) 150 mg/kg iv twice a week (300 IU/kg/week; low dose) for 4 weeks.

	25 infants (Group B) received packed washed erythrocyte transfusion, 10 to 15 mL/kg, during a 2 to 4 hour period when their Hb levels were less than 10 g/dL with signs and symptoms attributed to anaemia or who had a Hb level less than 8 g/dL even if asymptomatic. 16 infants (Group C) did not receive rHuEPO or erythrocyte transfusions (3 infants excluded from total 19, as they received erythrocyte transfusion later because of frequent episodes of apnoea). All infants received oral elemental iron 3 mg/kg/day (low dose) and vitamin E 5 mg/kg/day	
Outcomes	Mortality Side effects	
Notes	Unknown whether infants who had received blood transfusions prior to study entry were included or not. Strict guidelines for transfusions were not in place (transfusions were given based on frequent episodes of apnoea). We compared Groups A and C based on intention-to-treat in this systematic review	
<i>Risk of bias</i>		
Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	No information provided
Allocation concealment (selection bias)	Unclear risk	No information provided
Blinding (performance bias and detection bias) All outcomes	High risk	Blinding of intervention - no Blinding of outcome measurement - no
Incomplete outcome data (attrition bias) All outcomes	Low risk	Outcomes reported for all randomised infants
Selective reporting (reporting bias)	Unclear risk	The study was not entered into a trials registry and we could not tell if there were any deviations from the study protocol
Other bias	Low risk	Appeared free of other sources of bias

Corona 1998

Methods	Randomised, controlled clinical trial, single centre, Italy Study period: Not reported Funding sources: None reported Declarations of interest: None reported
Participants	60 preterm infants (BW < 1500 grams and < 33 weeks' PMA) Postnatal age (days): group A 10.1 ± 2; group B 9.5 ± 3; Group C 9.8 ± 2
Interventions	20 infants (group A) received rHuEPO (unnamed product) 150 IU/kg/week sc (low dose). 22 infants (group B) received 300 IU/kg/week sc. 18 infants (group C) received no treatment. All groups received oral iron 4 mg/kg/day.
Outcomes	Exposure of a proportion of infants to one or more red blood cell transfusions Total volume (mL/kg) of blood transfused per infant (no SD provided) Side effects
Notes	Unknown whether infants who had received blood transfusions prior to study entry were included or not. Infants were included after the first week of life the mean (SD) postnatal age was 10.1 (2) days in group A; 9.5 (3) in group B and 9.8 (2) in group C. Some infants may have been < 8 days old, but we included this study in the late EPO review as most infants were ≥ 8 days. Transfusion guidelines were in place. We combined the two groups of infants that received EPO (n = 42) and compared that combined group to the group (n = 18 infants) that did not receive any treatment

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	No information provided
Allocation concealment (selection bias)	Unclear risk	No information provided - infants were divided into 3 groups
Blinding (performance bias and detection bias) All outcomes	High risk	Blinding of intervention - no Blinding of outcome measurement - no
Incomplete outcome data (attrition bias) All outcomes	Low risk	Outcomes reported for all randomised infants
Selective reporting (reporting bias)	Unclear risk	The study was not entered into a trials registry and we could not tell if there were any deviations from the study protocol

Corona 1998 (Continued)

Other bias	Low risk	Appeared free of other bias

Donato 1996

Methods	Prospective, randomised, placebo-controlled trial, single centre Study period: Argentina, July 1991 to June 1993 Funding sources: This work was supported by Bio Sidus S.A. Declarations of interest: See funding resources
Participants	32 preterm infants with PMA < 34 weeks and BW < 1500 grams Age at enrolment was 21 to 35 days of life. Mean postnatal age at enrolment (days): group A. 27.0 ± 3.2; Group B 27.6 ± 3.6; Group C 29.7 ± 5.8; Group D 32.1 ± 7.7
Interventions	32 preterm infants were randomly assigned to receive: 1. Group A (n = 9) placebo (human seroalbumin) sc 2. Group B (n = 8) rHuEPO (HEMAX, Bio Sidus, S.A.) at dose of 50 IU/kg sc (150 IU/kg/week; low dose) 3. Group C (n = 8) rHuEPO at dose of 100 IU/kg sc (300 IU/kg/week; low dose) 4. Group D (n = 7) rHuEPO at dose of 250 IU/kg sc (750 IU/kg/week; high dose), sc 3 days per week during 8 consecutive weeks. All infants were given oral iron 6 mg/kg/day (high dose) and folic acid (2 mg/day) supplements, starting on day 15 of age and continuing during whole treatment period
Outcomes	Exposure of a proportion of infants to one or more red blood cell transfusions Average number of transfusions per infant during treatment Mortality Mean length of hospitalisation (days) Side effects Hypertension SIDS
Notes	Transfusion criteria were followed in this study. We combined the results for the three EPO groups (Group B, Group C and Group D; (total n = 23) regardless whether high or low dose of EPO was used and compared to the results for the placebo group (Group A; n = 9). In separate analyses, we compared the low dose EPO groups (Group B; n = 8 and group C; n = 8) to the placebo group (Group A) and the high dose EPO group (Group D; n = 7) to the placebo group (Group A; n = 9). All infants were given high dose of iron

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	No information provided

Donato 1996 (Continued)

Allocation concealment (selection bias)	Unclear risk	No information provided - "randomly assigned"
Blinding (performance bias and detection bias) All outcomes	Low risk	Blinding of intervention - yes - placebo-controlled Blinding of outcome measure assessment - yes
Incomplete outcome data (attrition bias) All outcomes	Low risk	Outcomes reported on all randomised infants
Selective reporting (reporting bias)	Unclear risk	The study was not entered into a trials registry and we could not tell if there were any deviations from the study protocol
Other bias	Low risk	Appeared free of other bias

Emmerson 1993

Methods	Randomised, double-blind, placebo-controlled trial, 1 centre in the UK Study period: Not stated Funding sources: This study was supported by Cilag Ltd. Declarations of interest: See founding sources
Participants	24 infants with GA between 27 and 33 weeks Postnatal age > 7 days Mean (SE) age (days) at first dose; EPO group 9 (0.4); Placebo group 8 (0.6) One infant in the EPO group was withdrawn at 14 days of age to enable transfer to another hospital for maternal reasons
Interventions	16 infants were randomly assigned to receive r-HuEpo sc (Eprex, Cilag, Ltd). The first 9 infants received 50 IU/Kg of EPO, the next 9 infants received 100 IU/kg, and the final 6 infants received 150 IU/kg of r-HuEpo or placebo (4% albumin) twice a week sc after 7 days of age twice weekly sc 300 IU/kg/week (low dose) into the buttock commencing after 7 days of age and administered until the infant was discharged home. All infants received iron (6.25 mg) in the form of ferrous glycine sulphate from four weeks of age (high dose)
Outcomes	Exposure of a proportion of infants to one or more red blood cell transfusions Volume transfused (mL/kg) Mortality Hospital stay SIDS Neutropenia
Notes	Transfusion guidelines were followed. Unknown if infants who had received transfusions prior to study entry were included or not

Emmerson 1993 (Continued)

	One infant in the EPO group was withdrawn at 14 days of age to enable transfer to another hospital for maternal reasons and was not included in the analyses	
<i>Risk of bias</i>		
Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	No information provided
Allocation concealment (selection bias)	Unclear risk	No information provided
Blinding (performance bias and detection bias) All outcomes	Low risk	Blinding of intervention - yes - placebo-controlled Blinding of outcome measure assessment - yes
Incomplete outcome data (attrition bias) All outcomes	Low risk	Outcomes reported on all randomised infants
Selective reporting (reporting bias)	Unclear risk	The study was not entered into a trials registry and we could not tell if there were any deviations from the study protocol
Other bias	Low risk	Appeared free of other bias

Giannakopoulou 1998a

Methods	Randomised controlled trial, single centre, Greece Study period: Period not stated Funding sources: None reported Declarations of interest: None stated
Participants	36 preterm infants with BWs 1000 to 1300 grams 32 preterm infants with BW < 1000 grams Postnatal age > 20 days Divided into 4 groups Groups 1 and 3 were controls and Groups 2 and 4 were treatment groups. One infant in Group 2 died and one infant from Group 1 was excluded because of severe infection
Interventions	24 infants received rHuEPO (Eprex, Cilag, Schaffhausen, Switzerland) from day 20 of life sc at a dose of 300 IU/kg body weight, 3 times a week (900 IU/kg/week; high dose) for 6 to 8 weeks (n = 24). 24 control infants did not receive any treatment. All received oral elemental iron 10 mg/kg/day (high dose), folic acid 1 mg/day and vitamin E 15 mg/day

Giannakopoulou 1998a (Continued)

Outcomes	Mortality Hypertension Neutropenia Side effects	
Notes	Unknown whether infants who had received blood transfusions prior to study entry were included or not Guidelines for blood transfusions were in place. Under Giannakopoulou 1998a, we reported on the 32 infants weighing < 1000 grams.	
<i>Risk of bias</i>		
Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	No information provided
Allocation concealment (selection bias)	Unclear risk	No information provided
Blinding (performance bias and detection bias) All outcomes	High risk	Blinding of intervention - no Blinding of outcome measures - no
Incomplete outcome data (attrition bias) All outcomes	Low risk	Outcomes reported for all randomised infants
Selective reporting (reporting bias)	Unclear risk	The study was not entered into a trials registry and we could not tell if there were any deviations from the study protocol
Other bias	Low risk	Appeared free of other bias

Giannakopoulou 1998b

Methods	See above
Participants	See above
Interventions	See above
Outcomes	See above
Notes	Under Giannakopoulou 1998b, we reported on the 36 infants weighing 1000 to 1300 grams.
<i>Risk of bias</i>	

Giannakopoulou 1998b (Continued)

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	No information provided
Allocation concealment (selection bias)	Unclear risk	No information provided
Blinding (performance bias and detection bias) All outcomes	High risk	Blinding of intervention - no Blinding of outcome measures - no
Incomplete outcome data (attrition bias) All outcomes	Low risk	Outcomes reported for all randomised infants
Selective reporting (reporting bias)	Unclear risk	The study was not entered into a trials registry and we could not tell if there were any deviations from the study protocol
Other bias	Low risk	Appeared free of other bias

Griffiths 1997

Methods	A multicentre, randomised, placebo-controlled, double-blind study, 4 NICUs in Yorkshire, the UK Study period: June 1993 to December 1994 Funding sources: One of the authors (GG) was funded by the Yorkshire Regional health Authority Declarations of interest: None reported
Participants	43 preterm infants with PMA < 32 weeks and/or BW < 1500 grams, requirement for mechanical ventilation and/or supplemental oxygen on day 7 to 14 after birth
Interventions	21 infants received R-HuEpo (Eprex; Cilag UK) EPO 480 IU/kg/week (low dose) sc and 21 infants received placebo (4% human albumin) sc. The first injection was given on the day of randomisation, and then twice weekly until the infant either (a) reached 40 weeks' PMA, (b) was transferred to another hospital (not in the study), (c) did not require any mechanical ventilation/oxygen for a period of more than a week, (d) had local adverse reactions/complications related to the trial solution, or (e) had PCV > 60%. All infants received oral iron (3.0 mL/kg/day) (low dose) from 4 week after birth
Outcomes	Mortality, BPD (at 36 weeks' PMA) SIDS Median number of blood transfusions
Notes	Infants who received blood transfusions prior to study entry were not excluded. Transfusion guidelines were in place. One infant who received treatment was ineligible and was subsequently withdrawn and excluded from the analysis

Griffiths 1997 (Continued)

