Neonatology

Meta-Analysis

Neonatology 2023;120:566–576
DOI: 10.1159/000530126

Received: October 31, 2022
Accepted: March 7, 2023
Published online: June 27, 2023

Effect of Early Erythropoietin on Retinopathy of Prematurity: A Stratified Meta-Analysis

Hendrik S. Fischer Nora J. Reibel Christoph Bührer Christof Dame

Department of Neonatology, Charité - Universitätsmedizin Berlin, Berlin, Germany

Keywords
Erythropoietin · Meta-analysis · Retinopathy of prematurity · Anemia of prematurity

Abstract

Background: Recombinant human erythropoietin (rhEPO) lost its role in minimizing red blood cell transfusion in very preterm infants after it had been associated with severe retinopathy of prematurity (ROP). Previous systematic reviews did not stratify ROP by gestation and birth weight (BW). ***Objectives:*** The aim of this study was to investigate the effect of early prophylactic rhEPO on ROP in a stratified meta-analysis of randomized controlled trials (RCTs). ***Methods:*** The databases EMBASE, MEDLINE, and the Cochrane Central Register of Controlled Trials were searched in January 2022 and complemented by citation searching. RCTs comparing early rhEPO treatment with no treatment or placebo were selected if they were published in a peer-reviewed journal and reported ROP outcomes. Previously unpublished data were requested from the study authors to allow stratified analyses by gestational age (GA) and BW. Data were extracted and analyzed using the standard methods of the Cochrane Neonatal Review Group. Prespecified outcomes were "ROP stage ≥3" (primary outcome) and "any ROP." ***Results:*** Fourteen RCTs, comprising 2,040 infants of <29 weeks of GA, were included for meta-analysis. Data syntheses showed no effects of rhEPO on ROP stage ≥3 or on any ROP, neither in infants of <29 weeks GA, nor in infants of <1,000 g BW, nor in any GA strata. The risk ratio (95% confidence interval) for ROP stage ≥3 in infants of <29 weeks of GA was 1.13 (0.84, 1.53), $p = 0.41$ (quality of evidence: moderate). ***Conclusions:*** The present meta-analysis detected no effects of early rhEPO on ROP in any comparison, but most stratified analyses were limited by low statistical power.

Introduction

Prevention and treatment of the anemia of prematurity are major challenges in neonatal intensive care. Over the past 30 years, clinical and translational research focused on three major strategies to tackle this problem: (1) reduction of diagnostic blood loss, (2) appropriate triggers for red blood cell (RBC) transfusion, and (3) supplemental treatment, in particular iron and recombinant human erythropoietin (rhEPO) [1]. Through the years, intervention studies of rhEPO were analyzed in a series of Cochrane reviews (2006–2020) [2–5]. Besides investigating the efficacy of rhEPO in reducing RBC transfusions (assessed by number or volume as well as donor exposure), one major objective of these analyses was to assess retinopathy of prematurity (ROP) as a secondary outcome measure.

karger@karger.com
www.karger.com/neo

Correspondence to:
Christof Dame, christof.dame@charite.de

Karger

This objective was justified by the biology of erythropoietin (EPO) and its receptor in the eye: during retinal development, EPO contributes to normal angiogenesis but may also lead to neovascularization and vascular leakage under ischemic or hypoxic conditions [6]. Retrospective cohort analyses suggested an association of rhEPO and risk of ROP with (1) the length (as total of 6 weeks) and (2) late initiation of treatment (after 20 days of age) [7, 8]. Both may have contributed to the concept of distinguishing the effect of early versus late initiation of rhEPO treatment in the series of Cochrane meta-analyses [2–5]. Uncertainty evolved when the initial Cochrane meta-analysis (2006) on early rhEPO use (initiation before 8 days after birth), its update in 2012, and finally a post hoc analysis in 2014, including all available studies regardless of age at initiation of rhEPO, reported an increased risk of ROP stage ≥3 [2, 3]. This led to the authors' conclusion that administration of rhEPO was not recommended [3]. Subsequently, the European Medicines Agency (EMA) and national regulatory authorities issued warnings that an increased risk of ROP due to rhEPO treatment could not be excluded. As suggested by a recent survey in European neonatal care units, these developments have caused a restrictive use of rhEPO in clinical routine, even in units that previously participated in randomized controlled trials (RCTs) showing beneficial effects of rhEPO on anemia of prematurity [9].

Subsequent updates of the Cochrane review published in 2017 and 2020, however, found no association between early rhEPO and the risk of ROP stage ≥3 [4, 5], in line with systematic reviews by others [10, 11] and recent RCTs [12, 13]. At first glance, the cumulative data appear to provide sufficient evidence against the hypothesis that treatment with rhEPO is associated with a risk of severe (stage ≥3) or any ROP. Upon a closer view, however, current analyses lack stratification of data on the inverse correlation of ROP with gestational age (GA) and birth weight (BW). In a recent study, the frequency of developing severe ROP in infants born at 24, 25, 26, 27, and 28 weeks was inversely correlated with 19.6%, 8.6%, 4.6%, 1.1%, and 0.8%, respectively [14]. Notably, the inclusion criteria for most meta-analyses were prematurity (<32/<37 weeks) and/or BW (<1,500 g/<2,500 g) [4, 5, 10, 11], which may lead to false conclusions concerning infants actually at risk.

To investigate whether in very preterm infants of <29 $^{0}/_{7}$ weeks of GA or in infants with a BW <1,000 g (P) early administration of rhEPO (I) versus placebo or no treatment (C) increases the risk of ROP (stage ≥3 or any stage) (O) and whether there are effects in specified GA groups, we performed a stratified meta-analysis using previously unpublished data from RCTs. Thus, this meta-analysis aimed to close a relevant gap in previous systematic reviews on the risk of ROP associated with early initiated rhEPO treatment.

Methods

Criteria for Considering Studies for This Meta-Analysis

RCTs that investigated the effect of early prophylactic rhEPO in preterm infants of <29 $^{0}/_{7}$ weeks of GA and/or <1,000 g BW were eligible. Treatment with rhEPO must have started before 8 days of age, regardless of dose, duration, and route of application. Included trials had to compare rhEPO administration with no treatment or placebo and had to report "any ROP" or "severe ROP" (defined as ROP stage ≥3) as outcomes. Only studies published in full in peer-reviewed journals were accepted. No language restrictions applied. The review protocol for the meta-analysis was registered on February 26, 2019, at the PROSPERO international prospective register of systematic reviews (registration CRD42019124053, available at https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42019124053). A revised protocol was submitted on February 24, 2023.

