



Erythropoietin Improves Poor Outcomes in Preterm Infants with Intraventricular Hemorrhage

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Abstract

Background Intraventricular hemorrhage (IVH) is a common complication in preterm infants that has poor outcomes, especially in severe cases, and there are currently no widely accepted effective treatments. Erythropoietin has been shown to be neuroprotective in neonatal brain injury.

Objective The objective of this study was to evaluate the protective effect of repeated low-dose recombinant human erythropoietin (rhEPO) in preterm infants with IVH.

Methods This was a single-blinded prospective randomized controlled trial. Preterm infants ≤ 32 weeks gestational age who were diagnosed with IVH within 72 h after birth were randomized to receive rhEPO 500 IU/kg or placebo (equivalent volume of saline) every other day for 2 weeks. The primary outcome was death or neurological disability assessed at 18 months of corrected age.

Results A total of 316 eligible infants were included in the study, with 157 in the rhEPO group and 159 in the placebo group. Although no significant differences in mortality ($p = 0.176$) or incidence of neurological disability ($p = 0.055$) separately at 18 months of corrected age were seen between the rhEPO and placebo groups, significantly fewer infants had poor outcomes (death and neurological disability) in the rhEPO group: 14.9 vs. 26.4%; odds ratio (OR) 0.398; 95% confidence interval (CI) 0.199–0.796; $p = 0.009$. In addition, the incidence of Mental Development Index scores of < 70 was lower in the rhEPO group than in the placebo group: 7.2 vs. 15.3%; OR 0.326; 95% CI 0.122–0.875; $p = 0.026$.

Conclusions Treatment with repeated low-dose rhEPO improved outcomes in preterm infants with IVH.

Trial Registration The study was retrospectively registered on ClinicalTrials.gov on 16 April 2019 (NCT03914690).

Juan Song and Yong Wang contributed equally to the manuscript.

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1 Introduction

Intraventricular hemorrhage (IVH) is one of the most common complications in preterm infants [1] and is associated with low birth weight and low gestational age [2–4]. IVH-induced brain injuries are caused not only by hematoma but also by neurotoxic compounds released from the hematoma [5]. Nearly 60% of very preterm infants with grade III–IV IVH develop severe neurodevelopmental outcomes [6, 7]. There are currently no specific treatments to prevent preterm infants with IVH from developing serious neurological disabilities [8]. However, a promising candidate is erythropoietin, which is commonly used for treating anemia in preterm infants [9].

Erythropoietin and its receptor are expressed in brain cells and play critical roles in promoting brain maturation, and recent studies showed that erythropoietin is a promising

Key Points

Erythropoietin is a promising neuroprotective drug for preterm infants, and treatment with repeated low-dose recombinant human erythropoietin (rhEPO) is well-tolerated.

Treatment with repeated low-dose rhEPO improved poor outcomes in preterm infants with intraventricular hemorrhage.

neuroprotective drug [10–15]. Animal experiments have shown that erythropoietin exerts its neuroprotective effects by reducing inflammation, oxidative stress, and apoptosis and by promoting the regeneration of neuronal stem cells and blood vessels [16–21]. Erythropoietin treatment had positive effects on short- and long-term neurological outcomes in a preclinical model of IVH [22], and some observational clinical studies also showed that erythropoietin treatment may improve neurodevelopmental outcomes in preterm infants with IVH [10, 23]. However, the use of erythropoietin remains controversial with regard to poor outcomes [9], and both the dose and the course of erythropoietin in preterm infants is uncertain [24, 25]. Many clinical follow-up studies on preterm infants primarily treated with low-dose recombinant human erythropoietin (rhEPO) for anemia of prematurity showed improved neurodevelopmental outcomes [10, 26–28]. Our previous study showed that prophylactic use of low-dose rhEPO 500 IU/kg decreased the incidence of IVH in preterm infants [24]. In this study, we aimed to assess whether treatment with repeated low-dose rhEPO 500 IU/kg improved poor outcomes, including mortality and neurological disability, in preterm infants with IVH.