<i>Risk of bias</i>		
Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	No information provided
Allocation concealment (selection bias)	Low risk	Stratified randomisation was used. Blinding was maintained throughout the study
Blinding (performance bias and detection bias) All outcomes	Low risk	Blinding of intervention - yes Blinding of outcome measure assessment - yes
Incomplete outcome data (attrition bias) All outcomes	Low risk	Outcomes reported on all randomised infants except 1 infant who received treatment but was ineligible and was subsequently withdrawn and excluded from the analysis
Selective reporting (reporting bias)	Unclear risk	The study was not entered into a trials registry and we could not tell if there were any deviations from the study protocol
Other bias	Low risk	Appeared free of other bias

Javier Manchon 1997

Methods	A multicentre, randomised, controlled study, 3 centres in Barcelona, Spain Study period: Not stated Funding sources: None reported Declarations of interest: None reported
Participants	Population: Preterm infants < 34 weeks' PMA, who at 28 days after birth had haemoglobin levels < 10.5 g/dL
Interventions	15 infants received 200 IU EPO/kg sc (unnamed product) 3 days a week for 4 weeks (600 IU/kg/week; high dose) and ferrous sulphate 4 mg/kg/day (low dose). 13 control infants did not receive placebo, EPO, or iron,
Outcomes	Exposure of a proportion of infants to one or more red blood cell transfusions between 30 and 60 days of age
Notes	Infants were included if they had received blood transfusions prior to randomisation. Transfusion guidelines were similar in all 3 centres (details not provided)
<i>Risk of bias</i>	

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	No information provided
Allocation concealment (selection bias)	Unclear risk	No information provided
Blinding (performance bias and detection bias) All outcomes	High risk	Blinding of intervention - no Blinding of outcome measure assessment - no
Incomplete outcome data (attrition bias) All outcomes	Low risk	Outcomes reported on all randomised infants
Selective reporting (reporting bias)	Unclear risk	The study was not entered into a trials registry and we could not tell if there were any deviations from the study protocol
Other bias	Low risk	Appeared free of other bias

Juul 2003

Methods	A prospective, blinded, placebo-controlled study, single centre, USA Study period: Period not stated Funding sources: Supported by the National Institutes of health, grants M01-RR-00037 and RR00082 Declarations of interest: None reported
Participants	36 preterm infants with BW between 700 to 1500 grams and receiving at least 30 mL/kg per day of enteral feeding. 32 infants completed the study. The mean (SD) PMA (weeks) at birth was 27.8 ± 1.8 in the EPO group and 28.8 ± 2.1 in the placebo group. Mean (SD) corrected PMA (weeks) at study entry was 31.6 ± 2.0 in the EPO group and 31.9 ± 1.6 in the placebo group Infants in the EPO group ranged from 2 to 8 weeks' postnatal age at study entry (with a median of 4 weeks), whereas placebo-treated infants ranged from 1 to 7.4 weeks' postnatal age (with a median of 2 weeks). Overall, the median age at study entry was 2.8 weeks of age
Interventions	15 infants received rEpo (unnamed product) 1000 IU/kg per day divided into 2 daily feedings (7000 IU/kg/week; high dose), for 14 days. 17 infants received placebo (D5W) for 14 days. All subjects received supplemental iron (iron dextran, 1.0 mg/kg/day administered in the hyperalimentation solution, or enteral ferrous sulphate 6 mg/kg per day (high dose))
Outcomes	Phlebotomy loss (mL) and PRBC transfusion volume (mL) Evidence of feeding intolerance and other adverse effects

Notes	This was the only study which used oral EPO. The results of this study were not included in the meta-analyses	
<i>Risk of bias</i>		
Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	No information provided
Allocation concealment (selection bias)	Unclear risk	No information provided
Blinding (performance bias and detection bias) All outcomes	Low risk	Blinding of intervention - yes Blinding of outcome measure assessment - yes
Incomplete outcome data (attrition bias) All outcomes	High risk	Information on 4 infants was missing. 36 infants were enrolled and 32 completed the study
Selective reporting (reporting bias)	Unclear risk	The study was not entered into a trials registry and we could not tell if there were any deviations from the study protocol
Other bias	Low risk	Appeared free of other bias

Kivivuori 1999

Methods	An open randomised study, four centres; three in Helsinki and one in Espoo, Finland Study period: Not stated Funding sources: None reported Declarations of interest: Cilag A.G. supplied the erythropoietin and Vifor International supplied parenteral iron preparation
Participants	45 VLBW infants (4 excluded - see notes) 41 infants included Birth weight ranged from 625 to 1470 grams. The infants were randomised to 3 groups. One group (n = 14) received rHuEPO and oral iron (mean PMA 30.1 ± 0.7 weeks), one group (n = 14) received rHuEPO and im iron (mean PMA 28.8 ± 0.5 weeks), one group (n = 13) received no rHuEPO and im iron (mean PMA 29.1 ± 0.6 weeks). Infants who were weaned from the respirator by 2 weeks of age were eligible
Interventions	14 infants received rHuEPO (Cilag A.G., Schaffhausen, Switzerland) 300 IU/kg sc 3 times/week, 900 IU/kg/week (high dose) sc and oral iron 6 mg/kg/day (high dose). 14 infants received 900 IU/kg/week of rHuEPO and weekly im iron 12 mg/kg (high dose).

Kivivuori 1999 (Continued)

	13 infants received im iron 12 mg/kg/week but no rHuEPO.	
Outcomes	Exposure of a proportion of infants to one or more red blood cell transfusions Side effects	
Notes	Unknown whether infants who had received blood transfusion prior to study entry were included or not. 4 infants were excluded after randomisation; 1 weighed > 1500 G, 1 had intestinal “obstipation”, 1 had highly elevated serum transferrin saturation (96%), 1 accidentally received a high dose of parenteral iron (43 mg/kg). We included the infants in the im iron group in the high-dose iron analyses. The transfusion policies (not stated) were the same in all hospitals	
<i>Risk of bias</i>		
Bias	Authors’ judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	No information provided
Allocation concealment (selection bias)	Unclear risk	No information provided
Blinding (performance bias and detection bias) All outcomes	High risk	Blinding of intervention - no; open randomised study Blinding of outcome measure assessment - no
Incomplete outcome data (attrition bias) All outcomes	High risk	Complete follow-up - no; 4 infants were excluded after randomisation (see above)
Selective reporting (reporting bias)	Unclear risk	The study was not entered into a trials registry and we could not tell if there were any deviations from the study protocol
Other bias	Low risk	Appeared free of other bias

Kumar 1998

Methods	Randomised, double-blind, placebo-controlled trial, single centre, USA Study period: Period not stated Funding sources: None reported Declarations of interest: None reported
Participants	30 infants (PMA < 32 weeks, BW < 1250 grams with anaemia of prematurity) Mean (SD) postnatal age (days) at entry was 40.3 (20.4) in the EPO group and 36.5 (16.6) in the placebo group

Interventions	300 IU/kg/dose of rHuEPO (unnamed product) sc injection twice a week (600 IU/kg/week; high dose) for 6 weeks (n = 15). Identical volume of placebo suspension (normal saline) (n = 15). Infants received elemental iron 6 mg/kg/day (high dose).
Outcomes	Exposure of a proportion of infants to one or more red blood cell transfusions Number of erythrocyte transfusions (per infant) Entry to discharge duration (days) Side effects
Notes	Infants who had received transfusions prior to randomisation were included. Transfusion guidelines were followed.

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	No information provided
Allocation concealment (selection bias)	Unclear risk	No information provided; infants were randomly assigned
Blinding (performance bias and detection bias) All outcomes	Low risk	Blinding of intervention - yes Blinding of outcome measure assessment - yes
Incomplete outcome data (attrition bias) All outcomes	Low risk	Outcomes reported for all infants randomised
Selective reporting (reporting bias)	Unclear risk	The study was not entered into a trials registry and we could not tell if there were any deviations from the study protocol
Other bias	Low risk	Appeared free of other bias

Maier 2002

Methods	Randomised controlled study, 14 centres in four European countries (Belgium, France, Germany, Switzerland) Study period: May 1998 to June 1999 Funding sources: The study was supported by F. Hoffman-La Roche, Basel, Switzerland Declarations of interest: One of the authors (FG) was employed by F. Hoffman-La Roche
Participants	148 infants with BW 500 to 999 grams Exclusion criteria: cyanotic heart disease, major congenital malformation requiring surgery, administration of investigational drug during pregnancy, PMA $\geq$ 30 completed weeks

	The early EPO group started treatment at 3 to 5 days of age and the late EPO group 3 weeks later (at 24 to 26 days of age)
Interventions	Infants were randomised to 3 groups (see notes for the control group). In the early EPO group, 74 infants (median and quartiles for PMA; 26 (25 to 28) weeks for BW 778 (660, 880) grams) received 250 IU/kg iv or sc of rHuEPO on Mondays, Wednesdays and Fridays (NeoRecormon, F. Hofman-La Roche, Basel Switzerland) (750 IU/week iv or sc, high dose) starting at days 3 - 5 of life. In the late EPO group, 74 infants received the same treatment 3 weeks later. Treatment in both groups continued until days 65 to 68 of life. rHEPO was given iv in both groups as long as the infant had an iv line in place and sc thereafter. The late EPO group received sham injections until EPO was given. Enteral iron 3 mg/kg/day was given to all infants from days 3 - 5 and was increased at days 12 - 14 to 6 mg/kg/day and to 9 mg/kg/day at days 24 - 26 of life (high dose) A third group (control group, n =71) was included in this trial
Outcomes	Exposure of a proportion of infants to one or more red blood cell transfusions Donor exposure Mortality during hospital stay NEC IVH PVL ROP Days in oxygen Days in NICU Days in hospital
Notes	Sample size calculation was performed. Transfusion guidelines were followed. Industry funded (F. Hoffman-La Roche, Basel Switzerland) 24 (32%) of the infants in the early EPO group and 23 (31%) in the late EPO group received 1 - 3 transfusions before they entered the study. A third group (control group, n =71) was included in this trial. One infant in the control group was excluded from all evaluations because the parents withdrew consent a few hours after randomisation before the start of the treatment phase We compared the late EPO group (n = 74) to the control group (n = 71)

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	No information provided
Allocation concealment (selection bias)	Low risk	Numbered sealed envelopes
Blinding (performance bias and detection bias) All outcomes	Low risk	Blinding of intervention - yes Blinding of outcome-measure assessment - yes

Maier 2002 (Continued)

Incomplete outcome data (attrition bias) All outcomes	Low risk	One infant in the control group was excluded from all evaluations because the parents withdrew consent a few hours after randomisation before the start of the treatment phase
Selective reporting (reporting bias)	Unclear risk	The study was not entered into a trials registry and we could not tell if there were any deviations from the study protocol
Other bias	Low risk	Appeared free of other bias

Meyer 1994

Methods	Randomised, double-blind, placebo-controlled study, single centre, South Africa Study period: Not stated Funding sources: Funding for the study was obtained from Janssen's Pharmaceutical Declarations of interest: See funding resources
Participants	80 preterm infants (< 32 weeks' PMA, BW < 1500 grams), postnatal age 2 to 8 weeks
Interventions	40 infants received rHuEPO (Eprex) sc 3 times a week at a dose of 200 IU/kg/dose (600 IU/kg/week, high dose). The volume was increased by the equivalent of 50 IU/kg per dose if the Hct declined by 6% during any 2-week period during the trial, but was withheld if the Hct was > 45%. 40 infants received an identical volume of placebo. Infants received 3 mg/kg/day of iron (low dose).
Outcomes	Exposure of a proportion of infants to one or more red blood cell transfusions Sepsis NEC SIDS
Notes	Previous blood transfusion was not an exclusion criterion. Transfusion guidelines were followed.