Types of Outcome Measures

The primary outcome was ROP stage ≥3. The incidence of any ROP was investigated as a secondary outcome.

Search Methods for Identification of Studies

The literature search complied with the standard methods of the Cochrane Collaboration [15]. Two authors (H.F. and C.D.) independently searched the databases EMBASE, MEDLINE, and the Cochrane Central Register of Controlled Trials (CENTRAL) and searched for citations in the reference sections of previous reviews and trials. The full search was last updated on January 06, 2022. The search strategy applied a variety of free-text and indexed search terms to identify eligible RCTs (search concept [premature infant OR retinopathy of prematurity] AND erythropoietin AND randomized controlled trial). The detailed search strategy is available online as Supplementary Information (for all online suppl. material, see www.karger.com/doi/10.1159/000530126).

Assessment of Methodological Quality

As per protocol, risk of selection bias, performance bias, detection bias, attrition bias, reporting bias, and other risks of bias were assessed by two study authors (H.F. and N.R.) and classified as low, high, or unclear risk. Any differences were resolved by a third author (C.D.).

Data Collection and Analysis

Two study authors (H.F., C.D.) worked independently of each other to select trials and extract data. At each stage, discrepancies were resolved by a third author (C.B.). As per protocol, we contacted the corresponding authors of all eligible RCTs to request stratified data for infants of <24, 24, 25, 26, 27, and 28 weeks of GA and for infants of <1,000 g BW. RevMan Version 5.4.1 and SPSS version 29.0.0 (for Egger's regression test) were used for data analysis. The effects of rhEPO on primary and secondary outcomes

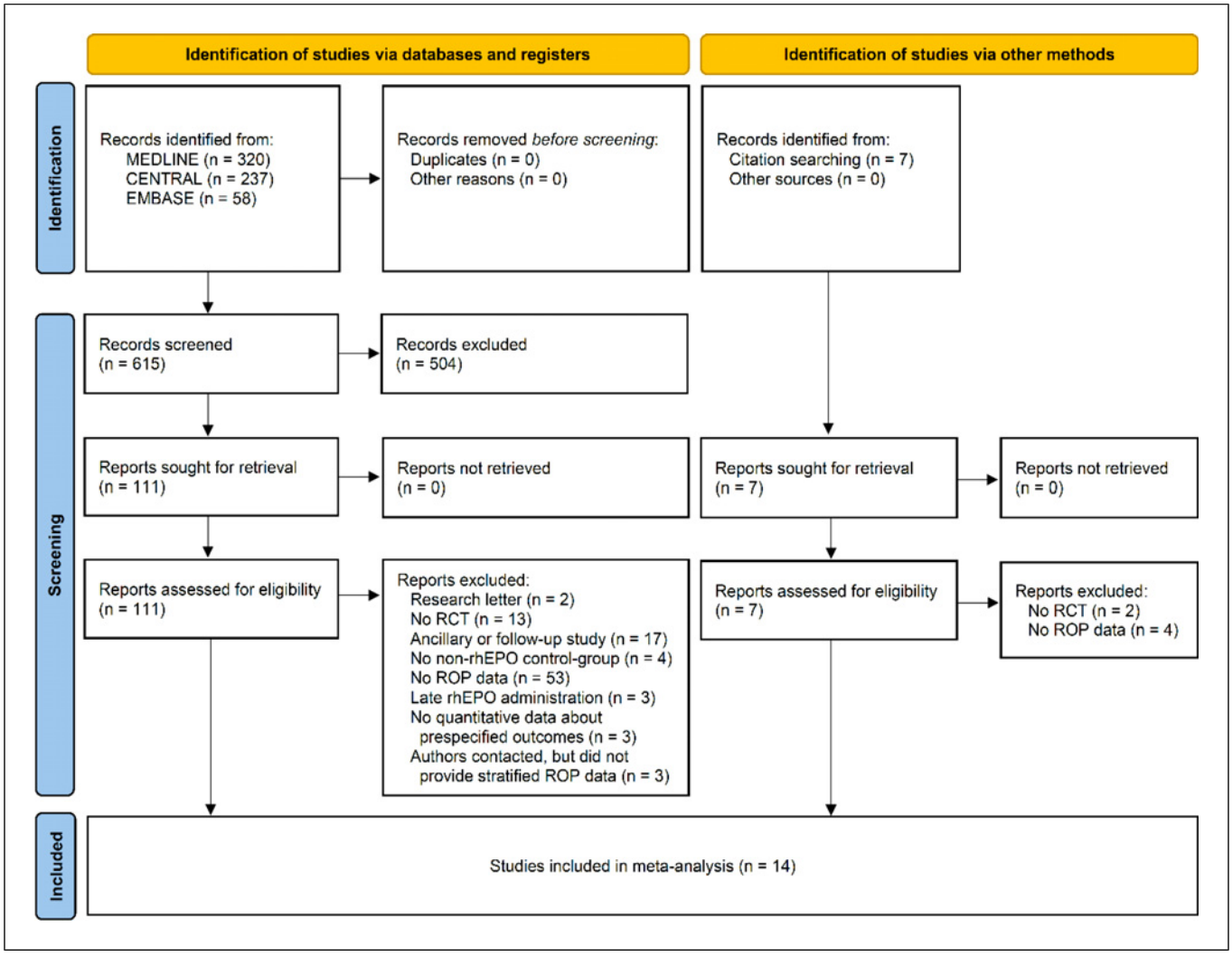

Fig. 1. Modified PRISMA 2020 flow diagram.

were calculated for infants <1,000 g BW, for infants of <29 weeks of GA, and separately for all GA strata, using the Mantel-Haenszel method for dichotomous outcomes in a random-effects model. Results were reported as risk ratios and 95% confidence intervals and displayed as forest plots. Statistical significance was defined as $p < 0.05$. Heterogeneity was estimated by using the χ^2 test and the I^2-value. To assess for publication bias, the treatment effects of rhEPO were plotted against standard error (log [odds ratio]) in funnel plots. The following non-pre-specified methods and analyses were added post hoc to enhance the understanding of the data: (1) Egger's regression test to assess for publication bias [16], (2) subgroup analyses of infants with 23–26 weeks of GA and 27–28 weeks of GA, (3) sensitivity analyses omitting all studies with a high risk of bias in at least one domain, (4) certainty of evidence assessment using the GRADE approach [17]. The meta-analysis was reported in accordance with the PRISMA statement [18] and accompanied by a PRISMA checklist.