2 Methods

2.1 Study Design and Participants

Between July 2014 and December 2017, preterm infants with a gestational age of ≤ 32 weeks who were diagnosed with IVH by cerebral ultrasound within 72 h after birth in the neonatal intensive care unit (NICU) of the Third Affiliated Hospital and Children's Hospital of Zhengzhou University were eligible for enrollment. IVH was classified as grade I–IV according to Papile et al. [29] as follows: grade I—blood in the periventricular germinal matrix regions or germinal matrix hemorrhage; grade II—blood within the lateral ventricular system without ventricular dilatation; grade III—blood acutely distending into the lateral ventricles; grade IV—blood within the ventricular system and parenchyma.

We excluded preterm infants with congenital cerebral malformation, chromosome disease, genetic metabolic disease, large venous thrombus, hypertension, or polycythemia and infants whose parents declined to participate or withdrew treatment. The study protocol was approved by the Ethics Committee of Zhengzhou University and Henan Medical Academy, and written informed consent was obtained from all parents. The study was retrospectively registered on ClinicalTrials.gov on 16 April 2019 (NCT03914690).

2.2 Sample Size

The sample size was calculated assuming an incidence of long-term neurological disability of 47% in very preterm infants [30] and that rhEPO treatment can reduce the incidence by 40%. Thus, 140 premature infants were needed in each group for a significance of 5% with 90% power, and a total of 308 infants were needed for a 10% loss to follow-up.

2.3 Randomization and Blinding

Each patient was randomly assigned to the rhEPO group or placebo group in a 1:1 ratio using a computer-based random number generator, and the group assignment for each eligible patient was concealed in a sealed envelope before the patients were included. The parents, neurologists, and examiners who performed the neurodevelopmental screening were blinded to the infants' group allocation. The neonatologists and nurses responsible for treatment in the NICU were not blinded.

2.4 Intervention

Infants in the rhEPO group received intravenous rhEPO 500 IU/kg immediately after diagnosis with IVH and then every other day for 2 weeks, whereas infants in the placebo group received an equivalent volume of saline. Infants in the two groups received the same nursing and treatment strategy except for rhEPO.

2.5 Data Collection

Cerebral ultrasound was performed through the anterior fontanelle within 72 h after birth and then performed once a week until the bleeding was stable (the absorption period). The day of IVH onset, degree, unilateral or bilateral IVH, and whether combined with periventricular leukomalacia (PVL) or lateral ventricular dilatation were recorded.

Baseline information were collected, including gestational age, birth weight, sex, delivery mode, Apgar score, mechanical ventilation, and maternal factors during the perinatal period. Complications during hospitalization such as retinopathy of prematurity (ROP) [31], necrotizing

enterocolitis (NEC) [32], and bronchopulmonary dysplasia (BPD) [33] were recorded. Infants who developed rhEPO-related side effects, including large venous thrombus, polycythemia, or hypertension, were immediately taken off the rhEPO treatment and given emergency therapy.

2.6 Follow-up and Outcomes

All subjects were followed-up every 3 months until they were aged 18 months of corrected age to undergo measurement of body weight and length and a standardized physical examination of the nervous system. Two experienced examiners evaluated the Mental Development Index (MDI) score at 18 months of corrected age using the Bayley Infant Development Scale (2nd edition). Deafness was defined as hearing loss that needed external hearing aids [34]. Blindness was defined as a best corrected visual acuity of < 0.05 or a sight radius $< 10^\circ$ [34]. Neurological disability was defined as surviving with one or more of the following complications: cerebral palsy, MDI score < 70 , blindness, or deafness [24]. The primary outcome was death or neurological disability at 18 months of corrected age.

2.7 Statistical Analysis

The data were analyzed using SPSS v23.0. We used Mann–Whitney U test to compare the quantitative data including gestational age, birth weight, Apgar score, and onset day of IVH between the two groups. We used the chi-squared test to compare the count data including sex, delivery mode, maternal and neonatal complications, and mortality and long-term neurological disability between the two groups. Multivariate logistic regression was performed to adjust for the effect of sociodemographic and perinatal risk factors on poor outcomes. Fisher's exact test and the Mantel–Haenszel test were used for subgroup analysis and interaction analysis.