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	A computerised random numbers generator was used.
Allocation concealment (selection bias)	Unclear risk	No information provided

Meyer 1994 (Continued)

Blinding (performance bias and detection bias) All outcomes	Low risk	Blinding of intervention - yes Blinding of outcome measure assessment - yes
Incomplete outcome data (attrition bias) All outcomes	Low risk	Three infants were early withdrawals from the trial. One infant in the placebo group completed 4 days in the trial before features of necrotising enterocolitis became apparent. The infant died one week later; the entry data were included in the analysis. A second infant from the placebo group completed 39 days in the study before dying from intraventricular haemorrhage associated with a previous ventriculoperitoneal shunt. One infant in the treatment group developed group B streptococcal septicaemia and meningitis at the age of 30 days; the symptoms began on day 19 of the study. The infant subsequently died of complications at one year of age. The observations obtained for the last 2 infants up to the time of the intercurrent event were included in the results
Selective reporting (reporting bias)	Unclear risk	The study was not entered into a trials registry and we could not tell if there were any deviations from the study protocol
Other bias	Low risk	Appeared free of other bias

Pollak 2001

Methods	Randomised, unmasked, controlled study, single centre study conducted in Vienna, Austria Study period: August 1995 to May 1997 Funding sources: The study was supported by the March and Dimes Birth defects Foundation (Grant FY 95-0220); by Vifor International, St Gallen, Switzerland; by Janssen-Cilag Pharma, Vienna, Austria, by the Verein zur Förderung der wissenschaftlichen Forschung auf dem Gebiet der Neonatologie und Kinderintensivmedizin; and by the red Bull Company, Salzburg, Austria Declarations of interest: See funding resources
Participants	38 preterm infants with PMA < 31 weeks and weight < 1300 grams at birth Infants who had received red blood cell transfusions 3 days before study entry or during the study were excluded. 9 infants were disqualified (see 'Notes'). Age at entry > 7 days

Interventions	The 21-day study consisted of a 3-day run-in baseline period during which 9 mg/kg/day of iron poly maltose complex (IPC; Ferrum Hausmann Syrup, Vifor International, St Gallen, Switzerland) supplementation was administered to all participants in all groups. This was followed by an 18-day treatment period during which participants received: 1) the same oral iron supplementation dose alone (oral iron group, n = 9); 2) 300 IU/kg/day (> 600 IU/kg/week; high dose) of r-HuEPO (Erypo, Janssen-Cilag Pharma, Vienna, Austria) as an iv bolus infusion administered at 3-day intervals along with the same oral iron supplement as the oral iron group (EPO + oral iron group, n = 10); or 3) 2 mg of iv iron sucrose/kg/day (Venofer, Vifor International) diluted in 0.9% of sodium chloride to a final concentration of 2 mg/mL and infused daily over 2 hours (iv iron + EPO group, n = 10). To maintain comparability of iron intake among the 3 groups, this last group also received EPO and oral iron in an identical manner as the EPO + oral iron group
Outcomes	Mortality Sepsis BPD at 36 weeks' postmenstrual age ROP (stage not provided) Hospital stay
Notes	Transfusion guide lines were in place. Of the 38 study participants who began the study, 9 were disqualified during the treatment period (3 infants with sepsis or sepsis-like episodes, 2 with NEC, 2 who received blood transfusions, and 2 for a protocol violation) In a secondary analysis, a diagnostic nomogram was used by Kasper et al to characterise and differentiate iron status in anaemic VLBW infants (Pollak 2001). In this secondary study no outcomes of interest for this review were included

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	No information provided
Allocation concealment (selection bias)	Low risk	Randomisation was performed by drawing sequentially numbered sealed envelopes in blocks of 6 for each stratification group
Blinding (performance bias and detection bias) All outcomes	High risk	Unmasked trial
Incomplete outcome data (attrition bias) All outcomes	Low risk	Of the 38 study participants who began the study, 9 were disqualified during the treatment period (see above) Disqualified infants were replaced by the next eligible infant in the stratification group

Pollak 2001 (Continued)

Selective reporting (reporting bias)	Unclear risk	The study was not entered into a trials registry and we could not tell if there were any deviations from the study protocol
Other bias	Low risk	Appeared free of other bias

Reiter 2005

Methods	Randomised, controlled study, single centre, the USA Study period: Not stated Funding sources: Supported by Grant Number M01 RR000069 General Clinical Research Centers Program National Centers for Research Resources NIH Declarations of interest: No conflict of interests with any of the authors	
Participants	60 preterm infants with PMA at birth < 32 weeks, Hct $\leq$ 28%, < 48 weeks' conceptual age or 5 months chronological age	
Interventions	30 infants received EPO (Eogen alpha, Amgen Inc., Thousand Oaks, CA) at 300 IU/kg per day sc for 10 days (2100 IU/kg/week; high dose) and oral elemental iron at 6 mg/kg/day (high dose). The control group (n = 30) received only supplemental iron in the same dose. 7 infants in the EPO group and 5 in the control group had received EPO prior to study entry	
Outcomes	Exposure of a proportion of infants to one or more red blood cell transfusions Volume of red blood cells transfused (mL/kg)	
Notes	Transfusion guidelines were in place and followed.	

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	A clinical research nurse assigned infants to a study group using a random number table
Allocation concealment (selection bias)	Unclear risk	It was uncertain whether there was concealed allocation to the two study groups. A clinical research nurse assigned infants to a study group using a random number table
Blinding (performance bias and detection bias) All outcomes	High risk	There was no placebo.
Incomplete outcome data (attrition bias) All outcomes	Low risk	Three infants were withdrawn from the initial data analysis: 1 from the EPO group and 2 from the control group because post-

Reiter 2005 (Continued)

		treatment laboratory variables were not obtained
Selective reporting (reporting bias)	Unclear risk	The study was not entered into a trials registry and we could not tell if there were any deviations from the study protocol
Other bias	Low risk	Appeared free of other bias

Rocha 2001

Methods	Randomised, controlled study, single centre study in Brazil Study period: March 1995 to December 1996 Funding sources: None reported Declarations of interest: None reported
Participants	45 preterm infants with PMA $\leq$ 33 weeks, BW $\leq$ 1550 grams, postnatal age 10 to 35 days
Interventions	In Group 1, 15 infants received daily doses of 100 IU/kg of EPO sc (unnamed product) (700 IU/kg/week - high dose); in Group 2, 15 infants received 350 IU/kg of EPO sc twice weekly (700 IU/kg/week - high dose); in Group 3, 15 infants did not receive EPO. Groups 1 and 2 were given iron (ferrous sulphate) 3 mg/kg/day enterally and this was increased to 6 mg/kg/day in the second week of treatment. In Group 3, iron supplementation was initiated around the 30th day of life
Outcomes	Short-term side effects Mean number of blood transfusions per infant, but no SD provided Excessive blood transfusions defined as $\geq$ 2 blood transfusions per infant (this was not an outcome identified in our protocol)
Notes	Although stated to be a randomised controlled trial in the abstract, this was uncertain as in the text, the authors wrote: "...each of the patients was systematically placed into one of the three existing groups...". Three infants (one from group 2 and two from group 3) were not included due to intercurrent diseases (2 cases of severe sepsis and one case of necrotising enterocolitis). The authors did not state in which groups the specific diseases occurred. The name of the manufacturer of EPO was not provided. "Data collection was interrupted after a sample size calculation revealed that the number of patients studied so far would have to be at least doubled in order to obtain a statistical difference between the two groups of preterm infants who used EPO in different posology". The authors were satisfied when "a statistical difference regarding excessive blood transfusions was found between the two treated groups and the control group". It is unknown whether infants who had received blood transfusions prior to study entry were included or not. Transfusion guidelines were followed.

Rocha 2001 (Continued)

<i>Risk of bias</i>		
Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	No information provided
Allocation concealment (selection bias)	Unclear risk	"...each of the patients were systematically placed in one of the three existing groups" (for groups, see interventions above)
Blinding (performance bias and detection bias) All outcomes	High risk	No placebo was used.
Incomplete outcome data (attrition bias) All outcomes	Low risk	Three infants (one from group 2 and two from group 3) were not included due to intercurrent diseases (2 cases of severe sepsis and one case of necrotising enterocolitis)
Selective reporting (reporting bias)	Unclear risk	The study was not entered into a trials registry and we could not tell if there were any deviations from the study protocol
Other bias	High risk	We had serious concerns about possible 'other bias' as noted under 'Notes' above

Romagnoli 2000

Methods	Randomised, double-blind, controlled clinical trial, single centre, Rome Italy, 3-year period ending December 1998
Participants	230 infants with gestational age < 30 weeks and 31 to 34 weeks with RDS and requiring mechanical ventilation. Infants were 7 days old, when randomised Study period: Not reported Funding sources: None reported Declarations of interest: None declared
Interventions	115 infants received EPO (unnamed product) 300 IU/kg sc, 3 times a week (900 IU/kg/week, high dose) from the 2nd to the 7th week and iron 1 mg/kg/day iv (high dose). 115 infants did not receive EPO, placebo, or iron.
Outcomes	Use of one or more red blood cell transfusions Number of blood transfusions per infant ROP (all stages, stages 1 - 2, 3 - 4, and the need for cryotherapy) NEC IVH > grade III - IV Chronic lung disease at 28 days

	Sepsis	
Notes	It was not stated whether infants who had received blood transfusions prior to study entry were included. Protocol was used to administer transfusions during study period. We obtained information from Dr Romagnoli stating that the mean (SD) age when the treatment started was 10 ($\pm$ 1) days. We therefore moved this study from the early EPO to the late EPO review	
<i>Risk of bias</i>		
Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Random number
Allocation concealment (selection bias)	Low risk	Opening of numbered, sealed envelopes, on the 7th day of life
Blinding (performance bias and detection bias) All outcomes	High risk	Blinding of intervention: no Blinding of outcome-measure assessment: no (the outcome of ROP was)
Incomplete outcome data (attrition bias) All outcomes	Low risk	Complete follow-up: yes
Selective reporting (reporting bias)	Unclear risk	The protocol for the study was not available to us and therefore we could not ascertain if there were deviations from the protocol. This study was reported as a 'Research letter' format allowing for a limited number of details
Other bias	Unclear risk	This study was reported in a 'Research letter' format allowing only for a few details. We obtained information from the Editorial office of the European Journal of Pediatrics, that 'Research letters' are peer-reviewed

Rønnestad 1995

Methods	Randomised, double-blind, placebo-controlled trial, single centre, Norway Study period: Period not stated Funding sources: The study was financially supported by the National Public Health Society Declarations of interest: Recombinant human erythropoietin was provided by Cilag, Norway
Participants	24 preterm infants, < 32 weeks' gestational age, between 14 and 22 days of age
Interventions	24 preterm infants were randomly assigned, to receive sc either rHuEPO (Eprex, Cilag) (n = 12) 150 IU/kg 3 times per week (450 IU/kg/week; low dose) or placebo (n = 12). Treatment was started between day 14 and 22 and continued for 6 weeks or alternatively until haemoglobin concentration exceeded 13.0 g/100 mL after 4 weeks of treatment. All infants were fed their own mother's milk 150 - 180 mL/kg/day, supplemented with a bovine milk protein and electrolyte fortifier. Additionally, they received vitamin E 25 mg/kg/day, a multivitamin formula (Multibionata) 0.5 mL/day and folic acid 100 mcg/day. Iron supplementation 4 mg/kg/day (low dose) as ferrous fumarate was started at study entry
Outcomes	Neutropenia
Notes	

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	No information provided
Allocation concealment (selection bias)	Unclear risk	No information provided - infants were randomly assigned
Blinding (performance bias and detection bias) All outcomes	Low risk	Blinding of intervention - yes; a placebo-treated group was in place Blinding of outcome measure assessment - yes
Incomplete outcome data (attrition bias) All outcomes	Low risk	Outcomes reported on all randomised infants
Selective reporting (reporting bias)	Unclear risk	The study was not entered into a trials registry and we could not tell if there were any deviations from the study protocol
Other bias	Low risk	Appeared free of other bias

