Decrease in Incidence of Bronchopulmonary Dysplasia with Erythropoietin Administration in Preterm Infants: A Retrospective Study

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Key Words

Erythropoietin · Bronchopulmonary dysplasia · Ventilator days · Oxygen days · Prematurity · Neonates · Anemia of prematurity

Abstract

Background: Despite advances in clinical care, the incidence of bronchopulmonary dysplasia (BPD) remains high in premature infants. Erythropoietin (EPO) is used for the treatment of anemia of prematurity (AOP) to decrease blood transfusion needs. EPO has been shown to mobilize circulating endothelial progenitor cells and to enhance lung repair in animal models. **Objective:** To determine whether EPO treatment for AOP was associated with a reduced incidence of BPD in premature infants. **Methods:** This retrospective study was performed on all live-born neonates with birth weights from 500 to 1,500 g and gestational age (GA) from 22 to 32 weeks admitted from 1994 to 2002. Infants who received EPO and those who did not receive EPO were compared for incidence of BPD and other morbidities. **Results:** Of 478 patients, 297 received EPO before 36 weeks' postmenstrual age (group 1) and 181 did not receive EPO (group 2). Group 1 was of similar birth weight but lower GA than

group 2. The incidence of BPD was lower in group 1 than group 2 (26 vs. 36%, $p = 0.03$); after adjusting for significant risk factors, the adjusted odds ratio for BPD was 0.50 (95% CI 0.32, 0.79), $p = 0.0028$. The BPD rate was much lower when EPO was initiated before 4 weeks of age (16%) as compared to later initiation (44%). **Conclusions:** This study shows an association between EPO treatment and reduced incidence of BPD in preterm infants, particularly when EPO treatment was initiated within the first 4 weeks of life.

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Introduction

Advances in perinatal management have improved the survival of preterm infants, however bronchopulmonary dysplasia (BPD) remains a major morbidity in very low birth weight (VLBW) infants [1–4].

Prematurity, lung injury, inflammation, and yet unknown factors contribute to processes that hinder lung development causing BPD [4, 5]. These processes are recognized as abnormal alveolar and vascular changes during a critical stage of postnatal lung development. Steroid therapy used for prevention or treatment of BPD matures

the epithelial compartment of the lung, but may impair microvascular development [4]. Long-term follow-up has recognized a rise in cerebral palsy and a decline in neurodevelopmental outcome in infants treated with dexamethasone (DEX) [6–8]. Therefore, finding alternative treatments that improve both the vascular and epithelial growth in postnatal premature lungs could potentially reduce the incidence of BPD.

Preterm infants have low endogenous erythropoietin (EPO) levels. As a result, to decrease blood transfusions, EPO was administered for treatment of anemia of prematurity (AOP) [9–11]. Treatment with EPO, which has the potential to mobilize endothelial progenitor cells (EPC) in animal models [12, 13], has been shown to partially reverse the histological features of BPD in rodents [14]. Additionally, decreased levels of circulating EPC were demonstrated in neonatal rodent models of BPD [15]. In adults with acute lung injury, increased EPC are associated with improved survival [16] likely due to enhanced repair of the pulmonary endothelium. High levels of EPC in cord blood have been associated with a reduced incidence of BPD in preterm infants [17].

We hypothesized that VLBW preterm infants who had received EPO for AOP would have had a lower incidence of BPD determined as oxygen need at 36 weeks' postmenstrual age (PMA). Secondly, we assessed whether there was any association between EPO administration and other neonatal morbidities such as ventilator days, oxygen days, intraventricular hemorrhage (IVH) and retinopathy of prematurity (ROP). Lastly, we assessed whether the age at initiation of EPO treatment influenced the BPD outcome.

Patients and Methods

Study Setting

The study population was comprised of all live-born neonates with a birth weight (BW) from 500 to 1,500 g and 22–32 weeks' gestational age (GA) admitted to our neonatal intensive care unit from 1994 to 2002. The local institutional review board approved the study.

The indications for EPO treatment were: to decrease early blood transfusions in infants <1,000 g during the first days of life or to decrease late transfusions at 4–8 weeks of life in infants <1,500 g. EPO was given by subcutaneous injections 250–300 IU/kg/dose 3 times a week for 2 weeks and reticulocytes were checked weekly during the treatment. Additional courses of 2 weeks' treatment were considered if hematocrit was low or reticulocytes count was below 5%.