3 Results

3.1 Characteristics of Preterm Infants

During the study period, a total of 939 preterm infants ≤ 32 weeks gestational age were admitted to the NICUs, and 370 were diagnosed with IVH within 72 h after birth. In total, 54 infants were excluded (five had polycythemia, one had Down syndrome, three had genetic metabolic diseases, and 45 declined to participate). A total of 316 eligible preterm infants were randomly divided into the placebo group (159 infants) or the rhEPO group (157 infants) (Fig. 1). The gestational age range was 25⁺⁴–32 weeks in the rhEPO group, and 25⁺⁵–32 weeks in the placebo group. The median age of

IVH onset was 2 days (quartile range 2–3). In the total group of 316 infants, the number of those with grade I, II, III, and IV IVH was 133 (42.1%), 162 (51.3%), 10 (3.2%), and 11 (3.2%), respectively, with 89 (28.2%) having unilateral IVH and 227 (71.8%) having bilateral IVH. There was no significant difference between the two groups at the beginning of the study ($p > 0.05$) (Table 1). In total, 42 infants were lost to follow-up, and 274 (86.7%) infants attended the assessment at 18 months of corrected age (Fig. 1).

3.2 Mortality and Neurological Outcomes at 18 Months of Corrected Age

A total of 249 survivors (124 in the placebo group and 125 in the rhEPO group) with IVH were followed up to 18 months of corrected age. During the study period, 16 infants in the placebo group died (11 died during hospitalization and five died during follow-up; six died of BPD and severe pneumonia, four died of severe pneumonia, three died of NEC, two died of sepsis, and one died of respiratory distress syndrome [RDS]) and nine in the rhEPO group died (four during hospitalization and five during follow up; three died of BPD and severe pneumonia, three died of severe pneumonia, two died of RDS, and one died of multiple organ failure). The median (quartile range) age of death was 57.5 (38.3–71.5) days in the placebo group and 40.0 (22.5–48.0) days in the rhEPO group, with no significant difference between the two groups ($p > 0.05$). Among the 16 infants who died in the placebo group, the incidence of IVH grade I, II, III, and IV was 5 (31.3%), 9 (56.3%), 1 (6.3%), and 1 (6.3%), respectively. Among the nine patients who died in the rhEPO group, the incidence of IVH grade I, II, III, and IV was 4 (44.4%), 4 (44.4%), 0, and 1 (11.1%), respectively.

The mortality and the incidence of neurological disability at 18 months of corrected age were not significantly different between the two groups ($p > 0.05$). However, significantly fewer infants had poor outcomes (death and neurological disability) in the rhEPO group (20/134 [14.9%]) than in the placebo group (37/140 [26.4%]) (relative risk [RR] 0.488; 95% confidence interval [CI] 0.267–0.895; $p = 0.019$). The incidence of MDI score < 70 was also significantly lower in the rhEPO group than in the placebo group (7.2 vs. 15.3%; RR 0.429; 95% CI 0.186–0.989; $p = 0.043$). There was no significant difference in the incidence of cerebral palsy ($p = 0.231$), deafness ($p = 0.566$), or blindness ($p = 0.154$) between the two groups (Table 2).

The sociodemographic and perinatal risk factors of preterm infants may affect their outcomes, and multivariate logistic regression was performed to adjust for these factors. Maternal education, family economic status, gestational age, birth weight, sex, premature rupture of membrane, mechanical ventilation > 7 days, maternal hypertension, placental abruption, caesarean section, severe asphyxia, grade III–IV

Fig. 1 Study flow. Schematic flowchart showing the number of participants and the procedure of assigning patients to recombinant human erythropoietin or placebo and follow-up to 18 months of corrected age. Preterm infants with grade I–IV intraventricular hemorrhage were enrolled in the study. *IVH* intraventricular hemorrhage, *rhEPO* recombinant human erythropoietin