Samanci 1996

Methods	Randomised, double-blind, controlled clinical trial, single centre, University of Istanbul, Turkey Study period: September 1993 to March 1994 Funding sources: None reported Declarations of interest: Recombinant human erythropoietin was manufactured by Cilag AG, Switzerland and provided by Güler Pharmaceutical Corp, Istanbul, Turkey
Participants	24 preterm infants with PMA $\leq$ 32 weeks, BW of $\leq$ 1250 grams and postnatal age at the first dose was 2 to 4 weeks
Interventions	12 infants received r-HuEPO (Cilag AG, Switzerland, provided by Güler Pharmaceutical Corp, Istanbul, Turkey) at a dose of 200 IU/kg, sc, 3 times weekly (600 IU/kg/week, high dose), for 4 weeks. 12 infants received an equivalent volume of placebo sc, 3 times weekly, for 4 weeks. All infants received oral supplements of elemental iron (3 mg/kg/day) (low dose) and vitamin E, 25 IU/day, during the study period
Outcomes	Exposure of a proportion of infants to one or more red blood cell transfusions Number of blood transfusions per infant IVH NEC Side effects
Notes	Infants who received erythrocyte transfusions before study entry were excluded. Guidelines for erythrocyte transfusions were developed and followed

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	A random number table was used.
Allocation concealment (selection bias)	Unclear risk	No information provided
Blinding (performance bias and detection bias) All outcomes	Low risk	A placebo was used.
Incomplete outcome data (attrition bias) All outcomes	Low risk	Outcomes reported on all infants randomised
Selective reporting (reporting bias)	Unclear risk	The study was not entered into a trials registry and we could not tell if there were any deviations from the study protocol
Other bias	Low risk	Appeared free of other bias

Shannon 1991

Methods	Multicentre, randomised, double-blind, controlled clinical trial, 3 centres, USA Study period: Study period not stated Funding sources: Supported by a grant from the R. W. Johnson Pharmaceutical Research Institute, and by grants from the National Institutes of Health (DK 32094 and M01RR01271, Pediatric Clinical Research Center) Declarations of interest: None reported
Participants	20 preterm infants, including 2 pairs of twins Participants were stratified at entry into 2 groups: a) 'large' (BW 901 to 1250 g) and b) 'small' (BW $\leq$ 900 g). 10 small and 10 large babies were entered into the trial. Within these two subgroups, 5 infants each were randomly assigned to receive either r-HuEPO or placebo. Postnatal age: 10 to 35 days
Interventions	10 infants received iv injections of r-HuEPO (Amgen) at a dose of 100 IU/kg, twice each week (200 IU/kg/week; low dose) for 6 weeks. 10 infants received iv injections of identical volume of placebo twice each week for 6 weeks. All infants received 3 mg/kg/day of oral iron (low dose) and continued at the discretion of the attending physician. All infants received supplemental vitamin E (5 IU/day).
Outcomes	Exposure of a proportion of infants to one or more red blood cell transfusions Mortality NEC Hypertension Neutropenia Side effects
Notes	Unknown whether infants who had received transfusions prior to randomisation were included or not. Infants with more complicated courses had large amounts of blood removed for laboratory tests. Transfusions were administered at the discretion of the attending physician

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	No information provided
Allocation concealment (selection bias)	Unclear risk	No information provided; infants were randomly assigned
Blinding (performance bias and detection bias) All outcomes	Low risk	A placebo was used.

Shannon 1991 (Continued)

Incomplete outcome data (attrition bias) All outcomes	Low risk	Outcomes reported for all infants randomised
Selective reporting (reporting bias)	Unclear risk	The study was not entered into a trials registry and we could not tell if there were any deviations from the study protocol
Other bias	Low risk	Appeared free of other bias

Shannon 1992

Methods	A double-blind, randomised, pilot study, single centre, USA Study period: October to December 1991 Funding sources: Supported by a grant from the R. W. Johnson Pharmaceutical Research Institute, and by grants from the National Institutes of Health (DK 32094 and M01RR01271, Pediatric Clinical Research Center) Declarations of interest: None reported	
Participants	8 preterm infants with PMA < 33 weeks and BW < 1250 grams Postnatal age (range) 8 - 28 days	
Interventions	4 infants received sc injections of r-HuEPO (unnamed product) at a dose of 100 IU/kg, 5 times a week (500 IU/kg/week; high dose). 4 infants received identical volume of placebo suspension, 5 times a week. Oral iron was started in all infants at 3 mg/kg/day (low dose), divided in 3 doses and given between feedings. The iron dose was increased to 6 mg/kg/day for infants who were tolerating full caloric feedings. Infants also received 1 mL/day of multivitamins and vitamin E (15 IU per day)	
Outcomes	Exposure of a proportion of infants to one or more red blood cell transfusions Major adverse events	
Notes	This was a pilot study. Unclear if infants were included if they had received transfusions prior to randomisation. Guidelines for red blood cell transfusions were in place.	

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	No information provided
Allocation concealment (selection bias)	Unclear risk	No information provided; infants were randomly assigned

Shannon 1992 (Continued)

Blinding (performance bias and detection bias) All outcomes	Low risk	An identical-looking placebo was used.
Incomplete outcome data (attrition bias) All outcomes	Low risk	Outcomes reported on all infants randomised
Selective reporting (reporting bias)	Unclear risk	The study was not entered into a trials registry and we could not tell if there were any deviations from the study protocol
Other bias	Low risk	Appeared free of other bias

Shannon 1995

Methods	Multicentre, randomised, double-blind, controlled clinical trial, 11 centres, USA Study period: October 1991 to October 1993 Funding sources: This work was supported, in part, by the R.W. Johnson Pharmaceutical Research Institute. Additional support was provided by Clinical Research Center grants from the National Institutes of Health to the University of California, San Francisco (Grant M01-RR-01271), to Yale University (Grant M01-RR-06022), to the University of Iowa (Grant M01-RR-00059), to the University of New Mexico (Grant M01-RR-00997), and to Stanford University (Grant M01-RR-00070). Further support came from National Institutes of Health Grant HD-14426 at Stanford; from Presbyterian/St.Luke's Community Foundation Grant 9017 to Dr. Brown; from the Children's Miracle Network Telethon at the University of Iowa; and from the Chief, Navy Bureau of Medicine and Surgery, Washington, D.C., at the Naval Medical Center San Diego (Clinical Investigation Program S-91-083) Declarations of interest: Marlene Brim and Robert I. Abels are employees of the R. W. Johnson Pharmaceutical Research Institute and own stock in its parent company, Johnson and Johnson, Inc
Participants	157 preterm infants with PMA < 31 weeks with BW $\leq$ 1250 grams Mean (SD) age (days) at study entry; EPO group 22.9 $\pm$ 10.1; placebo group 24.1 $\pm$ 9.9
Interventions	Sc injection of rHuEPO (unnamed product) at a dose of 100 IU/kg, or an identical volume of placebo suspension, were given from Monday through Friday (500 IU/kg/week; high dose) for 6 weeks or until the infants were ready to be discharged home. Doses of rHuEPO (or placebo) were adjusted weekly according to changes in body weight. There were 77 infants in the rHuEPO group and 80 infants in the placebo group. Participants received oral iron supplements at study entry to achieve a total enteral intake of 3 mg/kg/day of elemental iron (low dose). Total iron intake was increased to 6 mg/kg/day when the infants tolerated full caloric feeding enterally. Infants also received 15 IU of supplemental vitamin E and an additional 1 mL/day of an enteral multivitamin preparation

Outcomes	Exposure of a proportion of infants to one or more red blood cell transfusions Mean number of erythrocyte transfusions per infant Mortality Sepsis NEC Threshold ROP Hypertension SIDS Side effects	
Notes	Infants who had received blood transfusions prior to study entry were included. Guidelines for blood transfusions were developed. This study reports 4 post-discharge infants deaths among 125 infants followed until they were at least 6 months old. Three placebo-treated infants died; 2 of probable SIDS and one from aspiration pneumonia. The only late infant death in an EPO-treated infant occurred at 11 months of age from late NEC	
<i>Risk of bias</i>		
Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Computer-generated randomisation was used.
Allocation concealment (selection bias)	Low risk	The personnel responsible for administrative support, oversight, and monitoring of the study, the study chair and all investigators were masked to treatment group assignments throughout the study
Blinding (performance bias and detection bias) All outcomes	Low risk	A placebo was used.
Incomplete outcome data (attrition bias) All outcomes	Low risk	Outcomes reported for all infants randomised
Selective reporting (reporting bias)	Unclear risk	The study was not entered into a trials registry and we could not tell if there were any deviations from the study protocol
Other bias	Low risk	Appeared free of other bias

Whitehall 1999

Methods	Single centre, randomised controlled clinical trial, single centre study in Australia Study period: August 1992 to March 1993 Funding sources: None reported Declarations of interest: None declared
Participants	42 infants with PMA $\leq$ 32 weeks Postnatal age 14 days Exclusion criteria: major congenital anomaly, primary haematological disease, hypertension, seizures, failure to obtain consent
Interventions	10 infants with BW $\leq$ 1000 grams and 12 infants with BW > 1000 grams received 400 IU/kg of EPO (EPREX, Janssen-Cilag) sc every second day x 10 doses (high dose). 10 infants with BW $\leq$ 1000 grams and 10 infants with BW > 1000 grams received no placebo. Both groups received 3 mg/kg/day of iron (low dose) increased to 6 mg/kg/day (high dose), as tolerated
Outcomes	Total volume (mL/kg) of blood transfused Number of transfusions per infant Mortality during hospital stay
Notes	Unknown whether infants were included if they had received blood transfusion prior to study entry. Guidelines for blood transfusions were in place.

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Computer-generated random numbers were used.
Allocation concealment (selection bias)	Low risk	Sealed coded envelopes were used.
Blinding (performance bias and detection bias) All outcomes	High risk	No placebo was used.
Incomplete outcome data (attrition bias) All outcomes	Low risk	Outcomes reported for all randomised infants
Selective reporting (reporting bias)	Unclear risk	The study was not entered into a trials registry and we could not tell if there were any deviations from the study protocol
Other bias	Low risk	Appeared free of other bias

Yamada 1999a

Methods	Single centre, randomised controlled clinical trial, Japan Study period: Not stated Funding sources: Not stated Declarations of interest: Not stated
Participants	55 infants with BW 1000 to 1499 grams and PMA < 33 weeks and postnatal age < 40 days
Interventions	28 infants received 200 IU/kg of EPO (unnamed product) sc twice a week (400 IU/kg/week, low dose) for 8 weeks and oral iron (3 mg/kg/day). 27 infants in the control group received no treatment or placebo
Outcomes	Exposure of a proportion of infants to one or more red blood cell transfusions The total volume (mL) of blood transfused per infant Number of transfusions per infant
Notes	Unknown if infants who had received transfusions prior to enrolment were excluded or not. Conservative red blood cell transfusion guidelines were followed

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	No information provided
Allocation concealment (selection bias)	Unclear risk	Randomly assigned
Blinding (performance bias and detection bias) All outcomes	High risk	No placebo was used.
Incomplete outcome data (attrition bias) All outcomes	Low risk	Outcomes reported for all randomised infants
Selective reporting (reporting bias)	Unclear risk	The study was not entered into a trials registry and we could not tell if there were any deviations from the study protocol
Other bias	Low risk	Appeared free of other bias

