Clinical and Experimental Obstetrics & Gynecology

Clin. Exp. Obstet. Gynecol. **2025**; 52(3): 27183
https://doi.org/10.31083/CEOG27183

Original Research

Hemoglobin Levels on Day 3 of Life as Early Predictors of Bronchopulmonary Dysplasia in Preterm Infants

Ronghe Zhu[1,†], Shi Wang[2,†], Cuie Chen[3], Ya Ling[1], Qiu Wang[1], Chaosheng Lu[1], Yuanyuan Sun[4,*]

[1]Department of Pediatrics, The First Affiliated Hospital of Wenzhou Medical University, 325000 Wenzhou, Zhejiang, China
[2]Department of Anaesthesia, Women's Hospital, School of Medicine, Zhejiang University, 310002 Hangzhou, Zhejiang, China
[3]Department of Pediatrics, Yiwu Maternity and Children Hospital, 322000 Yiwu, Zhejiang, China
[4]Department of Pediatrics, The Quzhou Affiliated Hospital of Wenzhou Medical University (Quzhou People's Hospital), 324000 Quzhou, Zhejiang, China
*Correspondence: 61132318@qq.com (Yuanyuan Sun)
[†]These authors contributed equally.
Academic Editor: Paolo Ivo Cavoretto
Submitted: 29 October 2024 Revised: 7 February 2025 Accepted: 26 February 2025 Published: 20 March 2025

Abstract

Backgrounds: Bronchopulmonary dysplasia (BPD) is a major contributor to mortality in extremely preterm infants. Therefore, it is essential to identify effective clinical prognostic indicators of BPD and implement early interventions. The objective of this study was to evaluate the predictive value of erythrocyte-related indices, such as hemoglobin (Hb) and hematocrit (Hct), for BPD. **Methods**: This retrospective cohort study included 413 neonates with a gestational age (GA) of < 32 weeks who were admitted to The First Affiliated Hospital of Wenzhou Medical University between January 2019 and January 2024. Maternal and infant characteristics were recorded, and Hb and Hct levels were measured on days 1, 3, 14 and 28 of life (DOL1, DOL3, DOL14, and DOL28, respectively). **Results**: Compared to non-BPD patients (n = 170), BPD patients (n = 218) had a lower GA ($p < 0.001$), birth weight ($p < 0.001$), 1-minute postnatal Apgar scores ($p < 0.001$) and 5-minute postnatal Apgar scores ($p < 0.001$). However, they exhibited higher rates of intubation in the delivery room ($p < 0.001$), surfactant treatment ($p < 0.001$), diuretic treatment ($p < 0.001$), caffeine treatment ($p < 0.001$), postnatal steroid use ($p < 0.001$), hemodynamically significant patent ductus arteriosus (hsPDA) ($p < 0.001$), intraventricular hemorrhage (IVH) ($p = 0.001$), retinopathy of prematurity (ROP) ($p < 0.001$), duration of invasive mechanical ventilation (IMV) $\geq$1 week ($p < 0.001$), and number of packed red blood cell (PRBC) transfusions ($p < 0.001$). In addition, BPD patients received PRBC transfusions earlier ($p = 0.004$), had lower Hb levels on DOL1 ($p = 0.001$), DOL3 ($p < 0.001$) and DOL14 ($p < 0.001$), but higher levels on DOL28 ($p = 0.003$). They also had lower Hct levels on DOL1 ($p = 0.004$), DOL3 ($p < 0.001$) and DOL14 ($p < 0.001$), but higher levels on DOL28 ($p = 0.001$). An Hb level of $\leq$150 g/L on DOL3 (DOL3-Hb) was an early predictor for BPD (adjusted odds ratio (OR) = 3.222, $p = 0.002$), with high sensitivity (69.72%) and specificity (78.24%). The number of PRBC transfusions was also a significant risk factor for BPD (adjusted OR = 4.436, $p < 0.001$). **Conclusions**: Significant predictors of BPD included DOL3-Hb 150 g/L and the number of PRBC transfusions, with DOL3-Hb serving as an early predictor.

Keywords: erythrocyte-related indices; hemoglobin; hematocrit; bronchopulmonary dysplasia; predictor

1. Introduction

Bronchopulmonary dysplasia (BPD) was initially described by Northway *et al.* [1] as a chronic lung disease in premature infants, requiring mechanical ventilation and oxygen therapy for neonatal respiratory distress syndrome (NRDS). In recent years, the survival rate for extremely premature infants has significantly increased due to advances in obstetrical and neonatal care, such as antenatal steroid treatment [2], surfactant therapy [3], oxygen saturation target, caffeine, and mechanical ventilation strategies [4]. Meanwhile, promising therapies are being transferred from bench to bedside, including mesenchymal stromal cells (MSCs), insulin-like growth factor 1/binding protein-3 (IGF-1/IGFBP-3), and interleukin 1 receptor (IL-1R) antagonist (anakinra) [5]. Nevertheless, there has not been a decrease in the incidence of BPD [6], with an estimated prevalence ranging from 10.8% to 37.1% among preterm neonates born at 24 to 31^{+6} weeks gestational age (GA) and a birth weight (BW) of <1500 g [7].

BPD is characterized by a large and simplified alveolar structure, and a reduced and dysmorphic vascular bed [8]. The pathogenesis of BPD is multifactorial and includes immature lungs, NRDS, barotrauma, volutrauma, oxygen toxicity, sepsis, inflammation and gene specificity. BPD is associated with long-term pulmonary morbidities, such as reduced lung function, increased risk of emphysema, high incidence of wheezing, impaired growth and mental development, as well as cerebral palsy [9,10]. Despite extensive research, the ability to predict which infants will develop BPD early in life remains challenging. A regres-

sion model that improves the accuracy of predicting BPD in preterm infants was recently proposed [11]. This model includes mechanical ventilation lasting >7 days, frequent apnea episodes, the time taken to achieve full enteral nutrition, and serum biomarkers such as the level of mediator complex subunit 1 (MED1) and peroxisome proliferator-activated receptor gamma coactivator-1alpha (PGC-1α) on day 1 of life (DOL1). Several models have also included tracheal biomarkers, blood tests, and lung ultrasound, but these have yet to be externally validated [12]. Currently, however, there are no reliable indicators for the early diagnosis of BPD, nor for predicting its severity or treatment response. It is therefore important to identify accurate and robust predictors of BPD.