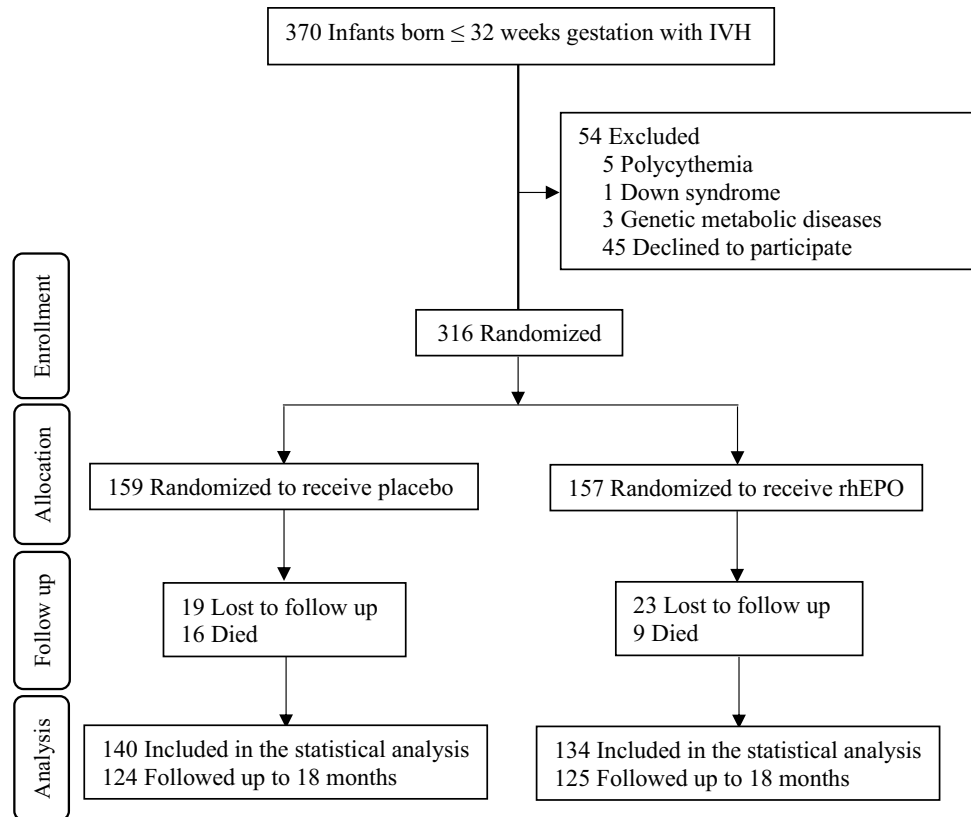


Table 1 Baseline information of preterm infants with intraventricular hemorrhage in the placebo and recombinant human erythropoietin groups

Characteristic	Placebo (<i>n</i> = 159)	rhEPO (<i>n</i> = 157)	<i>p</i> value
Gestational age, weeks	29.6 (28.6–30.6)	29.4 (28.4–30.3)	0.997
Birth weight, g	1250 (1050–1400)	1200 (1030–1350)	0.948
Male	96 (60.4)	88 (56.1)	0.436
SGA	30 (18.9)	37 (23.6)	0.307
Premature rupture of membrane	59 (37.1)	62 (39.5)	0.663
Maternal hypertension	32 (20.1)	25 (15.9)	0.331
Placental abruption	6 (3.8)	11 (7.0)	0.203
Caesarean section	80 (50.3)	91 (58.0)	0.173
5-Minute Apgar	8 (7–9)	8 (8–9)	0.688
IVH onset, days	2 (2–3)	2 (1–3)	0.951
Bilateral IVH	111 (69.8)	116 (73.9)	0.421
IVH (grade III–IV)	14 (8.8)	7 (4.5)	0.121
Post-hemorrhagic ventricular dilation	7 (4.4)	5 (3.2)	0.571
Early onset sepsis	18 (11.3)	16 (10.2)	0.856
Chorioamnionitis	26 (16.4)	29 (18.5)	0.658
Number of transfusions	2 (1–4)	2 (1–4)	0.249