Transfusion triggers levels were: (1) hematocrit <35% or hemoglobin <12 g/dl for patients on mechanical ventilation requiring $\text{FiO}_2 > 40\%$ or with hypotension; (2) hematocrit <30% or he-

moglobin <10 g/dl for patients on mechanical ventilation and requiring $\text{FiO}_2 < 40\%$, patients requiring $\text{FiO}_2 > 40\%$, unexplained apnea, 10 or 2 spells per day requiring bag-mask resuscitation, unexplained heart rate >165/min for 2 days, poor weight gain (<10 g/kg/day over 1 week), unexplained lethargy, and (3) hematocrit <25% in asymptomatic patients.

We excluded infants with major congenital anomalies, infants born <22 weeks' gestation, those who died before 36 weeks' PMA and cases in which EPO was initiated at 36 weeks' PMA or beyond, or when pulmonary outcomes data were missing.

Study Design

This study was a retrospective observational cohort study to assess the impact of EPO administration on BPD as well as other neonatal morbidities in VLBW premature infants. The patients were placed in group 1 (received EPO before 36 weeks' PMA) and in group 2 (did not receive EPO). The infants were selected from 1994 until 2002 as the use of EPO was discontinued at the end of 2002 secondary to lack of clear benefits [9] and concerns of its possible association with ROP [10].

Data Collection

Data collected from the neonatal database included BW, GA, gender, type of delivery and Apgar scores at 1, 5 and 10 min. Maternal factors such as clinical chorioamnionitis, pregnancy-induced hypertension, diabetes and prenatal steroid administration were collected. Neonatal factors such as sepsis (documented as clinical sepsis or with positive blood culture), pneumonia, pneumothorax, patent ductus arteriosus (PDA), PDA requiring surgical ligation, IVH, ROP highest grade and any surgery performed, days of mechanical ventilation, days of supplemental oxygen, need for supplemental oxygen or mechanical ventilation at 28 days and 36 weeks' PMA, length of hospital stay and administration of pulmonary medications (surfactant, vitamin A and DEX) as well as the final outcome (discharge home or death) were also evaluated. Babies who received 3 or more consecutive days of DEX treatment were counted as DEX-treated. BPD was determined at 36 weeks' PMA as need for oxygen supplementation to maintain pulse oximeter saturation above 94%. Lastly, we recorded the age at initiation of EPO and the number of doses administered. Patients' medical records were reviewed when data elements were missing from the database.

Statistical Analyses

Our primary objective was to compare the incidence of BPD in groups 1 and 2. Univariate analysis was performed to assess the difference in the incidence of BPD with each risk factor between groups 1 and 2 using the χ^2 test or the two-sided Fisher's exact test to compare proportions and the two-sample Student t test or the Wilcoxon rank-sum test to compare means wherever appropriate. Risk factors that were significant at $p < 0.05$ from the univariate analysis were entered in the multivariate logistic regression analysis to derive the adjusted odds ratio (OR) and 95% confidence interval (CI) for BPD rate between groups 1 and 2. For secondary outcomes measured on a continuous scale, we used analysis of covariance. Statistical significance was set at $p < 0.05$. All statistical analyses were performed using SAS 9.1 software (SAS Institute, Cary, N.C., USA).

Fig. 1. Inclusion and exclusion of patients.

Results

During the study period, 762 patients were identified with a BW between 500 and 1,500 g and 682 were ≤ 32 weeks' GA. We excluded 94 patients who died prior to 36 weeks' PMA, 65 patients for incomplete data due to being transferred from or referred to other facilities, and 45 patients who received EPO at or after 36 weeks' PMA. Of the 478 patients included in this study, 297 received EPO (group 1) and 181 did not (group 2) (fig. 1). The clinical characteristics are presented in table 1. BW and Apgar scores were similar between the two groups. The patients in group 1 had a lower GA (28 ± 2 vs. 29 ± 3 weeks, $p = 0.006$) than group 2 and a higher incidence of maternal diabetes (8.5 vs. 3.4%, $p = 0.034$). There was no difference in surfactant, vitamin A or DEX use, no difference in the incidence of pneumothorax, PDA, sepsis, highest grade of ROP or ROP-requiring surgery, rates of IVH grade 2 or higher, number of patients who required a blood transfusion or the number of transfusions between the two groups.