Anemia is common among preterm infants and can result in tissue hypoxia, anaerobic metabolism and the accumulation of lactic acid, as well as giving rise to inflammation [13]. Hemoglobin (Hb) and hematocrit (Hct) levels are significant erythrocyte-related indices that can be derived from routine hematologic parameters. Decreased levels of these markers are characteristic of anemia. Previous studies have reported that lower Hb or Hct levels were closely related to BPD. Duan *et al.* [14] reported that preterm infants with BPD had lower Hct level than those without BPD on days 1, 7, 14 and 21 after birth, and that early anemia within 14 days after birth was a risk factor for BPD. These researchers also found that Hb $\leq$155 g/L within the first 3 days of life was a significant risk factor for BPD [15]. However, the correlation between Hb and Hct levels and BPD at different time points remains unclear. Moreover, studies on the pre-delivery Hb and Hct levels of the mothers of BPD infants have yet to be reported.

Hence, the objective of this study was to evaluate the predictive value for BPD of erythrocyte-related indices, such as Hb and Hct, in preterm infants on days 1, 3, 14 and 28 of life (DOL1, DOL3, DOL14 and DOL28, respectively).

2. Materials and Methods

2.1 Study Population

This retrospective cohort study included 413 neonates with GA $\leq$32 weeks who were admitted to The First Affiliated Hospital of Wenzhou Medical University within one hour after birth, from January 2019 to January 2024. Excluded from the study were preterm infants with chromosomal anomalies, genetic disorders, congenital heart disease, inherited metabolic disease, or who died before 36 weeks postmenstrual age (PMA) or were missing clinical data due to surgery.

2.2 Data Collection

Detailed data was collected on all mothers and infants. Maternal information included age, mode of delivery, complications, administration of prenatal steroids (four doses of 6 mg of dexamethasone given intramuscularly at 12-h intervals), prenatal magnesium sulfate, premature rupture of membranes, and chorioamnionitis. Infant data included GA, BW, sex, Apgar scores (measured at 1 and 5 minutes postnatal), intubation in the delivery room, small for gestational age (SGA) status, surfactant treatment [poractant alpha-Curosurf® (1122246, Chiesi Farmaceutici S.p.A, Parma, Italy), with an initial dose of 200 mg/kg, followed by 100 mg/kg for subsequent doses] [16], caffeine treatment [caffeine citrate injection (21827, Chiesi Farmaceutici S.p.A, Parma, Italy), a 20 mg/kg intravenous loading dose, followed by a maintenance dose of 5 to 10 mg/kg, once per day], diuretics treatment [hydrochlorothiazide tablets (22043011, Changzhou Pharmaceutical Factory, Changzhou, Jiangsu, China), 1 to 2 mg/kg, twice per day. Spironolactone tablets (T22N069, Hangzhou Minsheng Pharmaceutical Co.,Ltd., Hangzhou, Zhejiang, China), 1 to 2 mg/kg, twice per day], postnatal steroid use [dexamethasone sodium phosphate injection (52106112, TianJin KingYork Group Hubei TianYao Pharmaceutical Co.,Ltd., Xiangyang, Hubei, China), cumulative dose of 0.89 mg/kg over 10 days] [17], nosocomial sepsis (indicated by positive blood cultures and systemic symptoms), nosocomial pneumonia, hemodynamically significant patent ductus arteriosus (hsPDA), intraventricular hemorrhage (IVH), retinopathy of prematurity (ROP), timing of the first packed red blood cell (PRBC) transfusion, total number of PRBC transfusions, duration of invasive mechanical ventilation, and length of hospital stay. BPD was diagnosed when supplemental oxygen was required for more than 28 days, with severity assessed according to the oxygen concentration needed at 36 weeks PMA or at discharge (whichever came first): mild BPD (breathing ambient air), moderate BPD (<30% oxygen level), and severe BPD ($\geq$30% oxygen or positive pressure support) [18]. HsPDA was defined as the narrowest ductal diameter in the pulmonary artery >1.5 mm, along with at least two of the following criteria: left atrial-to-aortic root ratio >1.5, ductal pulsating blood flow, or retrograde/absent diastolic flow in the anterior cerebral artery or descending aorta [19]. IVH was identified through transfontanelle ultrasonography and classified according to the Papile classification [20].

Anemia in the neonatal period (<28 days postnatal) was defined as venous Hct <40% [21]. PRBC transfusions were conducted as per the standard guidelines [22]: (1) Hct <35% in neonates with continuous positive airway pressure/intermittent mandatory ventilation, mean airway pressure $\geq$6 cmH_2O and FiO_2 >30%; (2) Hct <30% in neonates with continuous positive airway pressure and/or intermittent mandatory ventilation plus mechanical ventilation, mean airway pressure <6 cmH_2O and FiO_2 <30%; significant apnea or bradycardia (requiring air bag and mask ventilation 6 times in 12 h, or twice in 24 h when receiving methylxanthine); significant tachycardia or tachypnea (heart rate >180 beats/min for 24 h; respiratory rate >80 breaths/min for 24 h); and slow weight gain (<10 g/day

over 4 days with ≥100 kcal/kg/day intake). A total volume of 15 mL/kg was used for PRBC transfusions. This was administered as two separate transfusions over 4 h each.

Peripheral venous blood samples were collected from the radial vein of patients on DOL1 (within 2 h of birth), DOL3, DOL14 and DOL28, in accordance with the diagnostic and treatment protocols in our center. Blood samples were collected on DOL1 for routine assessment, on DOL3 to confirm and evaluate the infection, and on DOL14 and DOL28 to assess anemia, thyroid function, vitamin D levels, liver and kidney function, and other indicators. Additionally, Hb and Hct values were obtained from the mother in the 24 h period prior to delivery. A complete blood count was conducted using an XN-350 instrument (SYSMEX, Japan).

2.3 Statistical Analysis

Statistical analyses were performed using SPSS 23.0 (SPSS Inc., Chicago, IL, USA). Continuous variables were reported as the mean ± standard deviation (SD) if they followed a normal distribution, or as medians (interquartile range) if they did not. Categorical variables were presented as frequencies and percentages. The Kolmogorov-Smirnov test was used to assess the normality of the data. For continuous variables, either the independent sample *t*-test or the Mann-Whitney U test was applied, while the Chi-square test was used for categorical variables. The sensitivity and specificity of Hb and Hct (on DOL1, DOL3, DOL14, ad DOL28) for BPD, and of Hb and Hct on DOL3, were determined by receiver operating characteristic (ROC) curves. Multivariate logistic regression analysis was performed to identify independent risk factors for BPD. A p-value < 0.05 was considered to be statistically significant.