Data are presented as *n* (%) or median (quartile range) unless otherwise indicated

The *p* value was calculated using Mann–Whitney U test or the chi-squared test to compare the placebo and rhEPO groups. *p* < 0.05 was considered significantly different

IVH intraventricular hemorrhage, *rhEPO* recombinant human erythropoietin, *SGA* small for gestational age

Table 2 Poor outcomes with intraventricular hemorrhage at 18 months of corrected age

Outcome	Placebo	rhEPO	RR (95% CI)	<i>p</i> value
Primary outcome				
Death	16/140 (11.4)	9/134 (6.7)	0.558 (0.238–1.310)	0.176
Disability	21/124 (16.9)	11/125 (8.8)	0.473 (0.218–1.029)	0.055
Death + disability	37/140 (26.4)	20/134 (14.9)	0.488 (0.267–0.895)	0.019
Secondary outcome ^a				
Cerebral palsy	8/124 (6.5)	4/125 (3.2)	0.479 (0.141–1.635)	0.231
MDI < 70	19/124 (15.3)	9/125 (7.2)	0.429 (0.186–0.989)	0.043
Deafness	1/124 (0.8)	2/125 (1.6)	2.000 (0.179–22.344)	0.566
Blindness	2/124 (1.6)	0/125 (0)	0.984 (0.962–1.006)	0.154

Data are presented as *n/N* (%) unless otherwise indicated. Disability is defined as surviving infants with one or more of the following complications: cerebral palsy, MDI < 70, blindness, or deafness. *p* < 0.05 was considered statistically significant

CI confidence interval, MDI Mental Developmental Index, rhEPO recombinant human erythropoietin, RR relative risk

^aOne infant with more than one disability could be counted repeatedly

IVH, PVL, post-hemorrhagic ventricular dilation, sepsis, respiratory distress, NEC, and BPD were included as covariates. For death or neurological disability, after adjusting for confounding factors (mechanical ventilation > 7 days, grade III–IV IVH, PVL, and BPD), infants in the rhEPO group had lower odds ratios (ORs) than those in the placebo group: 14.9 versus 26.4%; OR 0.398; 95% CI 0.199–0.796; *p* = 0.009. For MDI score < 70, after adjusting for confounding factors (grade III–IV IVH and PVL), infants in the rhEPO group also had lower ORs than those in the placebo group: 7.2 versus 15.3%; OR 0.326; 95% CI 0.122–0.875; *p* = 0.026) (Table 3).

3.3 Subgroup Analysis

Subgroup analysis was performed using gestational age (< 28, 28–29^{6/7}, and 30–32 weeks), birth weight (< 1000, 1000–1499, and ≥ 1500), sex (males and females), and the grade of IVH (I–II and III–IV). Treatment with rhEPO reduced the incidence of death or neurological disability in infants born at 30–32 weeks of gestational age (*p* < 0.05), in infants with a birth weight of 1000–1499 g (*p* < 0.05), in girls (*p* < 0.05), and in infants with grade I–II IVH (*p* < 0.05) (Table 4), and treatment with rhEPO reduced the incidence of MDI score < 70 in infants born at 28–29^{6/7} weeks of gestational age (*p* < 0.05) and infants with a birth

weight of 1000–1499 g (*p* < 0.05) (Table 5). However, interaction analysis showed that gestational age, birth weight, sex, and degree of IVH had no significant interaction effect on rhEPO treatments in terms of improving neurological outcomes and mortality (Table 4) and MDI score (Table 5) in preterm infants.

3.4 Safety

Compared with infants in the placebo group, those in the rhEPO group had a significantly lower incidence of BPD (85/159 [53.5%] vs. 60/157 [38.2%]; RR 0.539; 95% CI 0.344–0.843; *p* = 0.007). The incidence of ROP (13/159 [8.2%] vs. 9/157 [5.7%]; RR 0.683; 95% CI 0.283–1.647; *p* = 0.393) and NEC (11/159 [6.9%] vs. 9/157 [5.7%]; RR 0.818; 95% CI 0.329–2.032; *p* = 0.665) between the control group and the rhEPO group were not significantly different. No adverse events were observed during the study period.