Yamada 1999b

Methods	Single centre, randomised controlled clinical trial, Japan Study period: Not reported Funding sources: Not stated Declarations of interest: Not stated
Participants	27 infants with BW 500 to 999 grams and PMA < 33 weeks and postnatal age < 40 days
Interventions	15 infants received EPO 200 IU/kg of EPO (unnamed product) sc twice a week (400 IU/kg/week, low dose) sc for 8 weeks and oral iron (3 mg/kg/day). 12 infants in the control group received no treatment or placebo
Outcomes	Exposure of a proportion of infants to one or more red blood cell transfusions The total volume (mL) of blood transfused per infant Number of transfusions per infant
Notes	Unknown if infants who had received transfusions prior to enrolment were excluded or not. Conservative red blood cell transfusion guidelines were followed

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	No information provided
Allocation concealment (selection bias)	Unclear risk	No information provided; randomly assigned
Blinding (performance bias and detection bias) All outcomes	High risk	No placebo was used.
Incomplete outcome data (attrition bias) All outcomes	Low risk	Outcomes for all randomised infants were reported.
Selective reporting (reporting bias)	Unclear risk	The study was not entered into a trials registry and we could not tell if there were any deviations from the study protocol
Other bias	Low risk	Appeared free of other bias

AGA=appropriate for gestational age

BPD = bronchopulmonary dysplasia

BW = birth weight

EPO = erythropoietin

GA = Gestational age

Hb = haemoglobin

Hct = haematocrit
 iv = intravenous (or intravenously)
 im = intramuscular (or intra muscularly)
 int. = international
 IU = units
 IVH = intraventricular haemorrhage
 NEC = necrotising enterocolitis
 NICU = neonatal intensive care unit
 PCV = packed cell volume (haematocrit)
 PMA = postmenstrual age
 PRBC = packed red blood cells
 PVL = periventricular leukomalacia
 RBC = red blood cell count
 RDS = respiratory distress syndrome
 ROP = retinopathy of prematurity
 rHuEPO = recombinant human erythropoietin
 sc = subcutaneous (or subcutaneously)
 SD = standard deviation
 SEM = standard error of the mean
 SIDS = sudden infant death syndrome
 TPN: total parenteral nutrition
 VLBW: very low birth weight

Characteristics of excluded studies *[ordered by study ID]*

Study	Reason for exclusion
Ahmadpour Kacho 2004	Published in abstract form only. Randomised controlled trial of erythropoietin versus no treatment in preterm low birth weight infants. The abstract lacked information on the age of the infants at the time of enrolment
Amin 2004	Published in abstract form only. The authors stated that this was a controlled clinical trial of erythropoietin versus conventional therapy in preterm very low birth weight infants. It was not clear if this was a randomised controlled trial or not
Badiee 2006	No outcomes of interest.
Bechensteen 1997	Both groups got EPO.
Messer 1993	This was not a randomised controlled trial. Non-randomised controls were used
Meyer 1996	All infants were treated with EPO.
Mohammadzadeh 2005	Both groups got EPO.
Ohls 1991	This study compared EPO with erythrocyte transfusion and not placebo

(Continued)

Ohls 2012	This study compared 2 dosing schedules; once a week versus 3 times a week of EPO in infants ≥ 7 days of age. It appeared that this study was published as an abstract in 2008 when a lower number of infants had been enrolled (14 in the abstract versus 20 in the full publication)
Pasha 2008	No outcomes of interest. "No subject from the study group required red blood cell transfusion". The authors did not state if any infant was transfused in the control group
Pathak 2003	Both groups got EPO.
Testa 1998	The study used historical controls.
Warwood 2005	This study was a dose-finding study of darbepoetin (longer-acting and more potent than EPO). Infants were randomised to 2 different doses of darbepoetin
Warwood 2011	No outcomes of interest for this review were presented apart from need for transfusion. One of the study subjects qualified for and received a blood transfusion. However, the authors did not state which group this infant belonged to
Widness 2006	No outcomes of interest for this review were presented.

EPO:erythropoietin

DATA AND ANALYSES

Comparison 1. Late initiation of EPO (8-28 days) vs. placebo or no intervention

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Use of one or more red blood cell transfusions (low and high dose of EPO)	21	1202	Risk Ratio (M-H, Fixed, 95% CI)	0.72 [0.65, 0.79]
2 Use of one or more red blood cell transfusions (high dose of EPO)	15	972	Risk Ratio (M-H, Fixed, 95% CI)	0.77 [0.68, 0.86]
2.1 High dose iron	6	318	Risk Ratio (M-H, Fixed, 95% CI)	0.74 [0.62, 0.88]
2.2 Low dose iron	9	654	Risk Ratio (M-H, Fixed, 95% CI)	0.78 [0.67, 0.90]
3 Use of one or more red blood cell transfusions (low dose of EPO)	7	239	Risk Ratio (M-H, Fixed, 95% CI)	0.53 [0.42, 0.67]
3.1 High dose of iron	3	77	Risk Ratio (M-H, Fixed, 95% CI)	0.50 [0.31, 0.79]
3.2 Low dose of iron	4	162	Risk Ratio (M-H, Fixed, 95% CI)	0.54 [0.41, 0.71]
4 Total volume (mL/kg) of red blood cells transfused per infant	5	197	Mean Difference (IV, Fixed, 95% CI)	-1.61 [-5.78, 2.57]
5 Number of red blood cell transfusions per infant	11	817	Mean Difference (IV, Fixed, 95% CI)	-0.22 [-0.38, -0.06]
6 Number of donors the infant was exposed to	2	165	Mean Difference (IV, Fixed, 95% CI)	0.45 [0.20, 0.69]
7 Mortality during initial hospital stay (all causes)	14	767	Risk Ratio (M-H, Fixed, 95% CI)	0.82 [0.49, 1.39]
8 Retinopathy of prematurity (all stages or stage not reported)	3	404	Risk Ratio (M-H, Fixed, 95% CI)	1.27 [0.99, 1.64]
9 Retinopathy of prematurity (stage ≥ 3)	3	442	Risk Ratio (M-H, Fixed, 95% CI)	1.73 [0.92, 3.24]
10 Proven sepsis	5	551	Risk Ratio (M-H, Fixed, 95% CI)	0.75 [0.52, 1.09]
11 Necrotising Enterocolitis $\geq$ Bell's stage 2	6	656	Risk Ratio (M-H, Random, 95% CI)	0.88 [0.45, 1.70]
12 Intraventricular haemorrhage all grades (or grade not specified)	4	454	Risk Ratio (M-H, Fixed, 95% CI)	0.87 [0.53, 1.42]
13 Periventricular leukomalacia	1		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
14 Bronchopulmonary dysplasia (supplementary oxygen at 28 days)	2	285	Risk Ratio (M-H, Fixed, 95% CI)	1.25 [1.00, 1.55]
15 Bronchopulmonary dysplasia (supplementary oxygen at 36 weeks' postmenstrual age)	3	216	Risk Ratio (M-H, Fixed, 95% CI)	0.89 [0.59, 1.35]
16 SIDS	6	363	Risk Ratio (M-H, Fixed, 95% CI)	1.06 [0.25, 4.52]
17 Neutropenia	6	164	Risk Ratio (M-H, Fixed, 95% CI)	0.28 [0.05, 1.54]
18 Hypertension	7	361	Risk Ratio (M-H, Fixed, 95% CI)	1.20 [0.46, 3.14]
19 Length of hospital stay (days)	2	55	Mean Difference (IV, Fixed, 95% CI)	-0.35 [-12.83, 12.13]

20 Use of one or more red blood cell transfusions (secondary analysis based on study quality)	21	1202	Risk Ratio (M-H, Fixed, 95% CI)	0.72 [0.65, 0.79]
20.1 High quality studies	6	417	Risk Ratio (M-H, Fixed, 95% CI)	0.83 [0.73, 0.95]
20.2 Studies of uncertain quality	15	785	Risk Ratio (M-H, Fixed, 95% CI)	0.63 [0.54, 0.73]
21 Use of one or more red blood cell transfusions (secondary analysis based on RBC transfusion guidelines)	19	1120	Risk Ratio (M-H, Fixed, 95% CI)	0.74 [0.67, 0.82]
21.1 Strict RBC transfusion guidelines	16	1023	Risk Ratio (M-H, Fixed, 95% CI)	0.76 [0.69, 0.85]
21.2 No or less strict RBC guidelines	3	97	Risk Ratio (M-H, Fixed, 95% CI)	0.25 [0.08, 0.77]

Analysis 1.1. Comparison 1 Late initiation of EPO (8-28 days) vs. placebo or no intervention, Outcome 1 Use of one or more red blood cell transfusions (low and high dose of EPO).

Review: Late erythropoiesis-stimulating agents to prevent red blood cell transfusion in preterm or low birth weight infants

Comparison: 1 Late initiation of EPO (8-28 days) vs. placebo or no intervention

Outcome: 1 Use of one or more red blood cell transfusions (low and high dose of EPO)

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Analysis 1.2. Comparison 1 Late initiation of EPO (8-28 days) vs. placebo or no intervention, Outcome 2 Use of one or more red blood cell transfusions (high dose of EPO).

Review: Late erythropoiesis-stimulating agents to prevent red blood cell transfusion in preterm or low birth weight infants

Comparison: 1 Late initiation of EPO (8-28 days) vs. placebo or no intervention

Outcome: 2 Use of one or more red blood cell transfusions (high dose of EPO)

Analysis 1.3. Comparison 1 Late initiation of EPO (8-28 days) vs. placebo or no intervention, Outcome 3 Use of one or more red blood cell transfusions (low dose of EPO).

Review: Late erythropoiesis-stimulating agents to prevent red blood cell transfusion in preterm or low birth weight infants

Comparison: 1 Late initiation of EPO (8-28 days) vs. placebo or no intervention

Outcome: 3 Use of one or more red blood cell transfusions (low dose of EPO)

Analysis 1.4. Comparison 1 Late initiation of EPO (8-28 days) vs. placebo or no intervention, Outcome 4 Total volume (mL/kg) of red blood cells transfused per infant.

Review: Late erythropoiesis-stimulating agents to prevent red blood cell transfusion in preterm or low birth weight infants

Comparison: 1 Late initiation of EPO (8-28 days) vs. placebo or no intervention

Outcome: 4 Total volume (mL/kg) of red blood cells transfused per infant

Analysis 1.5. Comparison 1 Late initiation of EPO (8-28 days) vs. placebo or no intervention, Outcome 5 Number of red blood cell transfusions per infant.

Review: Late erythropoiesis-stimulating agents to prevent red blood cell transfusion in preterm or low birth weight infants

Comparison: 1 Late initiation of EPO (8-28 days) vs. placebo or no intervention

Outcome: 5 Number of red blood cell transfusions per infant

Analysis 1.6. Comparison 1 Late initiation of EPO (8-28 days) vs. placebo or no intervention, Outcome 6 Number of donors the infant was exposed to.

Review: Late erythropoiesis-stimulating agents to prevent red blood cell transfusion in preterm or low birth weight infants

Comparison: 1 Late initiation of EPO (8-28 days) vs. placebo or no intervention

Outcome: 6 Number of donors the infant was exposed to

Analysis 1.7. Comparison 1 Late initiation of EPO (8-28 days) vs. placebo or no intervention, Outcome 7 Mortality during initial hospital stay (all causes).

Review: Late erythropoiesis-stimulating agents to prevent red blood cell transfusion in preterm or low birth weight infants

Comparison: 1 Late initiation of EPO (8-28 days) vs. placebo or no intervention

Outcome: 7 Mortality during initial hospital stay (all causes)

Analysis 1.8. Comparison 1 Late initiation of EPO (8-28 days) vs. placebo or no intervention, Outcome 8 Retinopathy of prematurity (all stages or stage not reported).

Review: Late erythropoiesis-stimulating agents to prevent red blood cell transfusion in preterm or low birth weight infants

Comparison: 1 Late initiation of EPO (8-28 days) vs. placebo or no intervention

Outcome: 8 Retinopathy of prematurity (all stages or stage not reported)

Analysis 1.9. Comparison 1 Late initiation of EPO (8-28 days) vs. placebo or no intervention, Outcome 9 Retinopathy of prematurity (stage ≥ 3).