3. Results

3.1 Demographic Characteristics

A total of 413 preterm infants with a GA ≤32 weeks were included in the study (Fig. 1). Seven infants who died within 72 h after stopping treatment due to family financial issues or to poor prognosis were excluded, as well as 13 infants who died from infections within 72 h. Five infants were excluded due to missing clinical data because of surgery for necrotizing enterocolitis (NEC). Of the remaining 388 infants, 218 (56.2%) were diagnosed with BPD and 170 (43.8%) without. The clinical characteristics of the two patient groups are shown in Table 1. Preterm infants with BPD had significantly lower GA ($p < 0.001$) and BW ($p < 0.001$) compared to non-BPD infants. The incidence of intubation in the delivery room ($p < 0.001$) was significantly higher in BPD patients, while their Apgar scores at 1 ($p < 0.001$) and 5 minutes postnatal ($p < 0.001$) were lower. Furthermore, BPD patients were treated more frequently with surfactant ($p < 0.001$), diuretics ($p < 0.001$), caffeine ($p < 0.001$), and postnatal steroids ($p < 0.001$) than non-BPD infants. The rates of hsPDA ($p < 0.001$), IVH ($p = 0.001$) and ROP ($p < 0.001$) were also higher in BPD patients compared to non-BPD patients. The frequency of invasive mechanical ventilation with duration ≥1 week ($p < 0.001$) was higher in BPD patients, and they received PRBC transfusions earlier ($p = 0.004$) and more often ($p < 0.001$). The length of hospital stay ($p < 0.001$) was also significantly longer for BPD patients compared to non-BPD patients. No significant differences were observed between BPD and non-BPD patients for gender, rates of SGA, nosocomial pneumonia, nosocomial sepsis, prenatal steroids, prenatal magnesium sulfate, maternal hypertension, maternal diabetes, chorioamnionitis, premature rupture of membranes, vaginal delivery, or maternal age.

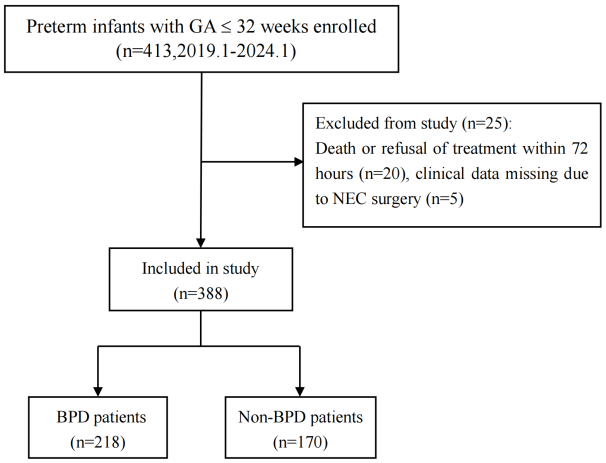

Fig. 1. Flow chart for this study. Abbreviations: GA, gestational age; NEC, necrotizing enterocolitis; BPD, bronchopulmonary dysplasia.

3.2 Hb and Hct Levels at Different Time Points

Patients with BPD exhibited significantly lower levels of Hb on DOL1 ($p = 0.001$), DOL3 ($p < 0.001$) and DOL14 ($p < 0.001$), but higher levels on DOL28 ($p = 0.003$), and had lower Hct levels on DOL1 ($p = 0.004$), DOL3 ($p < 0.001$) and DOL14 ($p < 0.001$), but higher levels on DOL28 ($p = 0.001$) (Table 2). No significant differences in maternal Hb and Hct levels were observed between preterm infants with and without BPD.

3.3 DOL3 Hb ≤150 g/L was an Early Predictor of BPD

ROC analysis was performed to assess the predictive value of Hb and Hct levels for the diagnosis of BPD. Cut-off values for Hb and Hct levels were calculated for DOL1, DOL3, DOL14, and DOL28. An Hb level on DOL3 (DOL3-Hb) of ≤150 g/L showed the highest sensitivity (69.72%) and specificity (78.24%), with a positive predictive value (PPV) of 80.42%. The combination of DOL3-Hb with DOL3-Hct revealed an area under the curve (AUC) of 0.702, sensitivity of 63.30%, and specificity of 76.47% (Tables 2,3, and Fig. 2).

Table 1. Comparison of clinical characteristics between BPD and non-BPD patients.

	BPD patients (n = 218)	Non-BPD patients (n = 170)	χ^2 or t or Z	p-value
Maternal age, years, median (P25, P75)	30 (27, 34)	30.5 (27, 34)	–1.340	0.180
Maternal pregnancy-induced hypertension, n (%)	52 (23.9%)	41 (24.1%)	0.004	0.952
Maternal diabetes, n (%)	51 (23.4%)	49 (28.8%)	1.472	0.225
Premature rupture of membranes, n (%)	73 (33.5%)	69 (40.6%)	2.076	0.150
Vaginal delivery, n (%)	100 (45.9%)	63 (37.1%)	3.045	0.081
Chorioamnionitis, n (%)	42 (19.3%)	26 (15.3%)	1.043	0.307
Prenatal steroids, n (%)	114 (52.3%)	99 (58.2%)	1.362	0.243
Prenatal magnesium sulfate, n (%)	76 (34.9%)	75 (44.1%)	3.442	0.064
Gestational age, weeks, median (P25, P75)	28.9 (27.7, 29.9)	31.0 (30.3, 31.6)	–12.689	<0.001
Birth weight, g, mean ± SD	1187 ± 277	1500 ± 254	11.444	<0.001
Male, n (%)	130 (59.6%)	90 (52.9%)	1.742	0.187
Small for gestational age, n (%)	29 (13.3%)	20 (11.8%)	0.205	0.651
Intubation in the delivery room, n (%)	100 (45.9%)	21 (12.4%)	50.005	<0.001
Apgar score at 1 min postnatal, median (P25, P75)	6 (5, 8)	8 (6, 9)	–4.719	<0.001
Apgar score at 5 min postnatal, median (P25, P75)	9 (8, 9)	9 (9, 10)	–5.727	<0.001
Surfactant treatment, n (%)	182 (83.5%)	85 (50.0%)	49.908	<0.001
Diuretics treatment, n (%)	122 (56.0%)	4 (2.4%)	125.188	<0.001
Caffeine treatment, n (%)	187 (85.8%)	73 (42.9%)	81.323	<0.001
Postnatal steroids, n (%)	49 (22.5%)	0 (0%)	43.734	<0.001
Invasive mechanical ventilation ≥1 week, n (%)	42 (19.3%)	2 (1.2%)	31.087	<0.001
Nosocomial pneumonia, n (%)	72 (33.0%)	52 (30.6%)	0.261	0.609
Nosocomial sepsis, n (%)	31 (14.2%)	15 (8.8%)	2.663	0.103
hsPDA, n (%)	104 (47.7%)	35 (20.6%)	30.552	<0.001
Intraventricular hemorrhage, n (%)	20 (9.2%)	2 (1.2%)	11.423	0.001
Retinopathy of prematurity, n (%)	24 (11.0%)	2 (1.2%)	14.771	<0.001
Number of PRBC transfusions, median (P25, P75)	2 (1, 4)	1 (0, 1)	–13.510	<0.001
Day of first PRBC transfusion, median (P25, P75)	14 (6, 27)	33 (26, 41)	–2.880	0.004
Length of hospital stay, days, mean ± SD	72.8 ± 19.4	43.2 ± 11.1	–18.870	<0.001