Results

The search identified 622 records of interest (PRISMA flow diagram, Fig. 1). After screening titles and abstracts, 118 reports were retrieved for full-text assessments, including 21 reports published in languages other than English (Bulgarian, Chinese, French, Italian, Japanese, Polish, Russian, Spanish, Turkish).

Excluded Studies

After the full-text assessments, 104 of 118 reports were excluded (Fig. 1), including 3 RCTs that were potentially eligible for our meta-analysis, but the study authors either did not respond to our requests for stratified data or the study data were not available

Table 1. Characteristics of included studies

References	Year	*n* (all infants)	*n* (<29 weeks)	GA, BW	Time point of intervention	Intervention	Recruitment
Fauchère et al. [19]	2008	45	8[a]	24 $^{0}/_{7}$ to 31 $^{6}/_{7}$,[a] any BW	<3 h of age	rhEPO 3,000 IU/kg IV at 3–6, 12–18, and 36–42 h after birth	2005–2006
Fauchère et al. [20]	2015	443	198	26 $^{0}/_{7}$ to 31 $^{6}/_{7}$, any BW	<3 h of age	rhEPO 3,000 IU/kg IV at 3–6, 12–18, and 36–42 h after birth	2005–2012
Haiden et al. [21]	2005	40	36	≤32 $^{6}/_{7}$, ≤800 g	2nd day of life	rhEPO 300 IU/kg/d IV with IV access, followed by rhEPO 700 IU/kg SC 3×/week, until discharge or 40 weeks of PMA	2000–2002
Juul et al. [22]	2020	936	936	24 $^{0}/_{7}$–27 $^{6}/_{7}$, any BW	<24 h of age	rhEPO 1000 IU/kg IV every 48 h for a total of 6 doses, followed by maintenance dose of 400 U/kg SC 3×/week, until 32 weeks of PMA	2013–2016
Maier et al. [23]	1994	241	71	Any GA, 750–1,499 g	3rd d of life	rhEPO 250 IU/kg SC 3×/week, until day 42 (for a total of 17 doses)	1991–1992
Maier et al. [24]	2002	145	136	Any GA 500–999 g	3rd d of life	rhEPO 250 IU/kg IV 3×/week as long as intravenous access was available; thereafter, rhEPO 250 IU/kg SC, until day 65–68 of life (only early rhEPO and placebo groups included in meta-analysis)	1998–1999
Ohls et al. [25]	2001	290	166	≤32 $^{6}/_{7}$, 401–1,250 g	24–96 h of age	rhEPO 400 IU/kg IV 3×/week (or SC when IV access was not available), until discharge, transfer, death, or 35 weeks of PMA	1997–1998
Ohls et al. [26]	2013	102	50	Any GA 500–1,250 g	≤48 h of age	rhEPO 400 IU/kg SC 3×/week until discharge, transfer to another hospital, death, or 35 weeks PMA (darbepoetin group not included in meta-analysis)	2006–2010
Peltoniemi et al. [27]	2017	39	21	≤30 $^{0}/_{7}$, 700–1,500 g	1st d after birth	rhEPO 250 IU/kg/d IV for 6 consecutive days	1998–2000
Sharafutdinova et al. [28]	2019	133	28	26 $^{0}/_{7}$–33 $^{6}/_{7}$, <1,500 g	Groups 1 and 2: 3rd day of life	Group 1: rhEPO 200 IU/kg SC 3×/week, for 6 weeks Group 2: rhEPO 400 IU/kg SC 3×/week, for 6 weeks (only early rhEPO and control groups included in meta-analysis)	2017–2019
Song et al. [29]	2016	743	99	≤32 $^{6}/_{7}$, any BW	<72 h of age	rhEPO 500 IU/kg IV every other day, for 2 weeks	2009–2013
Wang et al. [30]	2020	1,285	308	≤32 $^{6}/_{7}$, any BW	<72 h of age	rhEPO 500 IU/kg IV every other day, for 2 weeks	2014–2017
Yang et al. [31]	2018	81	31	≤32 $^{6}/_{7}$, <1,500 g	<72 h of age	rhEPO 500 IU/kg IV every other day, for 2 weeks	2016–2017
Yeo et al. [32]	2001	100	42	≤32 $^{6}/_{7}$, <1,500 g	5th day of life	rhEPO 250 IU/kg SC 3×/week, until day 40	1997–2000

BW, birth weight; GA, gestational age; IV, intravenous(ly); IU, international units; PMA, postmenstrual age; rhEPO, recombinant human erythropoietin; SC, subcutaneous(ly). [a]From the 2008 study, we reported only on infants born at < 26 weeks of GA as infants of ≥26 weeks of GA were included in the 2015 study.

anymore. All reports and the respective reasons for exclusion are detailed in online supplementary Table 1.

Included Studies

Table 1 shows the characteristics of the 14 RCTs included in our meta-analysis [19–32]. Although prophylactic

intravenous or subcutaneous rhEPO treatment started within the first week of life in all included RCTs, the exact timing, dosing, and duration of rhEPO varied considerably. While one study group applied early high-dose rhEPO within the first 42 h only [19, 20], other RCTs applied lower initial rhEPO doses in combination with a more sustained treatment lasting for several weeks.

Risk of Bias in the Included Studies

Selection bias: As shown in online supplementary Figure 1, random sequence generation and allocation concealment were sometimes incompletely reported (unclear risk). Two studies lacked allocation concealment (high risk) [30, 31] Performance bias: In 6 RCTs, parents and personnel were unblinded to the study intervention (high risk) [21, 28–32]. Detection bias: The ophthalmologists performing the ROP assessments were not blinded in 4 RCTs (high risk) [21, 30–32]. Attrition bias: Discrepancies in follow-up between rhEPO and control groups with regard to the ROP screening were detected in one study (unclear risk) [19]. Reporting bias: Most RCTs did not have their study protocols registered or were only registered after enrollment of the patients (unclear risk). One study was registered 6 months after enrollment was completed, and there was a discrepancy between the primary outcomes registered and those reported in the publication (high risk) [29]. A detailed documentation of the risk of bias assessment is available online (online suppl. Table 2).

Visual inspection of the respective funnel plots of the effects of rhEPO on ROP stage ≥3 or on any ROP in preterm infants of <29 weeks of GA or <1,000 g BW did not reveal significant asymmetries in the distribution of effect sizes around the mean, indicating a low risk of publication bias (online suppl. Fig. 2–5). Egger's regression testing confirmed the visual assessments: The respective coefficients of the intercept were close to zero, and *p* values ranged between 0.27 and 0.99 (online suppl. Table 3).