Review: Late erythropoiesis-stimulating agents to prevent red blood cell transfusion in preterm or low birth weight infants

Comparison: 1 Late initiation of EPO (8-28 days) vs. placebo or no intervention

Outcome: 9 Retinopathy of prematurity (stage ≥ 3)

Analysis 1.10. Comparison 1 Late initiation of EPO (8-28 days) vs. placebo or no intervention, Outcome 10 Proven sepsis.

Review: Late erythropoiesis-stimulating agents to prevent red blood cell transfusion in preterm or low birth weight infants

Comparison: 1 Late initiation of EPO (8-28 days) vs. placebo or no intervention

Outcome: 10 Proven sepsis

Analysis 1.11. Comparison 1 Late initiation of EPO (8-28 days) vs. placebo or no intervention, Outcome 11 Necrotising Enterocolitis $\geq$ Bell's stage 2.

Review: Late erythropoiesis-stimulating agents to prevent red blood cell transfusion in preterm or low birth weight infants

Comparison: 1 Late initiation of EPO (8-28 days) vs. placebo or no intervention

Outcome: 11 Necrotising Enterocolitis $\geq$ Bell's stage 2

Analysis 1.12. Comparison 1 Late initiation of EPO (8-28 days) vs. placebo or no intervention, Outcome 12 Intraventricular haemorrhage all grades (or grade not specified).

Review: Late erythropoiesis-stimulating agents to prevent red blood cell transfusion in preterm or low birth weight infants

Comparison: 1 Late initiation of EPO (8-28 days) vs. placebo or no intervention

Outcome: 12 Intraventricular haemorrhage all grades (or grade not specified)

Analysis 1.13. Comparison 1 Late initiation of EPO (8-28 days) vs. placebo or no intervention, Outcome 13 Periventricular leukomalacia.

Review: Late erythropoiesis-stimulating agents to prevent red blood cell transfusion in preterm or low birth weight infants

Comparison: 1 Late initiation of EPO (8-28 days) vs. placebo or no intervention

Outcome: 13 Periventricular leukomalacia

Analysis 1.14. Comparison 1 Late initiation of EPO (8-28 days) vs. placebo or no intervention, Outcome 14 Bronchopulmonary dysplasia (supplementary oxygen at 28 days).

Review: Late erythropoiesis-stimulating agents to prevent red blood cell transfusion in preterm or low birth weight infants

Comparison: 1 Late initiation of EPO (8-28 days) vs. placebo or no intervention

Outcome: 14 Bronchopulmonary dysplasia (supplementary oxygen at 28 days)

Analysis 1.15. Comparison 1 Late initiation of EPO (8-28 days) vs. placebo or no intervention, Outcome 15 Bronchopulmonary dysplasia (supplementary oxygen at 36 weeks' postmenstrual age).

Review: Late erythropoiesis-stimulating agents to prevent red blood cell transfusion in preterm or low birth weight infants

Comparison: 1 Late initiation of EPO (8-28 days) vs. placebo or no intervention

Outcome: 15 Bronchopulmonary dysplasia (supplementary oxygen at 36 weeks' postmenstrual age)

Analysis 1.16. Comparison 1 Late initiation of EPO (8-28 days) vs. placebo or no intervention, Outcome 16 SIDS.

Review: Late erythropoiesis-stimulating agents to prevent red blood cell transfusion in preterm or low birth weight infants

Comparison: 1 Late initiation of EPO (8-28 days) vs. placebo or no intervention

Outcome: 16 SIDS

Analysis 1.17. Comparison 1 Late initiation of EPO (8-28 days) vs. placebo or no intervention, Outcome 17 Neutropenia.

Review: Late erythropoiesis-stimulating agents to prevent red blood cell transfusion in preterm or low birth weight infants

Comparison: 1 Late initiation of EPO (8-28 days) vs. placebo or no intervention

Outcome: 17 Neutropenia

Analysis 1.18. Comparison 1 Late initiation of EPO (8-28 days) vs. placebo or no intervention, Outcome 18 Hypertension.

Review: Late erythropoiesis-stimulating agents to prevent red blood cell transfusion in preterm or low birth weight infants

Comparison: 1 Late initiation of EPO (8-28 days) vs. placebo or no intervention

Outcome: 18 Hypertension

Analysis 1.19. Comparison 1 Late initiation of EPO (8-28 days) vs. placebo or no intervention, Outcome 19 Length of hospital stay (days).

Review: Late erythropoiesis-stimulating agents to prevent red blood cell transfusion in preterm or low birth weight infants

Comparison: 1 Late initiation of EPO (8-28 days) vs. placebo or no intervention

Outcome: 19 Length of hospital stay (days)

Analysis 1.20. Comparison 1 Late initiation of EPO (8-28 days) vs. placebo or no intervention, Outcome 20 Use of one or more red blood cell transfusions (secondary analysis based on study quality).

Review: Late erythropoiesis-stimulating agents to prevent red blood cell transfusion in preterm or low birth weight infants

Comparison: 1 Late initiation of EPO (8-28 days) vs. placebo or no intervention

Outcome: 20 Use of one or more red blood cell transfusions (secondary analysis based on study quality)

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Analysis 1.21. Comparison 1 Late initiation of EPO (8-28 days) vs. placebo or no intervention, Outcome 21 Use of one or more red blood cell transfusions (secondary analysis based on RBC transfusion guidelines).

Review: Late erythropoiesis-stimulating agents to prevent red blood cell transfusion in preterm or low birth weight infants

Comparison: 1 Late initiation of EPO (8-28 days) vs. placebo or no intervention

Outcome: 21 Use of one or more red blood cell transfusions (secondary analysis based on RBC transfusion guidelines)

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ADDITIONAL TABLES

Table 1. Transfusion guidelines

Reference	Indications
Akisu 2001	Guidelines for transfusions were not presented.
Al-Kharfy 1996	The indications for transfusion were 1) shock, 2) cumulative loss of $\geq 10\%$ of the blood volume in 72 hours or less when further blood sampling is expected, 3) $\text{Hb} < 130 \text{ g/L}$ in acutely ill neonates with cardiorespiratory disease, and 4) $\text{Hb} < 80$ to 100 g/L with clinical signs of anaemia. A volume of 15 mL/kg was recommended for each transfusion
Atasay 2002	Criteria for blood transfusion (10 mL/kg packed red cells) were as follows: a $\text{Hct} < 30\%$ when signs and symptoms attributed to anaemia including persistent tachycardia (180 beats/min for 24 hours), frequent apnoea with bradycardia and daily weight gain $< 10 \text{ g/kg}$ despite optimal protein and caloric intake (3.5 g/kg , 100 kcal/kg/day). Infants were transfused with a Hct of 35% to 40% if they received more than 40% oxygen or ventilation therapy
Bader 1996	Criteria for blood transfusion (10 mL/kg of red cells) were as follows: a) a $\text{Hct} < 25\%$, b) an increased frequency of apnoeic events which required either stimulation or aminophylline therapy, c) changes in heart rate patterns, e.g. an increase in frequency of bradycardia ($< 80 \text{ beats/min}$) or tachycardia ($> 180 \text{ beats/min}$, d) failure of weight gain of $> 10 \text{ g/kg/day}$ despite an optimal caloric intake of $> 120 \text{ kcal/day}$ and e) lethargy without evidence of sepsis

Table 1. Transfusion guidelines (Continued)

Basiri 2015	Transfusion guidelines for preterm infants included: (1) asymptomatic infants with Hct < 21% and reticulocytes < 2%; (2) infants with haematocrit < 31% and hood O ₂ < 36% or mean airway pressure < 6 cm H ₂ O by continuous positive airway pressure (CPAP) or intermittent mandatory ventilation (IMV) or > 9 apnoeic and bradycardic episodes per 12 hours or 2/24 hours requiring bag- and mask ventilation while on adequate methylxanthine therapy or heart rate > 18/min or respiratory rate > 80/min sustained for 24 hours or weight gain of < 10 g/day for 4 days on 100 kcal/kg/day or having surgery; (3) infants with haematocrit < 36% and requiring > 35% O ₂ or mean airway pressure of 6 to 8 cm H ₂ O by CPAP or IMV.
Bechensteen 1993	Indications of blood transfusions were: 1) Hb < 80 g/L or 2) otherwise at the discretion of the clinician caring for the infant according to symptoms and signs
Bierer 2009	Very specific transfusion criteria were in place for infants on mechanical ventilation, for infants who required supplemental oxygen and for infants on room air
Chen 1995	Transfusions were given because of frequent and prolonged apnoea
Corona 1998	Transfusions were considered based on the clinical condition (pallor, tachycardia, tachypnoea, apnoea with or without bradycardia, poor weight gain, difficulties with sucking) and haematological parameters (Hb < 70 g/L, Hct < 26%, with low reticulocyte counts)
Donato 1996	During the first week of life, infants were given transfusions of packed red blood cells for replacement when blood drawn for analysis was in excess of 8 mL/kg of body weight; fresh whole blood was given if signs attributable to hypovolaemia or anaemia developed. Subsequently, infants with heart rate > 180 beats/min, severe apnoea/bradycardia or poor weight gain (< 10 grams/day in spite of a 100 calories/day intake during 5 consecutive days) were transfused if the Hct was < 25% (or < 30% if oxygen or mechanical ventilation was required); asymptomatic infants were transfused only when a central Hct < 23% was reached
Emmerson 1993	The decision to give a blood transfusion to a study infant was made by the medical staff of the neonatal unit who were blinded to the randomisation. The unit policy at the time of the study was to transfuse a preterm infant who had a Hb < 100 g/L and who had symptoms consistent with those caused by anaemia. The symptoms and signs of anaemia included poor feeding, tachycardia, tachypnoea, apnoea, and pallor. Infants with a Hb < 80 g/L were transfused even if asymptomatic
Giannakopoulou 1998a; Giannakopoulou 1998b	Indications for blood transfusion were a Hb < 80 g/L or otherwise at the discretion of the physician treating the infants according to symptoms and signs
Griffiths 1997	Infants were transfused if they were ventilated and/or oxygen-dependent with a Hb of < 120 g/L, had clinically symptomatic anaemia, or were asymptomatic with a Hb of < 70 g/L. Infants were transfused if they were ventilated and/or oxygen-dependent with a Hb of < 120 g/L, had clinically symptomatic anaemia, or were asymptomatic with a haemoglobin of < 70 g/L

Table 1. Transfusion guidelines (Continued)