SD, standard deviation; hsPDA, hemodynamically significant patent ductus arteriosus; PRBC, packed red blood cell.

Table 2. Hb and Hct levels at different time points in BPD and non-BPD patients.

Parameters	BPD patients (n = 218)	Non-BPD patients (n = 170)	t	p-value
Hb, g/L				
DOL1	163.47 ± 22.2	170.74 ± 21.02	3.272	0.001
DOL3	145.20 ± 23.42	160.16 ± 22.91	6.425	<0.001
DOL14	121.14 ± 19.0	131.13 ± 18.5	5.199	<0.001
DOL28	112.5 ± 17.3	107.3 ± 15.8	–3.037	0.003
Maternal	110.2 ± 13.5	112.2 ± 14.4	1.440	0.151
Hct, %				
DOL1	49.0 ± 6.0	50.7 ± 5.8	2.862	0.004
DOL3	43.2 ± 6.6	47.1 ± 6.3	5.788	<0.001
DOL14	35.9 ± 5.5	38.7 ± 5.3	4.999	<0.001
DOL28	33.5 ± 5.1	31.8 ± 4.6	–3.441	0.001
Maternal	33.4 ± 3.9	32.8 ± 3.8	1.634	0.103

Hb, hemoglobin; Hct, hematocrit; DOL, day of life.

3.4 DOL3-Hb ≤150 g/L and the Number of PRBC Transfusions were Associated With Increased Risk of Developing BPD

The factors associated with BPD in premature infants included GA, BW, Apgar scores at 1 and 5 minutes postnatal, intubation in the delivery room, surfactant treatment, caffeine treatment, duration of invasive mechanical ventilation ≥1 week, hsPDA, DOL3-Hb ≤150 g/L, and the number of PRBC transfusions. After adjusting for significant risk factors, multivariate logistic regression analysis indicated that DOL3-Hb ≤150 g/L and the number of PRBC transfusions were independently related to an increased risk

Table 3. Diagnostic performance of Hb and Hct levels at different time points for the prediction of BPD.

Parameters	Cut-off value	Sensitivity	Specificity	PPV	NPV	Accuracy	AUC (95% CI)	*p*-value
Hb, g/L								
DOL1	158	40.83	76.88	68.99	50.19	56.44	0.603 (0.552–0.652)	0.0004
DOL3	150	69.72	78.24	80.42	66.83	73.45	0.700 (0.652–0.745)	<0.0001
DOL14	111	37.61	88.24	80.39	52.45	59.79	0.650 (0.600–0.697)	<0.0001
DOL28	112	48.17	67.65	65.63	50.44	56.70	0.585 (0.534–0.634)	0.0034
Hct, %								
DOL1	49.6	56.88	61.76	65.61	52.76	59.02	0.593 (0.542–0.642)	0.0014
DOL3	44.1	58.26	75.29	75.15	58.45	65.72	0.679 (0.631–0.726)	<0.0001
DOL14	35.0	50.00	74.71	71.71	53.81	60.82	0.643 (0.593–0.691)	<0.0001
DOL28	35.7	30.73	84.12	71.28	48.64	54.12	0.593 (0.542–0.642)	0.0012
Hb and Hct on DOL3	150 and 44.1	63.30	76.47	77.53	63.33	69.07	0.702 (0.653–0.747)	<0.0001

PPV, positive predictive value; NPV, negative predictive value; AUC, area under the curve; CI, confidence interval.