4 Discussion

Preterm infants with IVH are susceptible to poor neurodevelopmental outcomes, including cerebral palsy, intellectual deficits, deafness, and blindness [35]. Although administration of antenatal corticosteroids and postnatal prophylactic

Table 3 Multivariate analysis for poor outcomes at 18 months of corrected age

Outcome	Placebo	rhEPO	<i>p</i> value	Odds ratio (95% CI)
Death + disability	37/140 (26.4)	20/134 (14.9)	0.009	0.398 (0.199–0.796)
MDI < 70	19/124 (15.3)	9/125 (7.2)	0.026	0.326 (0.122–0.875)

Data are presented as *n/N* (%) unless otherwise indicated. Poor outcomes are defined as infants with death or disability

CI confidence interval, MDI Mental Developmental Index, rhEPO recombinant human erythropoietin

Table 4 Subgroup interaction analysis for recombinant human erythropoietin on neurological disability or death

Subgroups	Placebo	rhEPO	RR (95% CI)	Interaction <i>p</i> value
Sex				
Male	23/84 (27.4)	14/78 (17.9)	0.580 (0.274–1.230)	0.465
Female	14/56 (25.0)	6/56 (10.7)*	0.360 (0.127–1.019)	
Total			0.488 (0.267–0.895)	
Gestational age				
< 28 weeks	6/19 (31.6)	5/21 (23.8)	0.677 (0.168–2.730)	0.694
28–29 ^{6/7} weeks	18/72 (25.0)	10/66 (15.2)	0.536 (0.227–1.264)	
30–32 weeks	13/49 (26.5)	5/47 (10.6)*	0.330 (0.107–1.014)	
Total			0.488 (0.267–0.895)	
Birth weight, g				
< 1000	7/18 (38.9)	6/19 (31.6)	0.725 (0.187–2.809)	0.683
1000–1499	24/102 (23.5)	12/96 (12.5)*	0.464 (0.217–0.991)	
≥ 1500	6/20 (30.0)	2/19 (10.5)	0.275 (0.048–1.579)	
Total			0.488 (0.267–0.895)	
Degree of IVH				
Grade I–II	30/126 (23.8)	17/128 (13.3)*	0.490 (0.255–0.943)	0.487
Grade III–IV	7/14 (50.0)	3/6 (50.0)	1.000 (0.148–6.772)	
Total			0.488 (0.267–0.895)	

Data are presented as *n/N* (%) unless otherwise indicated. The *p* value is for the interaction analysis in subgroups using the Mantel–Haenszel test. *p* < 0.05 was considered statistically significant

CI confidence interval, IVH intraventricular hemorrhage, rhEPO recombinant human erythropoietin, RR relative risk

**p* < 0.05 using Fisher's exact test to compare the placebo group and the rhEPO group

indomethacin is associated with a reduction in preterm infants with IVH [36, 37], no specific therapies or drugs are available for improving long-term neurological outcomes in preterm infants with IVH. In the current study, we showed that treatment with repeated low-dose rhEPO 500 IU/kg improved the incidence of poor outcomes in preterm infants with IVH.

Death and neurological disorders are poor outcomes for preterm infants [38], so we analyzed both in our study when evaluating whether rhEPO could improve poor outcomes in preterm infants with IVH. Multivariate analysis showed that treatment with rhEPO could reduce the incidence of death or neurological disability in preterm infants with IVH, which was consistent with findings from previous observational studies [10, 23]. Our previous study indicated that repeated low-dose rhEPO decreased the incidence of grade III–IV IVH in preterm infants [24], and this study added evidence that treatment with low-dose rhEPO could improve rates of death or the neurological outcomes of IVH. The incidence of death declined after treatment with rhEPO (11.4 vs. 6.7%) but without significant difference. One possible reason might be the small sample size (16 vs. 9 infant deaths). Another reason might be that some of the infants died from other diseases, such as NEC or sepsis, rather than IVH-induced brain