Effects of Intervention

The summary of findings for the main comparisons and their GRADE assessment are shown in Table 2 (corresponding forest plots: Fig. 2, 3). rhEpo had no effects on ROP stage ≥3 in preterm infants of <29 weeks of GA (P = 0.41) or in preterm infants <1,000 g BW (P = 0.20). Because criteria for optimal information size were not met, the certainty of evidence was downgraded for imprecision (quality of evidence: moderate). rhEPO had no effects on any ROP in preterm infants of <29 weeks of GA (P = 0.41) or in preterm infants <1,000 g BW (P = 0.51), quality of evidence: high. Table 3 summarizes the ROP data stratified by GA (corresponding forest plots: online Suppl. Fig. 6–9). We detected no effect of rhEPO on ROP stage ≥3 or any ROP in any of the gestational strata. Overall, statistical measures did not indicate heterogeneity, with an I^2-value of 0% in all main analyses (Fig. 2, 3) and a maximum I^2-value of 28% in the stratified analyses (online suppl. Fig. 6–9). The χ^2 test for heterogeneity ranged between P = 0.22 and P = 0.97. Sensitivity analyses omitting studies with a high risk of bias yielded results similar to those based on the primary analyses (online suppl. Fig. 10, 11).

Discussion

For the first time, this meta-analysis reports the effect of early rhEPO treatment on the risk of ROP, stratified by GA or BW. This approach considers a priori (i) the inverse correlation of ROP with GA (<29 weeks) [14, 33, 34], (ii) the inverse correlation with BW (<1,000 g), and (iii) early initiation of rhEPO (<8 days after birth), implying that rhEPO treatment covers both phase 1 (cessation of retinal vessel formation and maturation) and phase 2 (neovascularization) of ROP when given for 6 weeks or longer [7, 8]. By considering previously unpublished stratified data from 14 RCTs, this meta-analysis complements recent systematic reviews by others [5, 10, 11, 13].

Quality of Evidence

The included RCTs differed in quality. While three studies featured a low risk of bias in all domains [20, 22, 26], six studies were considered to harbor a high risk of bias in up to three domains (online suppl. Fig. 1) [21, 28–32]. The issue was addressed by sensitivity analyses, omitting the latter studies (online suppl. Fig. 10, 11). Because the results of the sensitivity analyses were similar or near-identical to the main analyses, we did not downgrade the certainty of evidence for risk of bias.

There were some concerns about unpublished data as 52 rhEPO studies (mostly conducted before 2000) were excluded because they did not report any ROP data. However, funnel plots and Egger's regression test were not indicative of publication bias (online suppl. Fig. 2–5; online suppl. Table 3).

Effects of rhEPO on ROP

Our meta-analysis did not detect any association between rhEPO and ROP in any analysis, but the certainty of this evidence depended on each analysis' sample size

Table 2. Summary of findings for the main comparisons

Early prophylactic rhEPO compared to placebo or no treatment to prevent ROP in preterm infants						
Patient or population: Preterm infants of <29 weeks of GA or with a BW <1,000 g BW, as specified below under "outcomes" Setting: Neonatal intensive care unit Intervention: Early prophylactic rhEPO Comparison: Placebo or no treatment						
Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	Participants (studies), *n*	Certainty of the evidence (GRADE)	Comments
	risk with placebo or no treatment	risk with early prophylactic rhEPO				
ROP stage ≥3 in infants of <29 weeks of GA (*primary outcome*)	study population		**RR 1.13** (0.84–1.53)	2,018 (13 RCTs)	⊕⊕⊕◯ **Moderate**[a]	b,c,d,e
	76 per 1,000	**86** per **1,000** (64–116)				
ROP stage ≥3 in infants with <1,000 g BW (*primary outcome*)	Study population		**RR 1.22** (0.90–1.65)	1,437 (13 RCTs)	⊕⊕⊕◯ **Moderate**[a]	b,c,d,e
	96 per 1,000	**117** per **1,000** (86–158)				
Any ROP in infants of <29 weeks of GA	Study population		**RR 0.96** (0.88–1.05)	2,009 (13 RCTs)	⊕⊕⊕⊕ **High**	c,d,e,f,g
	445 per 1,000	**428** per **1,000** (392–468)				
Any ROP in infants with <1,000 g BW	Study population		**RR 0.97** (0.90–1.06)	1,433 (12 RCTs)	⊕⊕⊕⊕ **High**	c,d,e,f,g
	555 per 1,000	**539** per **1,000** (500–589)				
GRADE working group grades of evidence High certainty: We are very confident that the true effect lies close to that of the estimate of the effect Moderate certainty: 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 Low certainty: Our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect Very low certainty: We have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect						

BW, birth weight; CI, confidence interval; GA, gestational age; rhEPO, recombinant human erythropoietin; RCTs, randomized controlled trials; ROP, retinopathy of prematurity; RR, risk ratio. [a]Downgraded one level for imprecision: Aiming at an alpha of 0.05 and a power of 0.8 to detect a relative risk reduction or relative risk increase of 25%, the total sample size was below the optimal information size. The CIs of the estimated absolute and relative effects included the possibility of appreciable harm. [b]Risk of bias: a sensitivity analysis omitting all studies with a high risk of bias in at least one domain produced a similar RR (95% CI). [c]Inconsistency: acceptable methodological heterogeneity of the included studies; no statistical heterogeneity detected. [d]Indirectness of evidence: study data originated from RCTs conducted in the target population. [e]Publication bias: undetected (funnel plot and Egger's regression test did not indicate small study bias/publication bias). [f]Imprecision: optimal information size criterion was met. CI overlapped no effect (RR of 1.0). The narrow CI excluded the possibility of important benefit or harm. [g]Risk of bias: a sensitivity analysis omitting all studies with a high risk of bias in at least one domain produced an almost identical RR (95% CI). *The risk in the intervention group (and its 95% CI) is based on the assumed risk in the comparison group and the relative effect of the intervention (and its 95% CI).