Three patients expired after 36 weeks' PMA, 2 from group 1 and 1 from group 2. Group 1 had 113 infants (38%) and group 2 had 60 infants (33%) < 28 weeks' gestation.

Pulmonary Outcomes

Several factors affected the incidence of BPD in preterm infants in univariate analyses, including GA, BW, sepsis, maternal diabetes, length of hospital stay, ventilator days, prenatal steroids, DEX, and vitamin A administration. After adjustments for significant differences in these risk factors, using multivariate logistic regression,

EPO had a statistically significant association with a decrease in BPD rate.

Table 1 shows that BPD rate was lower in group 1 than in group 2 (26 vs. 36%, $p = 0.030$; OR 0.64 (95% CI 0.43, 0.95), $p = 0.03$). After adjusting for differences in the significant risk factors, maternal diabetes and GA, the adjusted OR for BPD was 0.50 (95% CI 0.32, 0.79), $p = 0.0028$. Further, the adjusted mean ventilator days was significantly lower in group 1 than group 2 (mean 20 compared to 27 days, $p = 0.0005$). The adjusted mean oxygen days was also lower in group 1 (mean of 39 compared to 49 days, $p = 0.0002$). There was no difference in requirement for home oxygen between groups.

The incidence of BPD was 78% (73/94) in group 2 infants who received only DEX, 51% (63/124) among group 1 infants treated with both DEX and EPO, 11% (17/153) in infants who received neither EPO nor DEX, and 9% (15/173) in infants who only received EPO.

Age of Initiation of EPO and BPD Outcome

We observed that earlier initiation of EPO was associated with a lower incidence of BPD. We chose 4 weeks as a cut-off where the incidence of BPD seemed to change substantially. Infants in group 1 were divided into subgroups 1A and 1B with start of EPO treatment at < 4 weeks or > 4 weeks of age. The age at start of EPO was 36 ± 18 days for patients who developed BPD and 23 ± 14 days for patients who did not develop BPD ($p < 0.0001$). The BPD rate was 16% (29/186) in group 1A as compared to 44% (49/111) in group 1B ($p < 0.0001$). The median number of doses of EPO was 12 in patients who developed BPD and 9 in those who did not.

Table 1. Comparison of demographic and clinical characteristics between patients with and without EPO

	EPO group 1	No EPO group 2	p value
Number of patients	297	181	
Birth weight, g	1,038 ± 256	1,063 ± 281	0.29
Gestational age, weeks	28 ± 2	29 ± 3	0.0065*
Male	158 (53%)	107 (59%)	0.22
Apgar score at 1 min median (range)	6 (0, 9)	6 (1, 9)	0.43
Apgar score at 5 min median (range)	8 (2, 10)	8 (1, 10)	0.40
Apgar score at 10 min median (range)	7 (1, 9)	7 (2, 9)	0.80
Vaginal delivery	92 (32%)	62 (35%)	0.42
Prenatal steroids	84 (38%)	47 (35%)	0.65
Maternal PIH	67 (23%)	42 (24%)	0.91
Maternal diabetes	25 (9%)	6 (3%)	0.0341*
Maternal infection	55 (19%)	29 (16%)	0.54
Surfactant	179 (60%)	96 (53%)	0.13
Pneumothorax	22 (7%)	13 (7%)	1.00
Pneumonia	41 (14%)	36 (20%)	0.10
Sepsis	177 (60%)	108 (60%)	1.00
PDA	186 (63%)	105 (58%)	0.33
PDA ligation	36 (12%)	25 (14%)	0.67
Vitamin A	29 (10%)	23 (13%)	0.36
Dexamethasone	124 (42%)	65 (36%)	0.21
IVH grade >2	33 (29%)	21 (30%)	1.00
Patients with blood transfusion	258 (87%)	155 (86%)	0.89
Number of PRBC transfusions	4 ± 3	4 ± 3	0.45
Eye surgery for ROP	45 (15%)	24 (13%)	0.59
BPD	78 (26%)	65 (36%)	0.0305*
Length of hospital stay	66 ± 27 days	68 ± 40 days	0.62
Patients discharged alive	295	180	
Home oxygen	54 (18%)	36 (20%)	0.79

Mean ± SD for continuous parameters; number of cases (%) for dichotomous parameters. * $p < 0.05$.