injury itself. In addition to the anti-inflammatory, anti-oxidative, and anti-apoptotic effects of rhEPO and its promotion of nerve and vascular regeneration [39], rhEPO might also play a role in alleviating IVH-induced secondary injury due to the release of free iron and cell-free hemoglobin [40–42]. An experimental study showed that iron supplementation aggravated white-matter injury in newborn mice [43], and a clinical study found that unbound iron was elevated in the cerebral spinal fluid of preterm infants with white-matter damage [44]. These studies indicated that free iron plays an important role in premature brain injury [45, 46]. Erythropoietin increases iron utilization and reduces free iron oxidative toxicity [47], which might play a key role in preventing free-iron-induced brain injury. Experimental studies have shown that cell-free hemoglobin is distributed in areas of periventricular white matter [41], which induces inflammation and cell death in the choroid plexus [47]. Treatment with rhEPO promotes erythropoiesis and reduces free hemoglobin levels, which can reduce the toxicity of free hemoglobin.

In the subgroup analysis, interaction analysis showed that gestational age, birth weight, sex, and degree of IVH had no significant interaction effect on the ability of rhEPO treatments to improve poor outcomes. This means that

Table 5 Subgroup interaction analyses for recombinant human erythropoietin on Mental Development Index < 70

Subgroups	Placebo	rhEPO	RR (95% CI)	Interaction <i>p</i> value
Sex				
Male	12/75 (16.0)	7/72 (9.7)	0.565 (0.209–1.528)	0.361
Female	7/49 (14.3)	2/53 (3.8)	0.235 (0.046–1.193)	
Total			0.429 (0.186–0.989)	
Gestational age				
< 28 weeks	2/15 (13.3)	3/19 (15.8)	1.219 (0.176–8.423)	0.366
28–29 ^{6/7} weeks	9/64 (14.1)	2/60 (3.3)*	0.211 (0.044–1.019)	
30–32 weeks	8/45 (17.8)	4/46 (8.7)	0.440 (0.123–1.583)	
Total			0.429 (0.186–0.989)	
Birth weight, g				
< 1000	1/12 (8.3)	2/15 (13.3)	1.692 (0.135–21.270)	0.470
1000–1499	14/93 (15.1)	5/91 (5.5)*	0.328 (0.113–0.952)	
≥ 1500	4/19 (21.1)	2/19 (10.5)	0.441 (0.070–2.761)	
Total			0.429 (0.186–0.989)	
Degree of IVH				
Grade I–II	14/112 (12.5)	7/120 (5.8)	0.434 (0.168–1.118)	0.516
Grade III–IV	5/12 (41.7)	2/5 (40.0)	0.933 (0.111–7.820)	
Total			0.429 (0.186–0.989)	

Data are presented as *n/N* (%) unless otherwise indicated. The *p* value is for the interaction analysis in subgroups using the Mantel–Haenszel test. *p* < 0.05 was considered statistically significant

CI confidence interval, IVH intraventricular hemorrhage, rhEPO recombinant human erythropoietin, RR relative risk

**p* < 0.05 using Fisher's exact test to compare the placebo and rhEPO groups

rhEPO treatment could improve poor outcomes in all preterm infants with gestational age ≤ 32 weeks. However, the preterm infants in our study had a large range of gestational ages, from 25⁺⁴ to 32 weeks, and fewer infants aged < 28 weeks (40/274 [14.6%] at < 28 weeks, 138/274 [50.4%] at 28–29⁺⁶ weeks, and 96/274 [35.0%] at 30–32 weeks), which might complicate the interpretation of the neuroprotective role of rhEPO in different subgroups, especially in extremely preterm infants [13, 48]. Therefore, we conducted a further study focusing on preterm infants born at < 28 weeks of gestational age (NCT02745990) to further explore the protective effect of rhEPO.