Table 3. Stratified data per week of GA for the outcomes ROP stage ≥3 and any ROP

	ROP ≥3			Any ROP		
GA, weeks	studies	infants (%)	RR [95% CI]	studies	infants (%)	RR [95% CI]
23	4	3/17 (18)	4.17 [0.25–68.16]	4	9/17 (53)	1.07 [0.52–2.18]
24	7	55/257 (21)	1.22 [0.70–2.13]	7	211/257 (82)	1.01 [0.93–1.11]
25	10	44/318 (14)	1.13 [0.54–2.36]	11	210/321 (65)	1.05 [0.91–1.21]
26	11	24/394 (6)	1.41 [0.67–2.69]	12	201/398 (51)	1.08 [0.92–1.27]
27	12	21/529 (4)	0.94 [0.41–2.16]	12	156/529 (29)	0.95 [0.74–1.22]
28	11	15/503 (3)	0.95 [0.33–2.73]	11	78/487 (16)	0.75 [0.49–1.14]
23–26[a]	12	126/986 (13)	1.14 [0.76–1.70]	13	631/993 (64)	1.04 [0.96–1.13]
27–28[a]	12	36/1,032 (3)	0.83 [0.41–1.68]	12	234/1,016 (23)	0.88 [0.71–1.08]

CI, confidence interval; GA, gestational age; ROP, retinopathy of prematurity; RR, risk ratio. The number of studies contributing data; the number and percentage of infants with ROP ≥3 (left) or any ROP (right); RR to develop ROP ≥3 (left) or any ROP (right) when comparing rhEPO and control groups. [a]Exploratory analyses.

and event rate. Consequently, optimal information size for the primary outcome ROP stage ≥3 was not reached, and the quality of evidence downgraded to moderate, whereas the quality of evidence was high for the outcome "any ROP" (Table 2). Analyses in the gestational strata were mostly less informative with wide CIs due to limited sample size (Table 3). The wide publication period of the included RCTs (1994–2020) may explain a higher incidence of ROP stage ≥3 in some gestational strata in comparison to some but not all recent study populations [12, 14, 34]. This may reflect improved neonatal intensive care since the inverse correlation between ROP and GA was maintained [33, 34], whereas the lack of any association between rhEPO treatment and ROP was found in all strata.

The results of the present meta-analysis should be embedded in the context of previous work. In 2014, the authors of the Cochrane meta-analysis performed a *post hoc* analysis including all studies that reported on ROP stage ≥3 including early and late initiation of treatment, and there was an increased risk of ROP in the rhEPO group [3, 35]. Subsequently, Fang et al. [10] and Chou et al. [11] could not replicate this association in their meta-analyses on the effect of early or late rhEPO on ROP. Finally, the most recent Cochrane meta-analysis on early rhEPO (2020) investigated the risk of ROP ("all stages or stage not reported") and the risk of ROP stage ≥3, which included eight out of the 14 RCTs in our meta-analysis [20, 21, 23–27, 29]. There was no effect of rhEPO, neither on any ROP nor on ROP stage ≥3 [5]. In comparison to these four meta-analyses [3, 5, 11, 13], our meta-analysis not only included subsequently published RCTs [22, 28, 30, 31] but also benefited from direct communication with the corresponding authors. This allowed us to clarify questions of study design, to prevent overlaps in study populations, and to get further stratified data [19–32].

It should be noted that the authors of three included RCTs separately analyzed their data on ROP [22, 29, 30]. While the Preterm Erythropoietin Neuroprotection (PENUT) trial exclusively assessed infants with <1,000 g BW and <28 weeks of GA [12], the combined analysis of two RCTs from Zhengzhou University, China, also examined the effect of early rhEPO treatment on the risk of ROP stratified by GA (28–29 $^{6}/_{7}$ weeks vs. 30–31 $^{6}/_{7}$ weeks) or BW (1,000 g to 1,499 g vs. ≥1,500 g). Although this analysis is beyond our objective, it seems worth noting that early rhEPO treatment significantly reduced the risk of ROP stage 2 in infants with 28–29 $^{6}/_{7}$ weeks of GA and a BW of 1,000–1,499 g [13].

Implications of the Meta-Analysis

To our knowledge, the present meta-analysis appears to be the most comprehensive systemic review of the effect of rhEPO on ROP in very preterm infants published so far. The use of previously unpublished stratified data from RCTs allowed us to focus on preterm infants at high risk of ROP. As the meta-analysis did not indicate any association between rhEPO and ROP stage ≥3 or any ROP, this should have implications on the status of rhEPO in neonatology: First, it is time to withdraw warnings on the use of rhEPO in preterm infants. Second, early initiation, volume, and frequency of RBC transfusions are major risk factors for severe ROP and a poor neurodevelopmental outcome at 2 years of age [12, 36–38]. The significant reduction in multiple measures of rhEPO efficacy found by others (less transfused infants, less RBC transfusions, lower