Discussion

Our study is the first study to show a decrease in the incidence of BPD in preterm infants who received EPO for AOP. In previous reports, EPO was shown to decrease the number of RBC transfusions in preterm infants [11, 18–21]. Tempera et al. [20] reported an association between EPO treatment and a decrease in ventilator days and speculated on the potential to reduce BPD rates [20].

Previous randomized controlled trials of EPO for AOP have failed to demonstrate any significant difference in

rates of BPD [9–11]. These studies have reported BPD as a secondary outcome while primarily focusing on transfusion needs. They were not designed to detect a difference in BPD outcome. The study by Haiden et al. [21], in 40 infants with a BW 450–800 g and GA ≤ 32 weeks, reported a decrease in transfusions in the EPO group which also had a higher proportion of infants requiring mechanical ventilation. There was no significant difference in incidence of BPD overall (6/21 or 29% vs. 8/19 or 42%). Yet, the EPO group had a lower percentage of BPD among ventilated infants (6/17) compared to the control group (8/9). Further, a meta-analysis of these trials did not show any significant difference in BPD rates [19]. Our study included 478 patients, which is larger than published randomized studies that had <300 patients [11], and showed a significant difference after adjusting for risk factors for BPD.

The significant association of EPO and reduction of BPD could possibly be explained by several mechanisms. Non-hematopoietic properties of EPO [22] include mobilization of EPC from the bone marrow into the circulation, enhanced migration and proliferation of endothelial cells [12]. Animal studies have shown upregulation of EPO receptors in immature lungs [23]. It can be speculated from our results that the effect of EPO on reduction of BPD may be related to mobilization of EPC in preterm infants and enhanced repair of already damaged alveolar surfaces and pulmonary vascular endothelium.

Patients receiving both DEX and EPO had lower BPD rates than patients on DEX alone, but DEX was administered to preterm infants at highest risk for BPD needing mechanical ventilation after a week of life or to patients with established BPD. A possible mechanism may be related to maturation of the epithelial compartment by DEX while impairing microvascular development. Indeed, DEX treatment of neonatal rats was shown to inhibit alveolar development [24, 25] and reduce lung mid-kine expression, a proangiogenic growth factor present on alveolar type II cells [25]. EPO conversely favors vascular growth. We speculate that EPO usage may counteract the impairment in vascular development produced by DEX and protects against BPD.

The lowest incidence of BPD was observed in infants who received EPO within the first 4 weeks of life. Patients who were treated with EPO later did not benefit from this reduction. The total number of doses did not appear as important.

We found no significant difference in ROP requiring eye surgery between the two groups. Other studies also reported no increase in ROP with EPO treatment in preterm infants [18, 26]. However, Suk et al. [27] reported

increased ROP rates in preterm infants <1,000 g BW when EPO was administered after 20 days of age with more than 20 cumulative doses.

Our retrospective study has certain limitations. Although we adjusted for known confounding factors affecting the incidence of BPD, we cannot rule out other factors that may have an impact on the incidence of BPD. Our patients were not randomized for treatment with EPO, and the use of EPO was not uniform. The postnatal age at the start of EPO treatment or number of doses received was not standardized.

Conclusions

Our study findings suggest a possible association of EPO treatment within the first 4 weeks of life with a decrease in incidence of BPD in preterm infants. EPO

treatment was associated with a decrease in oxygen days and ventilator days. There was no difference in blood transfusions or ROP in preterm infants treated with EPO or not. These findings would need to be validated in future prospective clinical trials in patients at high risk for BPD.

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Disclosure Statement

The authors of have no conflicts of interest to disclose.

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Erratum

In the article by Rayjada N, Barton L, Chan LS, Plasencia S, Biniwale M, Bui KC: Decrease in Incidence of Bronchopulmonary Dysplasia with Erythropoietin Administration in Preterm Infants: A Retrospective Study. *Neonatology* 2012;102:287–292 (DOI: 10.1159/000341615), the following disclosure statement needs to be added:

K.C.B. has a close relation who owns Amgen stocks.