Javier Manchon 1997	Transfusion guidelines were similar in all three centres (details not provided)
Juul 2003	By NICU policy, on admission, infants weighing < 1000 grams at birth were assigned 1 unit of packed red blood cells divided into 8 aliquots. These aliquots were used for transfusions during the first month of life. The following transfusion guideline was used for infants of all birth weights: Transfusion is recommended for a Hct < 35% if the infant requires positive pressure with a mean airway pressure > 6 cm H₂O and requires > 35% oxygen. Transfusion is recommended for Hct < 30% if the infant requires oxygen (< 35% FIO₂), is receiving continuous positive airway pressure or intubated with mean airway pressure < 6 cm H₂O, if an infant has significant apnoea and bradycardia while receiving methylxanthines (> 9 episodes in 12 hours or 2 episodes in 24 hours requiring mask-and-bag ventilation), if the heart rate is > 180 beats/min or respiratory rate > 80/min and persists for 24 hours, if weight gain < 10 grams/day over 4 days despite adequate calories, or in the presence of sepsis. If the Hct is < 20%, no symptoms are necessary for transfusion. Transfusion volumes were standardised as follows: infants received an initial transfusion of 15 mL/kg over a period of 3 to 4 hours. A follow-up Hct was checked after 4 hours. If the Hct was < 30%, a second aliquot of 10 mL/kg was given If the Hct was between 30% and 35%, an additional 5 mL/kg was given
Kivivuori 1999	The Hct values were maintained at > 30% by red blood cell transfusions (10 mL/kg per time) in asymptomatic infants. In infants who had symptoms or signs of anaemia, red blood cells were transfused if the Hct value was < 40%. The transfusion policies were the same in all study hospitals
Kumar 1998	The need for erythrocyte transfusion was assessed by the clinicians caring for each infant and the decision to transfuse was made without consulting the study investigators. According to the practice in the NICU, infants received transfusion if a Hct level of < 27% was associated with one of the following signs and symptoms of anaemia: 1) frequent apnoea and bradycardia, defined as > 6 episodes in 12 hours or any episode requiring bag-and-mask ventilation, in an infant with therapeutic serum levels of theophylline; 2) persistent tachycardia, defined as > 180 beats/min for more than 12 hours; 3) poor weight gain (< 10 grams/day averaged over a 7-day period) despite adequate caloric intake; and 4) increasing oxygen requirement in infants with chronic lung disease despite optimum diuretic and bronchodilator therapy
Maier 2002	Infants with artificial ventilation or in > 40% of inspired oxygen were not transfused unless Hct dropped to < 40%. Spontaneously breathing infants were not transfused unless Hct dropped to < 35% during the first 2 weeks of life, 30% during the 3rd to 4th weeks, and 25% thereafter. Transfusion was allowed when life-threatening anaemia or hypovolaemia was assumed by the treating neonatologist, or surgery was planned. Twelve of the 14 centres used satellite packs of the original red cell pack to reduce donor exposure

Table 1. Transfusion guidelines (Continued)

Meyer 1994	Direct quote: “The need for blood transfusion was assessed by the attending neonatal physician and decisions were made independently of the investigators. The following guidelines were developed, based on existing literature and nursery practices: a. Hct < 30% and 1) Weight gain of < 10 grams/day averaged over 1-week period (infant tolerating full oral feeds and receiving adequate calories) 2) Three or more episodes of apnoea (respirations absent for 20 seconds) or bradycardia (heart rate of < 100 beats per minute) in a 24-hour period not due to other causes and not responsive to methylxanthine treatment 3) Tachycardia (> 170 beats/min) or tachypnoea (> 70 breaths/min) sustained over a 24-hour period or associated with acute cardiac decompression 4) A requirement for surgery. b. Development of a clinically significant patent ductus arteriosus (i.e. at least three of the following features: heart rate > 160 beats/min, brisk brachial and/or dorsalis pedis pulses, palpable precordial pulsation, systolic murmur, cardiomegaly on chest radiograph) c. Pulmonary disease and fractional inspired oxygen concentrations increasing by > 10% per week d. Systemic infection (either clinically suspected or proven on blood culture) associated with a sudden decrease in hematocrit e. Hematocrit of 22% or less and an absolute reticulocyte count of < 100 000 x 10⁹/L”.
Pollak 2001	Standard NICU transfusion criteria were used (authors refer to Shannon 1995; see below).
Reiter 2005	Conservative transfusion guidelines were in place and followed. Criteria for red blood cell transfusion in the acutely ill infant requiring mechanical ventilation or nasal continuous positive airway pressure included: phlebotomy loss of > 15% of blood volume associated with hypotension, or Hct < 30%. Criteria for red blood cell transfusion in the convalescent infant requiring no more than supplemental oxygen included: Hct < 28% with symptomatic anaemia (tachycardia, poor somatic growth, or metabolic acidosis) or Hct < 20%
Rocha 2001	The decision for blood transfusion within the whole study population was made by the assistant doctor of each newborn, and was based on the following criteria: Hct ≤ 20%, inadequate weight gain, 3 or more apnoea or bradycardia episodes within 24 hours, presurgical procedure requirement, disease associated with sudden Hct decline, restoration of the blood collected for lab exams, maintenance of Hct up to 30% associated with minimal ventilatory support requirement, and Hct up to 35% when ventilation requirements are greater. The assistant doctor who recommended blood transfusion did not know to which group the infant belonged
Romagnoli 2000	Infants on mechanical ventilation and/or on more than 30% of inspired oxygen received packed erythrocytes when their Hct levels dropped below 40%. Otherwise the transfusion was performed when the Hct fell below 35% from the 2nd to the 4th week of life and below 23% thereafter

Table 1. Transfusion guidelines (Continued)

Rønnestad 1995	Transfusions were given on the orders of the attending physician if Hb was < 90 g/L or otherwise as necessary according to signs and symptoms
Samanci 1996	The need for packed erythrocyte transfusions was judged by the attending neonatologist. Guidelines for erythrocyte transfusions were developed as follows: 1) Hct of $\leq 22\%$ and an absolute reticulocyte count of 100 000/microlitre; 2) Hct of $\leq 30\%$ and a) tachycardia (> 180 beats/min) and tachypnoea (> 70 breaths/min) persisting for 24 hours; or b) 3 or more episodes of apnoea or bradycardia in 24 hours, not due to other causes and not responsive to methylxanthine treatment; or c) average weight gain of < 10 grams/day over a 1-week period (infant tolerating full oral feed and receiving adequate calories); or d) undergoing surgery; 3) systemic infection associated with a sudden decrease in Hct
Shannon 1991	Transfusions were ordered by the clinicians caring for each infant without consulting the investigators. Written guidelines for erythrocyte transfusions were developed for the nursery 1 year before the start of the study. A copy of these guidelines was taped to the bed of each study infant. In general, babies who were otherwise well received transfusions only if they had a Hct $< 25\%$ and signs referable to their anaemia, such as slowing in rate of growth, persistent severe tachycardia and tachypnoea, or worsening of episodes of apnoea and bradycardia
Shannon 1992	See Shannon 1991 above.
Shannon 1995	Transfuse infants at Hct $\leq 20\%$: a) if asymptomatic with reticulocytes < 100 000/microlitre Transfuse infants at Hct $\leq 30\%$: a) if receiving $< 35\%$ supplemental hood oxygen, b) if on CPAP or mechanical ventilation with mean airway pressure < 6 cm H ₂ O, c) if significant apnoea and bradycardia are noted (9 episodes in 12 hours or 2 episodes in 24 hours requiring bag-and-mask ventilation) while receiving therapeutic doses of methylxanthines, d) if heart rate > 180 beats/min or respiratory rate > 80 breaths /min persists for 24 hours, e) if weight gain < 10 grams/day is observed over 4 days while receiving ≥ 100 kcal/kg/day, f) if undergoing surgery Transfuse for Hct $\leq 35\%$: a) if receiving $> 35\%$ supplemental hood oxygen, b) if intubated on CPAP or mechanical ventilation with mean airway pressure ≥ 6 to 8 cm H ₂ O. Do not transfuse: a) to replace blood removed for laboratory tests alone, b) for low Hct alone
Whitehall 1999	Guidelines for red cell transfusions for anaemia of prematurity were based on the existing policy in the nursery, generally adopted by the neonatologists. They were as follows: I. Transfuse infants at Hb 80 g/L, (a) If reticulocyte count is $< 4\%$ and (b) If receiving supplemental oxygen $> 30\%$ or (c) If unexplained recurrent apnoea/bradycardia is noted (> 1 to 2/hour) or (d) If persistent tachycardia (heart rate > 170 /min) or tachypnoea (respiratory rate > 60 /min) is noted, or (e) If there is failure to gain weight or successive weight loss on weekly recordings for 3 consecutive weeks. In absence of a clear evidence in the literature justifying red cell transfusions at a Hb of 80 g/L in otherwise asymptomatic neonates who are

Table 1. Transfusion guidelines (Continued)

	failing to thrive, it was decided that failure to gain weight or successive weight loss on weekly recordings for 3 consecutive weeks was a fair and substantial clinical indicator of the need to transfuse. II. Transfuse infants at Hb 100 g/L, (a) If receiving supplemental oxygen 30% and (b) needing intermittent mandatory ventilation or continuous positive airway pressure by nasal prongs for recurrent (> 1 to 2 per hour), apnoea/bradycardia with saturations < 90% on the pulse oximeter. III. Transfuse Infants at Hb 120 g/L, (a) If receiving mechanical ventilatory support with mean airway pressure 10 cm H₂O and supplemental oxygen 30% during the acute phase of illness after birth
Yamada 1999a	Conservative red blood cell transfusion guidelines were followed (details not presented, as we were unable to translate the information)
Yamada 1999b	Conservative red blood cell transfusion guidelines were followed (details not presented, as we were unable to translate the information)

CPAP: continuous positive airways pressure

FIO₂: fraction of inspired oxygen

H₂O: water

Hb: haemoglobin

Hct: haematocrit

IMV: intermittent mandatory ventilation

NICU: neonatal intensive care unit

O₂: oxygen

APPENDICES

Appendix I. Cochrane Neonatal standard search strategy

PubMed: ((infant, newborn[MeSH] OR newborn OR neonate OR neonatal OR premature OR low birth weight OR VLBW OR LBW or infan* or neonat*) AND (randomised controlled trial [pt] OR controlled clinical trial [pt] OR randomised [tiab] OR placebo [tiab] OR drug therapy [sh] OR randomly [tiab] OR trial [tiab] OR groups [tiab]) NOT (animals [mh] NOT humans [mh]))

Embase: ((exp infant) OR (infan* OR newborn or neonat* OR premature or very low birth weight or low birth weight or VLBW or LBW).mp AND (human not animal) AND (randomised controlled trial or controlled clinical trial or randomised or placebo or clinical trials as topic or randomly or trial or clinical trial).mp

CINAHL: (infan* OR newborn OR neonat* OR premature OR low birth weight OR VLBW OR LBW) AND (randomised controlled trial OR controlled clinical trial OR randomised OR placebo OR clinical trials as topic OR randomly OR trial OR PT clinical trial)

Cochrane Library: (infan* or newborn or neonat* or premature or preterm or very low birth weight or low birth weight or VLBW or LBW)

Appendix 2. Previous search methodology

For the original review (Aher 2006b), we searched the Cochrane Central Register of Controlled Trials (CENTRAL, *The Cochrane Library*, 2006, Issue 2) to identify relevant randomised and quasi-randomised controlled trials. We searched MEDLINE for relevant articles published from 1966 to November 2005 using the following MeSH terms or text words: (exp Erythropoietin/OR erythropoietin: .mp. OR rhuepo.mp.) AND (anaemia/OR exp anaemia, neonatal/) AND (blood transfusion/OR blood component transfusion/OR erythrocyte transfusion/) AND (infant, newborn/OR infant, low birth weight/OR infant, very low birth weight/OR infant, premature/OR exp Infant, Premature, Diseases) OR (neonate: OR prematur*: OR newborn:).mp. OR newborn infant [age limit]) AND (clinical trial.pt. OR Randomized Controlled Trials/OR (random: OR rct OR rcts OR blind OR blinded OR placebo:).mp. OR (review.pt. OR review, academic.pt.) AND human. We searched EMBASE from 1980 to November 2005 and CINAHL 1982 to November 2005 using the following MeSH terms or text words: (Erythropoietin/OR erythropoietin: OR epo OR epogen OR epoetin: OR (rhuepo).mp. AND (anaemia/OR exp anaemia, neonatal/) AND (blood transfusion/OR exp blood component transfusion/OR erythrocytes/) AND exp Infant, Premature, Diseases/OR infant, newborn/OR infant, low birth weight/OR infant, very low birth weight/OR infant, premature/OR (neonate: OR newborn: OR prematur*:.mp. OR newborn infant [age limit].

We ran the same search strategy in February 2010, March 2012, and in July 2013.

In addition, we performed manual searches of bibliographies and personal files, with no language restrictions. We handsearched abstracts published from the Pediatric Academic Societies' Meetings and the European Society of Pediatric Research Meetings (published in *Pediatric Research* or electronically) from 1980 to April 2006.