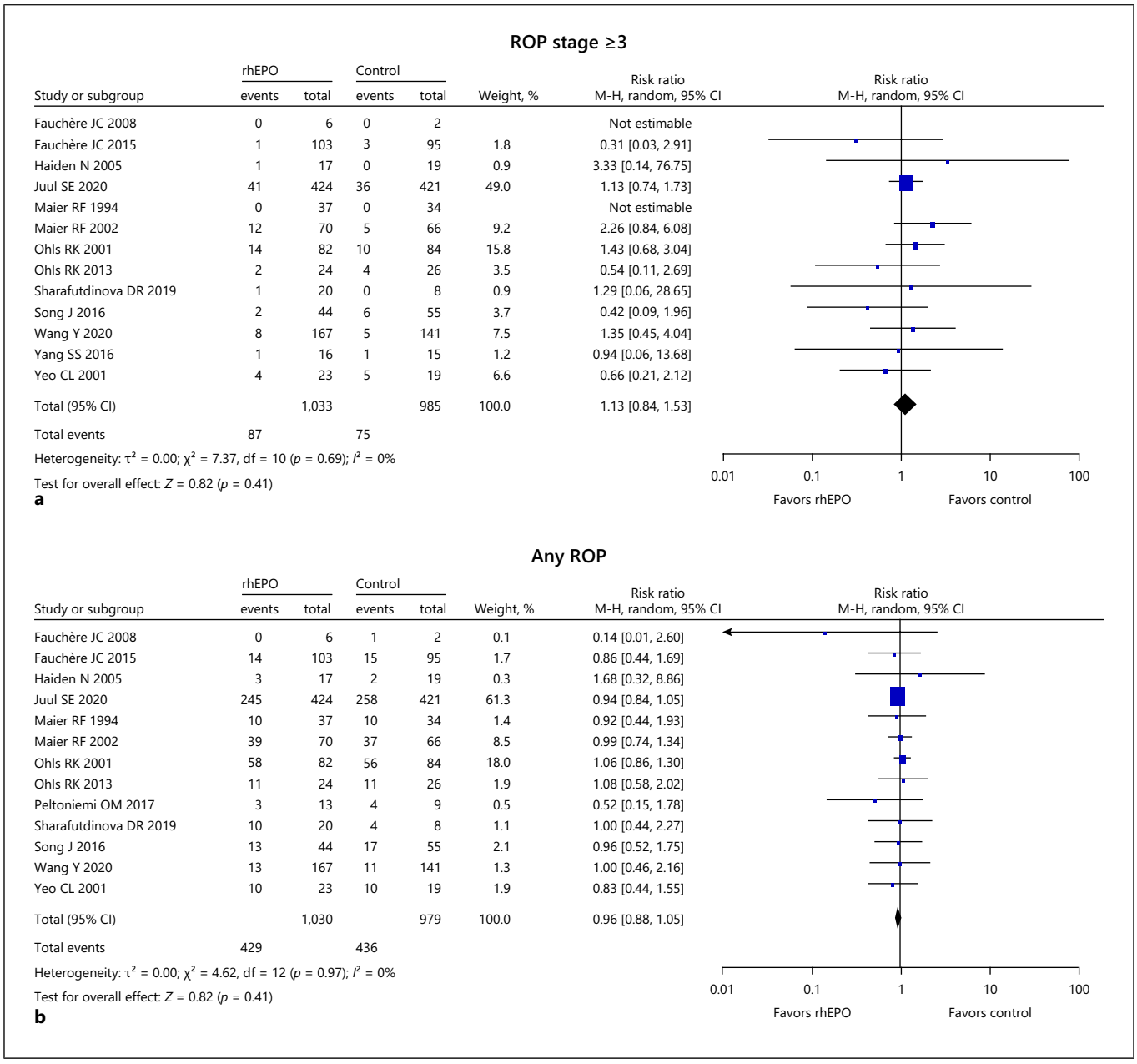

Fig. 2. Effects of rhEPO on the number of infants with ROP stage ≥3 (**a**) and any ROP (**b**), in infants of <29 $^{0}/_{7}$ weeks of GA.

transfusion volume) underlines potential benefits of rhEPO treatment [5, 13, 38]. Nevertheless, the most recent Cochrane meta-analysis (2020) recommended against rhEPO because of limited benefits as the number of transfusion per infant was only minimally reduced and the number of donors to whom transfused infants were exposed was not significantly reduced [5]. This interpretation of the data may not be universal, however, as these two measures depend on local transfusion policies (e.g., transfusion thresholds, volume per transfusion, use of satellite bags, storage time). Future research should focus on strategies optimizing the use of rhEPO or its analogues (e.g., darbepoetin) for patient blood management. This applies especially to dosage, frequency, and route of administration in preterm newborn infants, taking into consideration the specific pharmacokinetics of this group of patients [39]. Furthermore, appropriate iron supplementation should go

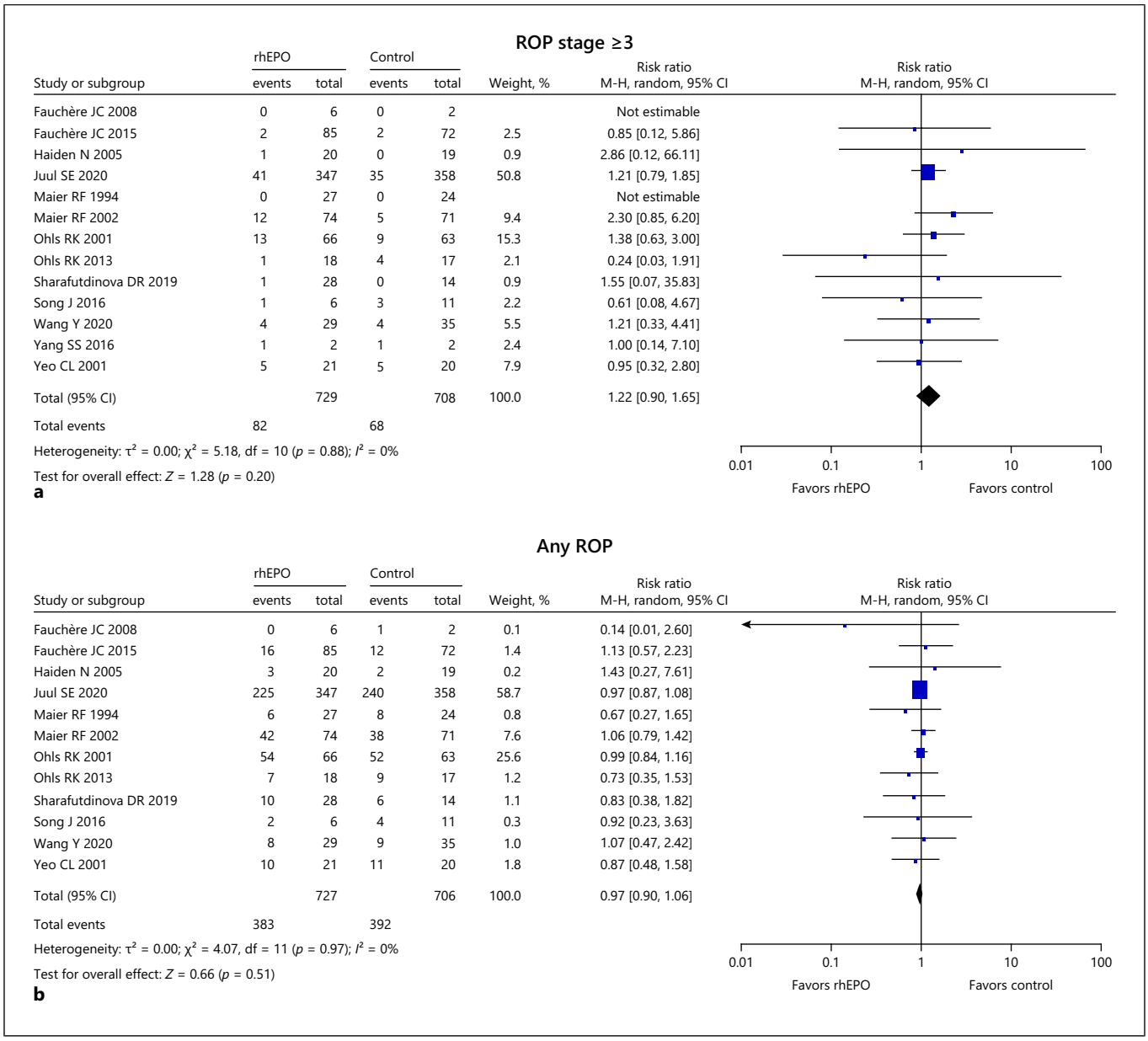

Fig. 3. Effects of rhEPO on the number of infants with ROP stage ≥3 (**a**) and any ROP (**b**), in infants with a BW <1,000 g.

alongside rhEPO administration. Finally, the question of using rhEPO after an early RBC transfusion should be addressed.