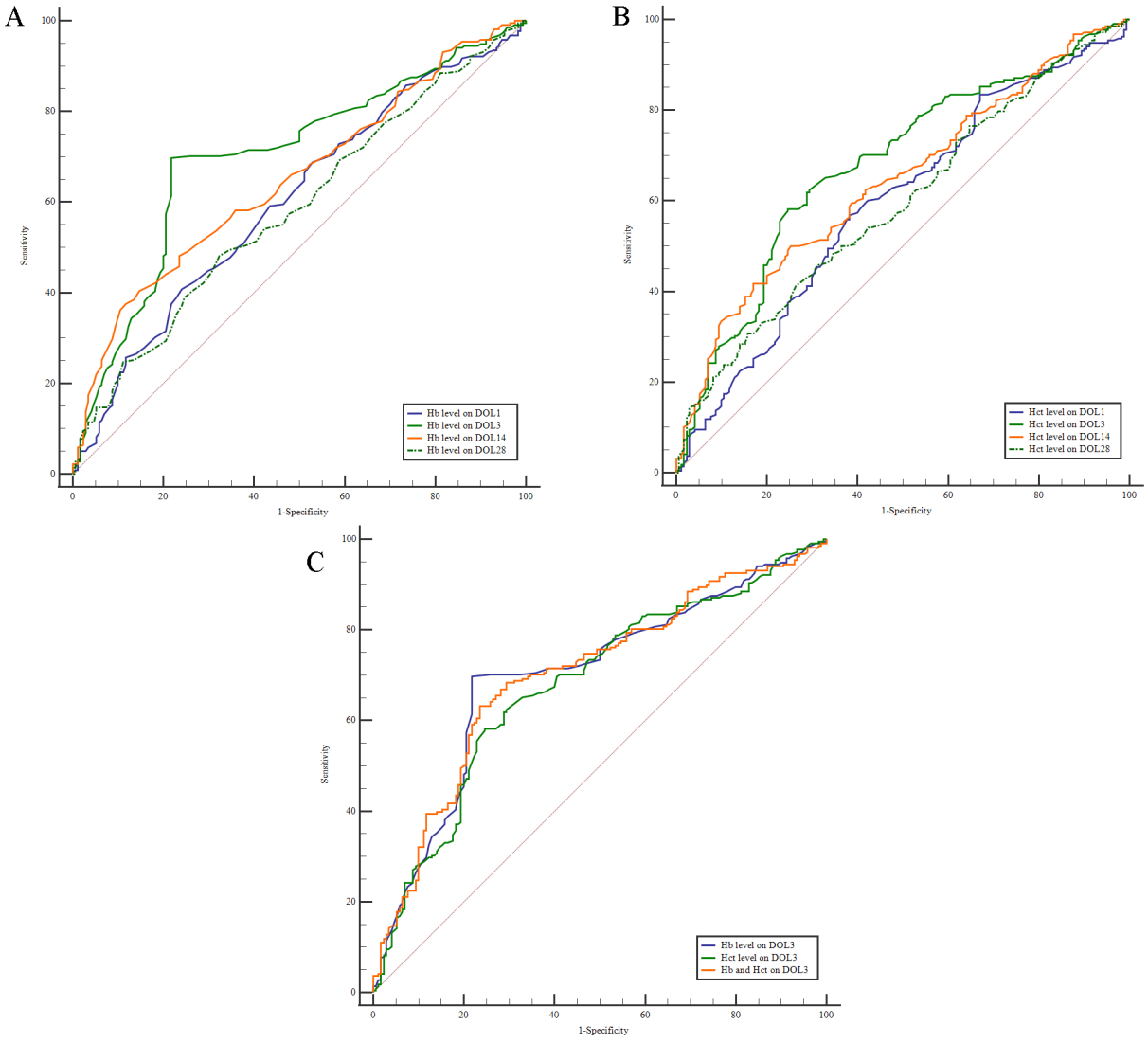

Fig. 2. Analysis of ROC curves. (A) ROC curve analysis was conducted to determine the cut-off value for the Hb level on DOL1, DOL3, DOL14, and DOL28 in relation to BPD. (B) ROC curve analysis was performed to determine the cut-off value for the Hct level on DOL1, DOL3, DOL14, and DOL28 in relation to BPD. (C) ROC curve analysis was conducted to determine the cut-off value for Hb and Hct levels on DOL3 in relation to BPD. ROC, receiver operating characteristic.

Table 4. Multiple logistic regression analysis of risk factors for BPD.

	β	SE	Wald	p-value	Adjusted OR	95% CI
Gestational age	–1.039	0.198	27.553	<0.001	0.354	0.240–0.521
Birth weight	0.002	0.001	3.503	0.061	1.002	1.000–1.003
Apgar score at 1 min postnatal	0.108	0.109	0.971	0.324	1.114	0.899–1.380
Apgar score at 5 min postnatal	–0.253	0.192	1.729	0.189	0.777	0.533–1.132
Intubation in the delivery room	0.653	0.545	1.439	0.230	1.922	0.661–5.590
Caffeine treatment	0.693	0.384	3.259	0.071	2.000	0.942–4.244
Duration of IMV ≥1 week	–1.202	1.070	1.262	0.261	0.301	0.037–2.448
Surfactant treatment	0.459	0.367	1.567	0.211	1.582	0.771–3.245
hsPDA	0.380	0.378	1.012	0.315	1.462	0.697–3.066
Number of PRBC transfusions	1.490	0.270	30.343	<0.001	4.436	2.611–7.538
(DOL3-Hb) ≤150 g/L	1.170	0.383	9.350	0.002	3.222	1.522–6.822

IMV, invasive mechanical ventilation; hsPDA, hemodynamically significant patent ductus arteriosus; PRBC, packed red blood cell; DOL3-Hb, the Hb level on the third day of life; SE, standard error; OR, odds ratio.

of BPD (Table 4). The adjusted odds ratio (OR) for DOL3-Hb ≤150 g/L in relation to BPD was 3.222 (95% CI: 1.522–6.822; $p = 0.002$), while for the number of PRBC transfusions the adjusted OR was 4.436 (95% CI: 2.611–7.538; $p < 0.001$).

4. Discussion

Although BPD is the most common clinical issue of prematurity, accurately predicting this condition at an early stage remains challenging. Clinical prediction models, echocardiogram measurements, lung function tests, epigenetic factors and various biomarkers have all been suggested as potential early indicators of BPD [23–25], but have yet to be consistently validated. Anemia of prematurity (AOP) is frequently observed in preterm infants, especially in those born at a GA ≤32 weeks [26]. Previous studies have indicated a link between AOP and complications in preterm infants. For instance, Patel *et al.* [27] demonstrated that severe anemia (Hb ≤80 g/L) was associated with a heightened risk of NEC in preterm infants with a BW <1500 g. Additionally, the presence of anemia at birth has been significantly associated with conditions such as hsPDA, severe intra-periventricular hemorrhage (IPVH), and an increased rate of mortality [21,28,29]. Duan *et al.* [14] found that low Hct level were associated with BPD. A retrospective analysis of 147 infants with GA ≤32 weeks revealed that those with BPD had lower Hb level during the first 3 days of life [15].