Sex differences have been noticed not only in adults but also in children, both in terms of the incidence of disease and therapeutic effects [49–51], and our previous animal studies showed sex differences in the immature brain after insult and treatment [52, 53]. These studies demonstrated that sex plays an important role in neuronal cell death and brain injury that is not related to sex hormone. Other studies further demonstrated that microglia activation and inflammatory responses are sex related [54–56]. Some studies showed that the effect of erythropoietin is even sex specific with regard to stimulating respiration in neonates [57] and regulating metabolism and hypothalamic inflammation [58]. In the current study,

we found that treatment with rhEPO reduced death and neurological disability more significantly in females than in males. However, interaction analysis showed the neuroprotective effect of rhEPO was not influenced by sex. This needs to be confirmed further with a greater number of infants.

Preterm infants of < 32 weeks gestational age are at high risk of ROP, and whether rhEPO, which has properties similar to those of vascular endothelial growth factor, is a risk factor for the development of ROP remains a concern [9, 59], even though some clinical studies showed the high-dose rhEPO or longer treatment durations did not increase the risk of ROP in extremely preterm infants [38, 60]. Our study showed that treatment with rhEPO did not increase the risk of ROP, which supports other studies that also reported that different dosages of rhEPO did not increase the incidence of ROP [59, 61, 62] in very low birth weight infants. Thus, again, rhEPO appears to be an effective and safe drug that does not increase ROP in preterm infants.

There are some limitations to this study. First, this trial was a follow-up study of the protective effect of rhEPO in terms of long-term outcomes in preterm infants with IVH born in 2014, which was retrospectively registered in 2019. Although this study was retrospectively registered, our group has been engaged in studies of this area since 2009

(NCT02036073, NCT03919500), and we found that repeated low-dose rhEPO was neuroprotective and safe for preterm infants. Second, the sample sizes of preterm infants with severe IVH (20/274 [7.3%]) and < 28 weeks of gestational age (40/274 [14.6%]) were small. Considering that preterm infants with severe IVH and < 28 weeks of gestational age are more likely to develop poor outcomes, further study focusing on extremely preterm infants with low-dose rhEPO for a long treatment duration is needed, even though recent studies with extremely preterm infants did not show that rhEPO is neuroprotective [60]. Third, the follow-up period to 18 months of corrected age was not long enough. Even though there was nothing abnormal in the imaging examination after the absorption of the hematoma, these patients probably experienced some neurological or psychological complications [63]. Such complications might not appear until school age or even later [64, 65], so longer follow-up to allow comprehensive assessments of neurodevelopment would be valuable [66].

5 Conclusions

This study showed that treatment with repeated low-dose rhEPO improved poor outcomes (mortality or neurodevelopmental outcomes) in preterm infants with IVH. Therefore, rhEPO might be a useful therapeutic option for improving long-term outcomes in preterm infants with IVH.

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Declarations

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Conflicts of interest Juan Song, Yong Wang, Falin Xu, Huiqing Sun, Xiaoli Zhang, Lei Xia, Shan Zhang, Kenan Li, Xirui Peng, Bingbing Li, Yaodong Zhang, Wenqing Kang, Xiaoyang Wang, and Changlian Zhu have no conflicts of interest that are directly relevant to the content of this article.

Ethics approval The study protocol was approved by the Ethics Committee of Zhengzhou University and Henan Medical Academy and was carried out in accordance with the Helsinki declaration.

Informed consent Written informed consent was obtained from all parents included in the study prior to any study-related activities.

Consent for publication All authors consent to the publication of the manuscript and accept the terms of related agreements.

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Data availability statement The datasets generated and/or analyzed during the current trial are available from the corresponding author on reasonable request.

Author contributions Study concept and design: CZ. Data acquisition: JS, YW, FX, HS, XZ, LX, SZ, KL, XP, BL, and YZ. Data analysis: JS and YW. Drafting the manuscript: JS and YW. Reviewing the manuscript: WK, XW, and CZ. All authors approved the final version of the manuscript and agreed to be accountable for the work described in the manuscript.

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