Third, other outcome measures of early rhEPO should also be considered. Although the most recent Cochrane analysis argues in favor of rhEPO based on reduced risks of intraventricular hemorrhage, periventricular leukomalacia, and necrotizing enterocolitis [5], data from subsequent studies including the PENUT [12, 22] trial are required to confirm such effects.

Limitations

Despite its unique concept and the implementation of previously unpublished stratified data, this meta-analysis was limited by the risk of imprecision. Aiming at an alpha of 0.05 and a power of 0.8 to detect a relative risk reduction or relative risk increase of 25%, the primary outcome and most stratified analyses failed to achieve optimal information size due to limited sample size and event rate. Therefore, the risk of committing a type II error was high in gestational strata with small sample

sizes, and the wide confidence interval in these analyses indicates the lack of power to exclude the possibility of appreciable harm. Small risks of publication bias or other bias in individual RCTs were minor issues, as already discussed above. Moreover, the design of the 14 RCTs included in this meta-analysis varied significantly, in particular concerning (1) timing, (2) dosing, (3) route of application, and (4) duration of rhEPO treatment (Table 1). The lack of any photographic documentation of the retinal vascularization could be another issue in almost all studies because it does not allow assessing and grading of the findings by independent investigators [40].

Conclusion

In conclusion, this stratified meta-analysis found no association between early prophylactic rhEPO treatment and ROP stage ≥3 (quality of evidence: moderate) or any ROP (quality of evidence: high) in infants of <29 weeks of GA or in infants with <1,000 g BW, even though these infants are at high risk of developing ROP. Besides, we detected no effects of rhEPO in any of the gestational strata, but most of these stratified analyses were not powered to exclude significant effects.

Acknowledgments

First, we thank all the study authors who provided stratified data and additional information about their trials: Dr. Sandra Juul, Dr. Robin Ohls, and the NRN Protocol Support Team of the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), and Drs. Jean-Claude Fauchère, Nadja Haiden, Rolf Maier, Outi Peltoniemi, Diana Sharafutdinova, Fa-Lin Xu, Cheo Lian Yeo, Juan Song, and Changlian Zhu. Moreover, we are grateful to Brigitte M. Koller (University of Zurich) and Boris Metze (Charité – Universitätsmedizin Berlin) for their support in the data sharing process and to Drs. Virgilio Carnielli and Wai-Tim Jim for providing information about their studies. We thank Lukas Bomke, Judith Hollnagel, Zhentang Zhang, and Christina Dame-Paasch and Drs. Makoto Kashiwabara, Paulina Aleksander, Nino Zurebiani, and Banu Orak for translating articles published in Bulgarian, Chinese (J.H. and Z.Z.), Italian, Japanese, Polish, Russian, and Turkish, respectively.

Statement of Ethics

All included data were obtained from randomized controlled clinical trials published in peer-reviewed journals and generally complied with the Declaration of Helsinki. Ethical considerations are detailed in the source publications.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Funding Sources

There was no funding for this study.

Author Contributions

Hendrik Fischer designed the meta-analysis; searched for relevant studies; extracted, assessed, and analyzed the data; and drafted the manuscript. Nora Reibel contributed to data extraction and assessment and drafted the manuscript. Christoph Bührer contributed to data extraction and assessment. Christof Dame conceptualized the meta-analysis, searched for relevant studies, corresponded with the authors of the trials, extracted and assessed the data, and drafted the manuscript. All the authors approved the final version as submitted and agree to be accountable for all aspects of the work.

Data Availability Statement

All data analyzed during this study are included in this article and its online supplementary material files. Further inquiries can be directed to the corresponding author.

References

1 Patel RM, Meyer EK, Widness JA. Research opportunities to improve neonatal red blood cell transfusion. Transfus Med Rev. 2016;30(4):165–73.
2 Ohlsson A, Aher SM. Early erythropoietin for preventing red blood cell transfusion in preterm and/or low birth weight infants. Cochrane Database Syst Rev. 2006 Jul 19(3):CD004863.
3 Ohlsson A, Aher SM. Early erythropoietin for preventing red blood cell transfusion in preterm and/or low birth weight infants. Cochrane Database Syst Rev. 2014 Apr 26(4):CD004863.
4 Ohlsson A, Aher SM. Early erythropoiesis-stimulating agents in preterm or low birth weight infants. Cochrane Database Syst Rev. 2017 Nov 16;11:CD004863.
5 Ohlsson A, Aher SM. Early erythropoiesis-stimulating agents in preterm or low birth weight infants. Cochrane Database Syst Rev. 2020 Feb 11;2:CD004863.
6 Caprara C, Grimm C. From oxygen to erythropoietin: relevance of hypoxia for retinal development, health and disease. Prog Retin Eye Res. 2012 Jan;31(1):89–119.