Maternal anemia during pregnancy is a known risk factor for anemia in extremely low birth weight (ELBW) infants (BW <1000 g) and extreme preterm infants. However, previous study has not emphasized the connection between maternal anemia and BPD [30]. In the present study, no significant correlations were found between maternal Hb and Hct levels and preterm infants, regardless of whether they developed BPD. We also compared Hb and Hct levels at various time points between preterm infants with or without BPD. BPD patients had lower Hb and Hct levels on DOL1, DOL3 and DOL14, but higher levels on DOL28, possibly due to their earlier PRBC transfusions (Table 1). Our findings suggest that DOL3-Hb ≤150 g/L could be an early predictor of BPD. Furthermore, the area under the curve (AUC) for Hb on DOL1, DOL3 and DOL14 was greater than that for Hct, indicating it has superior predictive value within the first 14 days of life. Combination of the Hb and Hct levels on DOL3 did not improve the predictive value for BPD. Multivariate regression analysis confirmed that DOL3-Hb ≤150 g/L was significantly associated with BPD (adjusted OR = 3.222, $p = 0.002$), highlighting the importance of monitoring Hb level in relation to BPD development. The potential mechanisms linking early anemia to BPD may include a reduction in the blood's oxygen-carrying capacity, which can lead to increased anaerobic metabolism and elevated lactic acid levels. Additionally, anemia may limit the oxygen supply to the brain's respiratory center, resulting in respiratory issues such as tachypnea, dyspnea, and apnea, which could prolong the need for supplemental oxygen or mechanical ventilation. Moreover, as preterm infants transition from the fetal environment to early life, their lungs must adapt to new conditions, and Hb plays a crucial role in this adaptation process. Lower Hb level may hinder normal lung function development, potentially contributing to the onset of BPD [31–34].

AOP is influenced by various factors, such as rapid postnatal growth, blood loss due to medical procedures, and deficiencies in essential micronutrients necessary for red blood cell production [35]. Several clinical practices have been implemented to reduce the severity of AOP. One such practice, delayed cord clamping (DCC), allows blood to transfer from the placenta to the newborn, thereby increasing Hb level and decreasing the need for PRBC transfusions [36]. However, a large randomized trial found that DCC (lasting at least 60 s) did not impact the incidence of BPD in infants born at GA ≤30 weeks [37], suggesting the effectiveness of DCC in reducing AOP should be

reassessed [38]. Phlebotomy losses are significant contributors to AOP, with ELBW infants losing about 11–22 mL/kg of blood weekly during the first 6 weeks of life [39]. The neonatal total circulating volume is approximately 80 mL/kg. Therefore, it is crucial to expand the range of analytes that can be measured using microsample point-of-care devices, to utilize placental blood for routine tests, and to implement neonatal sampling tubes to minimize blood loss.

Typically, the Hb level falls significantly during the first week after birth, leading to AOP [40]. PRBC transfusions are a key intervention for addressing AOP, with up to 40% of very low birth weight (VLBW) infants [41] and 90% of ELBW infants receiving PRBC transfusions during their hospital stay [42]. The Effects of Transfusion Thresholds on Neurocognitive Outcomes of Extremely Low-Birth-Weight Infants (ETTNO) trial and Transfusion of Prematures (TOP) trial have shown that receiving relatively low levels of Hb before transfusion, did not have any negative effect on the incidence of BPD [43,44]. However, numerous studies have reported that PRBC transfusions were an independent risk factor for BPD in preterm infants. Lee *et al*. [45] found that frequent PRBC transfusions in the first week of life and a higher total volume of PRBC transfusions were associated with an increased risk of BPD. We also found that PRBC transfusions were more frequent in patients with BPD compared to those without, suggesting it was a significant risk factor. Following PRBC transfusion, erythrocytes release heme, which is subsequently broken down and raises the serum iron level. Free radicals generated by the excess iron can lead to tissue damage. Since premature infants are particularly susceptible to oxidative damage [46], this oxidative stress and the resulting inflammatory cascade may provide an explanation for the development of BPD [47].

Recombinant human erythropoietin (rhEPO) has the ability to promote erythropoiesis and reduce the need for PRBC transfusions in AOP cases [48]. Bui *et al*. [49] reported that administration of rhEPO (250–300 U/kg) three times a week via intravenous or subcutaneous injection reduced the occurrence of BPD. Research on animals has demonstrated that erythropoietin (EPO) can stimulate angiogenesis [50], enhance alveolar development, reduce lung fibrosis during oxygen exposure, and inhibit transforming growth factor-β1 (TGF-β1) signaling pathways by mobilizing endothelial progenitor cells [51]. These findings support the use of EPO for extremely preterm infants who are at high risk for BPD.

The present work has several limitations. First, this was a retrospective study based on data from a single center, and lacks external validation. Larger prospective studies are required to better understand the role and clinical significance of Hb level in the onset and progression of BPD. Secondly, there may be a selection bias since we excluded some patients who either died or were not treated before being diagnosed with BPD. These preterm infants might also be at a higher risk for BPD due to intubation. Thirdly, Villar *et al*. [52] proposed that preterm birth can no longer be defined by GA alone since this approach fails to provide any pathophysiologic insights or assessment of specific risks. Premature infants are defined by GA <37 weeks in our research without considering their phenotypes. In the future study, we will incorporate new theories to define preterm birth and assess the outcomes of different phenotypes on BPD. Despite these limitations, our study is highly relevant to clinical practice and provides new insights that could assist in the management and prediction of outcomes for preterm infants.

5. Conclusions

In summary, this study demonstrated that patients with BPD had lower Hb and Hct levels up to DOL14. DOL3-Hb $\leq$150 g/L may be an early indicator for BPD. Additionally, we identified a link between the number of PRBC transfusions and a higher occurrence of BPD in premature infants. It is important to develop appropriate transfusion protocols and to consider treatments such as rhEPO to help prevent AOP.

Abbreviations

BPD, bronchopulmonary dysplasia; MSCs, mesenchymal stromal cells; IL-1R, interleukin 1 receptor; IGF-1/IGFBP-3, insulin-like growth factor 1/binding protein-3; Hb, hemoglobin; Hct, hematocrit; DOL, day of life; GA, gestational age; BW, birth weight; MED1, mediator complex subunit 1; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator-1alpha; hsPDA, hemodynamically significant patent ductus arteriosus; IVH, intraventricular hemorrhage; ROP, retinopathy of prematurity; PRBC, packed red blood cell; DOL3-Hb, Hb level on the third day of life; PMA, postmenstrual age; SGA, small for gestational age; AUC, area under the curve; ROC, receiver operating characteristic; IPVH, intra-periventricular hemorrhage; NEC, necrotizing enterocolitis; AOP, anemia of prematurity; VLBW, very low birth weight; DCC, delayed cord clamping; ELBW, extremely low birth weight; rhEPO, recombinant human erythropoietin; EPO, erythropoietin; TGF-β1, transforming growth factor-β1; SD, standard deviation; ETTNO, Effects of Transfusion Thresholds on Neurocognitive Outcomes of Extremely Low-Birth-Weight Infants; TOP, Transfusion of Prematures.