In July 2013, we conducted electronic searches of abstracts from the Annual Meetings of the Pediatric Academic Societies at Abstracts2View™ from 2000 to 2013. Additional searches in July 2013 included clinical trials registries (www.clinicaltrials.gov; www.controlled-trials.com; and who.int/ictrp).

Appendix 3. Risk of bias tool

For the 2012 and 2013 updates, one review author (AO) evaluated the following issues and entered them into the 'Risk of bias' table, and in 2013 and in 2018, the other review author (SA) checked the information.

We used the standard methods of Cochrane and Cochrane Neonatal to assess the methodological quality of the trials. For each trial, we sought information regarding the method of randomisation, blinding and reporting of all outcomes of all the infants enrolled in the trial. We assessed each criterion as being at a low, high, or unclear risk of bias. Two review authors separately assessed each study. Disagreements were resolved by discussion. We added this information to the table [Characteristics of included studies](#). We evaluated the following issues and entered the findings into the 'risk of bias' table:

1. Sequence generation (checking for possible selection bias). Was the allocation sequence adequately generated?

For each included study, we categorised the method used to generate the allocation sequence as:

- low risk (any truly random process e.g. random number table; computer random number generator);
- high risk (any non-random process e.g. odd or even date of birth; hospital or clinic record number); or
- unclear risk.

2. Allocation concealment (checking for possible selection bias). Was allocation adequately concealed?

For each included study, we categorised the method used to conceal the allocation sequence as:

- low risk (e.g. telephone or central randomisation; consecutively numbered sealed opaque envelopes);
- high risk (open random allocation; unsealed or non-opaque envelopes, alternation; date of birth); or
- unclear risk

3. Blinding of participants and personnel (checking for possible performance bias). Was knowledge of the allocated intervention adequately prevented during the study?

For each included study, we categorised the methods used to blind study participants and personnel from knowledge of which intervention a participant received. Blinding was assessed separately for different outcomes or class of outcomes. We categorised the methods as:

- low risk, high risk, or unclear risk for participants; and
- low risk, high risk, or unclear risk for personnel.

4. Blinding of outcome assessment (checking for possible detection bias). Was knowledge of the allocated intervention adequately prevented at the time of outcome assessment?

For each included study, we categorised the methods used to blind outcome assessment. Blinding was assessed separately for different outcomes or class of outcomes. We categorised the methods as:

- low risk for outcome assessors;
- high risk for outcome assessors; or
- unclear risk for outcome assessors.

5. Incomplete outcome data (checking for possible attrition bias through withdrawals, dropouts, protocol deviations). Were incomplete outcome data adequately addressed?

For each included study and for each outcome, we described the completeness of data including attrition and exclusions from the analysis. We noted whether attrition and exclusions were reported, the numbers included in the analysis at each stage (compared with the total randomised participants), reasons for attrition or exclusion, where reported, and whether missing data were balanced across groups or were related to outcomes. Where sufficient information was reported or supplied by the trial authors, we re-included missing data in the analyses. We categorised the methods as:

- low risk (< 20% missing data);
- high risk ($\geq$ 20% missing data); or
- unclear risk.

6. Selective reporting bias. Are reports of the study free of suggestion of selective outcome reporting?

For each included study, we described how we investigated the possibility of selective outcome reporting bias and what we found. For studies in which study protocols were published in advance, we compared prespecified outcomes versus outcomes eventually reported in the published results. If the study protocol was not published in advance, we contacted study authors to gain access to the study protocol. We assessed the methods as:

- low risk (where it is clear that all of the study's prespecified outcomes and all expected outcomes of interest to the review have been reported);
- high risk (where not all the study's prespecified outcomes have been reported; one or more reported primary outcomes were not prespecified outcomes of interest and are reported incompletely and so cannot be used; study fails to include results of a key outcome that would have been expected to have been reported); or
- unclear risk.

7. Other sources of bias. Was the study apparently free of other problems that could put it at a high risk of bias?

For each included study, we described any important concerns we had about other possible sources of bias (for example, whether there was a potential source of bias related to the specific study design or whether the trial was stopped early due to some data-dependent process). We assessed whether each study was free of other problems that could put it at risk of bias as:

- low risk;
- high risk;
- unclear risk.

If needed, we explored the impact of the level of bias through undertaking sensitivity analyses.

FEEDBACK

Feedback from Dr. R. Ohls, 27 March 2013

Summary

Our inclusion of the study by Dr. Romagnoli and co-workers (Romagnoli 2000) in the early EPO review was questioned by Dr. Robin Ohls, who suggested the study should be included in the late EPO review. We contacted Dr. Romagnoli and he informed us that the mean ($\pm$ SD) age of the infants when EPO treatment was started was 10 ± 1 days. We therefore moved the study to this late EPO review.

Reply

As expected, when the study by Romagnoli 2000 ($n = 230$) was added, the results of the meta-analyses changed. The outcome of BPD (defined as need for supplemental oxygen at 28 days of life) was now of borderline statistical significance with an increased risk in the EPO group. Because of the very high heterogeneity ($I^2 = 97\%$), the results should be interpreted with caution.

Our decision to divide the EPO studies into early and late based on initiating EPO treatment at the cut-off of ≤ 7 days of age for early and > 7 days for late treatment with EPO, although somewhat arbitrary, was chosen based on previously published meta-analyses (Garcia 2002; Kotto-Kome 2004) to allow us to compare the results between our reviews and previously published reviews.

In this update, there was a trend towards a non-significant increased risk of retinopathy (ROP) of prematurity.

The concern for an increased risk of ROP is real and because of the arbitrary cut-off age for early versus late EPO treatment, we decided post hoc to perform a secondary analysis including all studies that reported on ROP stage ≥ 3 regardless of the age at initiation of EPO treatment. This analysis early EPO review and showed an increased risk of ROP ≥ 3 when all studies of EPO regardless of age at initiation of the treatment were included in the analysis.

Contributors

Dr. R. Ohls

WHAT'S NEW

Date	Event	Description
26 September 2018	New citation required but conclusions have not changed	One additional study of small sample size was included in this update
26 September 2018	New search has been performed	New literature searches were run and data from one additional study were added to the review

HISTORY

Protocol first published: Issue 3, 2004

Review first published: Issue 3, 2006

Date	Event	Description
5 July 2013	New search has been performed	We searched the literature on July 1st, 2013, and identified no new trials for inclusion. However, one trial was moved from the review of <i>Early erythropoietin for preventing red blood cell transfusion in preterm and/or low birth weight infants</i> (Ohlsson 2012) to this review of later EPO treatment (see above).
5 July 2013	New citation required and conclusions have changed	This update was initiated following feedback from Dr. Robin Ohls. Our inclusion of the study by Dr. Romagnoli and co-workers (Romagnoli 2000) in the early EPO review was questioned by Dr. Robin Ohls, who suggested the study should be included in the late EPO review. We contacted Dr. Romagnoli and he informed us that the mean ($\pm$ SD) age of the infants when EPO

(Continued)

		treatment was started was 10 ± 1 days. We therefore moved the study to this Late EPO review As expected, when this study ($n = 230$) was added the results of the meta-analyses changed. The outcome of BPD (defined as need for supplemental oxygen at 28 days of life) is now of borderline statistical significance with an increased risk in the EPO group. Because of the very high heterogeneity ($I^2 = 97\%$) the results should be interpreted with caution Our decision to divide the EPO studies into early and late based on initiating EPO treatment at the cut-off of ≤ 7 days of age for early and > 7 days for late treatment with EPO, although somewhat arbitrary, was chosen based on previously published meta-analyses (Garcia 2002; Kotto-Kome 2004) to allow us to compare the results between our reviews and previously published reviews In this update there is a trend towards a non-significant increased risk of retinopathy (ROP) of prematurity The concern for an increased risk of ROP is real and because of the arbitrary cut-off age for early vs late EPO treatment we decided post hoc to perform a secondary analysis including all studies that reported on ROP stage ≥ 3 regardless of the age at initiation of EPO treatment. This analysis is included in the updated Early EPO review and shows an increased risk of ROP ≥ 3 when all studies of EPO regardless of age at initiation of the treatment were included in the analysis
10 May 2012	New citation required and conclusions have changed	The updated searches identified one additional trial for inclusion (20 additional enrolled infants) and identified some for exclusion With the inclusion of this trial there was no longer a significant reduction in the total volume (mL/kg) of blood transfused per infant
10 May 2012	New search has been performed	This updates the existing review "Late erythropoietin for preventing red blood cell transfusion in preterm and/or low birth weight infants" published in the Cochrane Database of Systematic Reviews (Aher 2012b) and updated in February 2010.
26 February 2010	New search has been performed	This review updates the existing review "Late erythropoietin for preventing red blood cell transfusion in preterm and/or low birth weight infants" published in the Cochrane Database of Systematic Reviews (Aher 2006b). Updated search found no new trials.

(Continued)

		No changes to conclusions.
25 September 2008	Amended	Converted to new review format.

CONTRIBUTIONS OF AUTHORS

Sanjay M Aher (SA) and Arne Ohlsson (AO) contributed equally to all sections of the protocol for this review. The literature search of databases was conducted with the help of an experienced librarian. Both review authors identified potentially eligible studies from the printouts and agreed on which trials to include; designed and agreed upon data collection forms; conducted quality assessments; and abstracted and compared data independently. One review author (AO) entered the data into RevMan 5 and the other review author (SA) checked for the data for accuracy. One review author (AO) wrote the full review and the other review author (SA) read and made changes. Both review authors made changes following feedback from the editors of the review group.

Sanjay M Aher (SA) and Arne Ohlsson (AO) wrote the original review.

The February 2010 update was conducted centrally by the Cochrane Neonatal Review Group staff (Yolanda Montagne, Diane Haughton, and Roger Soll). That update was reviewed and approved by AO, who completed the 'Risk of bias' tables.

The 2012 update was conducted by AO and approved by SA.

The update in 2013 was conducted by both authors (SA and AO).

The current update in 2018 was conducted by both authors (SA and AO).

DECLARATIONS OF INTEREST

None

SOURCES OF SUPPORT

Internal sources

- Mount Sinai Hospital, Toronto, Canada.

External sources

- Vermont Oxford Network, USA.

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DIFFERENCES BETWEEN PROTOCOL AND REVIEW

In our objectives, we stated that neonates enrolled between eight and 28 days of age were to be included. In the studies that we identified, some infants were less than 8 days at study entry and some were older than 28 days. When most of the enrolled infants were 8 days or older, we included the studies in this late EPO review and not in the early EPO review. The inclusion of these studies makes our review more comprehensive. The age at enrolment is stated for each study in the table [Characteristics of included studies](#). Hypertension was frequently reported by trial authors, and therefore it has been added as an outcome since the protocol was published.

We have made the following changes for this 2018 update:

- We changed the title from “Late erythropoietin for preventing red blood cell transfusion in preterm and/or low BW infants” to the current title in order to expand the interventions to include other erythropoiesis-stimulating agents.
- We added the methodology and plan for ‘Summary of findings’ tables and GRADE recommendations, which were not included in the original protocol, nor in previous versions of the review.

INDEX TERMS

Medical Subject Headings (MeSH)

Age Factors; Anemia, Neonatal [*prevention & control]; Bronchopulmonary Dysplasia [etiology]; Cause of Death; Drug Administration Schedule; Erythrocyte Transfusion [*statistics & numerical data]; Erythropoietin [*administration & dosage; blood]; Hematinics [*administration & dosage]; Hospital Mortality; Infant, Low Birth Weight [*blood]; Infant, Premature [*blood]; Randomized Controlled Trials as Topic; Retinopathy of Prematurity [etiology]; Time Factors

MeSH check words

Humans; Infant, Newborn