7 Brown MS, Barón AE, France EK, Hamman RF. Association between higher cumulative doses of recombinant erythropoietin and risk for retinopathy of prematurity. J AAPOS. 2006 Apr;10(2):143–9.
8 Suk KK, Dunbar JA, Liu A, Daher NS, Leng CK, Leng JK, et al. Human recombinant erythropoietin and the incidence of retinopathy of prematurity: a multiple regression model. J AAPOS. 2008 Jun;12(3):233–8.
9 Bolte K, Maier RF. Survey on clinical use and non-use of recombinant human erythropoietin in European neonatal units. J Perinat Med. 2020 Sep 25;48(7):744–50.
10 Fang JL, Sorita A, Carey WA, Colby CE, Murad MH, Alahdab F. Interventions to prevent retinopathy of prematurity: a meta-analysis. Pediatrics. 2016 Apr;137(4):e20153387.
11 Chou HH, Chung MY, Zhou XG, Lin HC. Early erythropoietin administration does not increase the risk of retinopathy in preterm infants. Pediatr Neonatol. 2017 Feb;58(1):48–56.
12 Mayock DE, Xie Z, Comstock BA, Heagerty PJ, Juul SE; Preterm Epo Neuroprotection PENUT Trial Consortium. High-dose erythropoietin in extremely low gestational age neonates does not alter risk of retinopathy of prematurity. Neonatology. 2020;117(5):650–7.
13 Sun H, Song J, Kang W, Wang Y, Sun X, Zhou C, et al. Effect of early prophylactic low-dose recombinant human erythropoietin on retinopathy of prematurity in very preterm infants. J Transl Med. 2020;18(1):397.
14 Gerull R, Brauer V, Bassler D, Laubscher B, Pfister RE, Nelle M, et al. Incidence of retinopathy of prematurity (ROP) and ROP treatment in Switzerland 2006-2015: a population-based analysis. Arch Dis Child Fetal Neonatal Ed. 2018 Jul;103(4):F337–F342.
15 Higgins JPT, Green S. Cochrane handbook for systematic reviews of interventions. Version 5.1.0 [updated March 2011]. In: The Cochrane collaboration. 2011. Available from: www.handbook.cochrane.org.
16 Egger M, Smith GD, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ. 1997 Sep 13;315(7109):629–34.
17 Schünemann H, Brożek J, Guyatt G, Oxman A. Handbook for grading the quality of evidence and the strength of recommendations using the GRADE approach The GRADE Working Group; 2013. Updated October 2013.
18 Page MJ, Moher D, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. PRISMA 2020 explanation and elaboration: updated guidance and exemplars for reporting systematic reviews. BMJ. 2021 Mar 29;372:n160.
19 Fauchère JC, Dame C, Vonthein R, Koller B, Arri S, Wolf M, et al. An approach to using recombinant erythropoietin for neuroprotection in very preterm infants. Pediatrics. 2008 Aug;122(2):375–82.
20 Fauchère JC, Koller BM, Tschopp A, Dame C, Ruegger C, Bucher HU, et al. Safety of early high-dose recombinant erythropoietin for neuroprotection in very preterm infants. J Pediatr. 2015 Jul;167(1):52–7.e1–3.
21 Haiden N, Cardona F, Schwindt J, Berger A, Kuhle S, Homoncik M, et al. Changes in thrombopoiesis and platelet reactivity in extremely low birth weight infants undergoing erythropoietin therapy for treatment of anaemia of prematurity. Thromb Haemost. 2005 Jan;93(1):118–23.
22 Juul SE, Comstock BA, Wadhawan R, Mayock DE, Courtney SE, Robinson T, et al. A randomized trial of erythropoietin for neuroprotection in preterm infants. N Engl J Med. 2020;382(3):233–43.
23 Maier RF, Obladen M, Scigalla P, Linderkamp O, Duc G, Hieronimi G, et al. The effect of epoetin beta (recombinant human erythropoietin) on the need for transfusion in very-low-birth-weight infants. European Multicentre Erythropoietin Study Group. N Engl J Med. 1994 Apr 28;330(17):1173–8.
24 Maier RF, Obladen M, Muller-Hansen I, Kattner E, Merz U, Arlettaz R, et al. Early treatment with erythropoietin beta ameliorates anemia and reduces transfusion requirements in infants with birth weights below 1000 g. J Pediatr. 2002 Jul;141(1):8–15.
25 Ohls RK, Ehrenkranz RA, Wright LL, Lemons JA, Korones SB, Stoll BJ, et al. Effects of early erythropoietin therapy on the transfusion requirements of preterm infants below 1250 grams birth weight: a multicenter, randomized, controlled trial. Pediatrics. 2001 Oct;108(4):934–42.
26 Ohls RK, Christensen RD, Kamath-Rayne BD, Rosenberg A, Wiedmeier SE, Roohi M, et al. A randomized, masked, placebo-controlled study of darbepoetin alfa in preterm infants. Pediatrics. 2013 Jul;132(1):e119–27.
27 Peltoniemi OM, Anttila E, Kaukola T, Buonocore G, Hallman M. Randomized trial of early erythropoietin supplementation after preterm birth: iron metabolism and outcome. Early Hum Dev. 2017 Jun;109:44–9.
28 Sharafutdinova DR, Balashova EN, Ionov OV, Kirtbaya AR, Golubtsova JM, Zubkov VV, et al. The recombinant human erythropoietin therapy for extremely and very low birth weight infants. Voprosy gematologii/onkologii i immunopatologii v pediatrii. 2019;18(2):75–82.
29 Song J, Sun H, Xu F, Kang W, Gao L, Guo J, et al. Recombinant human erythropoietin improves neurological outcomes in very preterm infants. Ann Neurol. 2016 Jul;80(1):24–34.
30 Wang Y, Song J, Sun H, Xu F, Li K, Nie C, et al. Erythropoietin prevents necrotizing enterocolitis in very preterm infants: a randomized controlled trial. J Transl Med. 2020 Aug 8;18(1):308.
31 Yang SS, Xu FL, Cheng HQ, Xu HR, Yang L, Xing JY, et al. Effect of early application of recombinant human erythropoietin on white matter development in preterm infants. Zhongguo Dang Dai Er Ke Za Zhi. 2018 May;20(5):346–51.
32 Yeo CL, Choo S, Ho LY. Effect of recombinant human erythropoietin on transfusion needs in preterm infants. J Paediatr Child Health. 2001 Aug;37(4):352–8.
33 Stoll BJ, Hansen NI, Bell EF, Walsh MC, Carlo WA, Shankaran S, et al. Trends in care practices, morbidity, and mortality of extremely preterm neonates, 1993–2012. JAMA. 2015 Sep 08;314(10):1039–51.
34 Bell EF, Hintz SR, Hansen NI, Bann CM, Wyckoff MH, DeMauro SB, et al. Mortality, in-hospital morbidity, care practices, and 2-year outcomes for extremely preterm infants in the US, 2013-2018. JAMA. 2022 Jan 18;327(3):248–63.
35 Aher SM, Ohlsson A. Late erythropoietin for preventing red blood cell transfusion in preterm and/or low birth weight infants. Cochrane Database Syst Rev. 2014;Apr 23(4):CD004868.
36 Lust C, Vesoulis Z, Jackups R Jr, Liao S, Rao R, Mathur AM. Early red cell transfusion is associated with development of severe retinopathy of prematurity. J Perinatol. 2019 Mar;39(3):393–400.
37 Hengartner T, Adams M, Pfister RE, Snyers D, McDougall J, Waldvogel S, et al. Associations between red blood cell and platelet transfusions and retinopathy of prematurity. Neonatology. 2020 Dec 08;117(5):1–7.
38 Vu PT, Ohls RK, Mayock DE, German KR, Comstock BA, Heagerty PJ, et al. Transfusions and neurodevelopmental outcomes in extremely low gestation neonates enrolled in the PENUT Trial: a randomized clinical trial. Pediatr Res. 2021 07;90(1):109–16.
39 Langer J, Obladen M, Dame C. Urinary loss of erythropoietin after intravenous versus subcutaneous epoetin-beta in preterm infants. J Pediatr. 2008 May;152(5):728–30.
40 Salcone EM, Johnston S, Van der Veen D. Review of the use of digital imaging in retinopathy of prematurity screening. Semin Ophthalmol. 2010Sep–Nov;25(5–6):214–7.