Availability of Data and Materials

All data reported in this paper will also be shared by the corresponding author upon request.

Author Contributions

YS and RZ designed the study. CC and SW analyzed the data and performed the statistical analysis. RZ and YL collected data. CL designed of the work. QW interpreted

of data of the work. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The study was approved by the Ethics Committee of the First Affiliated Hospital of Wenzhou Medical University (No.KY2021-R083) and followed the Helsinki Declaration. Written informed consent was obtained from parents when patients were admitted to hospital.

Acknowledgment

We would like to express our gratitude to all those who helped us during the writing of this manuscript. Thanks to all the peer reviewers for their opinions and suggestions.

Funding

All phases of this study were supported by Wenzhou Science and Technology Bureau, China (Y20210274); Zhejiang Medical Association clinical research fund project, China (2024ZYC-B51).

Conflict of Interest

The authors declare no conflict of interest.

References

[1] Northway WH, Jr, Rosan RC, Porter DY. Pulmonary disease following respirator therapy of hyaline-membrane disease. Bronchopulmonary dysplasia. The New England Journal of Medicine. 1967; 276: 357–368. https://doi.org/10.1056/NEJM196702162760701.

[2] McGoldrick E, Stewart F, Parker R, Dalziel SR. Antenatal corticosteroids for accelerating fetal lung maturation for women at risk of preterm birth. The Cochrane Database of Systematic Reviews. 2020; 12: CD004454. https://doi.org/10.1002/14651858.CD004454.pub4.

[3] Kribs A, Roberts KD, Trevisanuto D, O'Donnell C, Dargaville PA. Surfactant delivery strategies to prevent bronchopulmonary dysplasia. Seminars in Perinatology. 2023; 47: 151813. https://doi.org/10.1016/j.semperi.2023.151813.

[4] Principi N, Di Pietro GM, Esposito S. Bronchopulmonary dysplasia: clinical aspects and preventive and therapeutic strategies. Journal of Translational Medicine. 2018; 16: 36. https://doi.org/10.1186/s12967-018-1417-7.

[5] Durlak W, Thébaud B. BPD: Latest Strategies of Prevention and Treatment. Neonatology. 2024; 121: 596–607. https://doi.org/10.1159/000540002.

[6] Thébaud B, Goss KN, Laughon M, Whitsett JA, Abman SH, Steinhorn RH, *et al*. Bronchopulmonary dysplasia. Nature Reviews. Disease Primers. 2019; 5: 78. https://doi.org/10.1038/s41572-019-0127-7.

[7] Lui K, Lee SK, Kusuda S, Adams M, Vento M, Reichman B, *et al*. Trends in Outcomes for Neonates Born Very Preterm and Very Low Birth Weight in 11 High-Income Countries. The Journal of Pediatrics. 2019; 215: 32–40.e14. https://doi.org/10.1016/j.jpeds.2019.08.020.

[8] Coalson JJ. Pathology of new bronchopulmonary dysplasia. Seminars in Neonatology: SN. 2003; 8: 73–81. https://doi.org/10.1016/s1084-2756(02)00193-8.

[9] Nakanishi H, Uchiyama A, Kusuda S. Impact of pulmonary hypertension on neurodevelopmental outcome in preterm infants with bronchopulmonary dysplasia: a cohort study. Journal of Perinatology: Official Journal of the California Perinatal Association. 2016; 36: 890–896. https://doi.org/10.1038/jp.2016.108.

[10] Lin F, Dong H, Song Y, Zhang T, Qi J, Xiao X, *et al*. Effect of bronchopulmonary dysplasia on early intellectual development in preterm infants. Pediatrics International: Official Journal of the Japan Pediatric Society. 2017; 59: 691–697. https://doi.org/10.1111/ped.13257.

[11] Li M, Sun W, Fu C, Xu S, Wang C, Chen H, *et al*. Predictive value of serum MED1 and PGC-1α for bronchopulmonary dysplasia in preterm infants. BMC Pulmonary Medicine. 2024; 24: 363. https://doi.org/10.1186/s12890-024-03145-z.

[12] Oulego-Erroz I, Alonso-Quintela P, Terroba-Seara S, Jiménez-González A, Rodríguez-Blanco S. Early assessment of lung aeration using an ultrasound score as a biomarker of developing bronchopulmonary dysplasia: a prospective observational study. Journal of Perinatology: Official Journal of the California Perinatal Association. 2021; 41: 62–68. https://doi.org/10.1038/s41372-020-0724-z.

[13] Weiss G, Ganz T, Goodnough LT. Anemia of inflammation. Blood. 2019; 133: 40–50. https://doi.org/10.1182/blood-2018-06-856500.

[14] Duan J, Kong X, Li Q, Hua S, Zhang S, Zhang X, *et al*. Association between anemia and bronchopulmonary dysplasia in preterm infants. Scientific Reports. 2016; 6: 22717. https://doi.org/10.1038/srep22717.

[15] Duan J, Kong X, Li Q, Hua S, Zhang S, Feng Z, *et al*. Association between Hemoglobin Levels in the First 3 Days of Life and Bronchopulmonary Dysplasia in Preterm Infants. American Journal of Perinatology. 2016; 33: 998–1002. https://doi.org/10.1055/s-0036-1583189.

[16] Sweet DG, Carnielli V, Greisen G, Hallman M, Ozek E, Te Pas A, *et al*. European Consensus Guidelines on the Management of Respiratory Distress Syndrome - 2019 Update. Neonatology. 2019; 115: 432–450. https://doi.org/10.1159/000499361.

[17] Doyle LW, Davis PG, Morley CJ, McPhee A, Carlin JB, DART Study Investigators. Low-dose dexamethasone facilitates extubation among chronically ventilator-dependent infants: a multicenter, international, randomized, controlled trial. Pediatrics. 2006; 117: 75–83. https://doi.org/10.1542/peds.2004-2843.

[18] Jobe AH, Bancalari E. Bronchopulmonary dysplasia. American Journal of Respiratory and Critical Care Medicine. 2001; 163: 1723–1729. https://doi.org/10.1164/ajrccm.163.7.2011060.

[19] Polat TB, Celik IH, Erdeve O. Early predictive echocardiographic features of hemodynamically significant patent ductus arteriosus in preterm VLBW infants. Pediatrics International: Official Journal of the Japan Pediatric Society. 2016; 58: 589–594. https://doi.org/10.1111/ped.12915.

[20] Papile LA, Munsick-Bruno G, Schaefer A. Relationship of cerebral intraventricular hemorrhage and early childhood neurologic handicaps. The Journal of Pediatrics. 1983; 103: 273–277. https://doi.org/10.1016/s0022-3476(83)80366-7.

[21] Goobie SM, Faraoni D, Zurakowski D, DiNardo JA. Association of Preoperative Anemia With Postoperative Mortality in Neonates. JAMA Pediatrics. 2016; 170: 855–862. https://doi.org/10.1001/jamapediatrics.2016.1032.

[22] Roseff SD, Luban NLC, Manno CS. Guidelines for assessing appropriateness of pediatric transfusion. Transfusion. 2002; 42: 1398–1413. https://doi.org/10.1046/j.1537-2995.2002.00208.x.

[23] Jassem-Bobowicz JM, Klasa-Mazurkiewicz D, Żawrocki A, Stefańska K, Domżalska-Popadiuk I, Kwiatkowski S, *et al*. Prediction Model for Bronchopulmonary Dysplasia in Preterm

Newborns. Children (Basel, Switzerland). 2021; 8: 886. https://doi.org/10.3390/children8100886.

[24] Wang H, Yan D, Wu Z, Geng H, Zhu X, Zhu X. Predictive values of clinical data,molecular biomarkers, and echocardiographic measurements in preterm infants with bronchopulmonary dysplasia. Frontiers in Pediatrics. 2023; 10: 1070858. https://doi.org/10.3389/fped.2022.1070858.

[25] Ding L, Wang H, Geng H, Cui N, Huang F, Zhu X, *et al*. Prediction of Bronchopulmonary Dysplasia in Preterm Infants Using Postnatal Risk Factors. Frontiers in Pediatrics. 2020; 8: 349. https://doi.org/10.3389/fped.2020.00349.

[26] Ohlsson A, Aher SM. Early erythropoiesis-stimulating agents in preterm or low birth weight infants. Cochrane Database of Systematic Reviews. 2017; 11: CD004863. https://doi.org/10.1002/14651858.CD004863.pub5.

[27] Patel RM, Knezevic A, Shenvi N, Hinkes M, Keene S, Roback JD, *et al*. Association of Red Blood Cell Transfusion, Anemia, and Necrotizing Enterocolitis in Very Low-Birth-Weight Infants. JAMA. 2016; 315: 889–897. https://doi.org/10.1001/jama.2016.1204.

[28] Rocha G, Pereira S, Antunes-Sarmento J, Flôr-de-Lima F, Soares H, Guimarães H. Early anemia and neonatal morbidity in extremely low birth-weight preterm infants. The Journal of Maternal-fetal & Neonatal Medicine: the Official Journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstetricians. 2021; 34: 3697–3703. https://doi.org/10.1080/14767058.2019.1689948.

[29] Banerjee J, Asamoah FK, Singhvi D, Kwan AWG, Morris JK, Aladangady N. Haemoglobin level at birth is associated with short term outcomes and mortality in preterm infants. BMC Medicine. 2015; 13: 16. https://doi.org/10.1186/s12916-014-0247-6.

[30] Liao Z, Zhao X, Rao H, Kang Y. Analysis of correlative risk factors for blood transfusion therapy for extremely low birth weight infants and extreme preterm infants. American Journal of Translational Research. 2021; 13: 8179–8185.

[31] Kim YJ, Yoon SA. Risk factors associated with anemia of prematurity requiring red blood cell transfusion in very low birth weight infants: a retrospective study. BMC Pediatrics. 2024; 24: 623. https://doi.org/10.1186/s12887-024-05102-5.

[32] Von Kohorn I, Ehrenkranz RA. Anemia in the preterm infant: erythropoietin versus erythrocyte transfusion–it's not that simple. Clinics in Perinatology. 2009; 36: 111–123. https://doi.org/10.1016/j.clp.2008.09.009.

[33] Elguazzar S, Alaoui AM, Izgua AT. Evaluation of the practice of transfusion in the anemia in preterm infants. Revue Medicale De Bruxelles. 2013; 34: 4–11.

[34] Sallmon H, Sola-Visner M. Clinical and research issues in neonatal anemia and thrombocytopenia. Current Opinion in Pediatrics. 2012; 24: 16–22. https://doi.org/10.1097/MOP.0b013e32834ee5cc.

[35] Saito-Benz M, Flanagan P, Berry MJ. Management of anaemia in pre-term infants. British Journal of Haematology. 2020; 188: 354–366. https://doi.org/10.1111/bjh.16233.

[36] Rana N, Kc A, Målqvist M, Subedi K, Andersson O. Effect of Delayed Cord Clamping of Term Babies on Neurodevelopment at 12 Months: A Randomized Controlled Trial. Neonatology. 2019; 115: 36–42. https://doi.org/10.1159/000491994.

[37] Tarnow-Mordi W, Morris J, Kirby A, Robledo K, Askie L, Brown R, *et al*. Delayed versus Immediate Cord Clamping in Preterm Infants. The New England Journal of Medicine. 2017; 377: 2445–2455. https://doi.org/10.1056/NEJMoa1711281.

[38] Alan S, Arsan S. Prevention of the anaemia of prematurity. International Journal of Pediatrics & Adolescent Medicine. 2015; 2: 99–106. https://doi.org/10.1016/j.ijpam.2015.10.001.

[39] Hellström W, Forssell L, Morsing E, Sävman K, Ley D. Neonatal clinical blood sampling led to major blood loss and was associated with bronchopulmonary dysplasia. Acta Paediatrica (Oslo, Norway: 1992). 2020; 109: 679–687. https://doi.org/10.1111/apa.15003.

[40] Christensen RD, Henry E, Bennett ST, Yaish HM. Reference intervals for reticulocyte parameters of infants during their first 90 days after birth. Journal of Perinatology: Official Journal of the California Perinatal Association. 2016; 36: 61–66. https://doi.org/10.1038/jp.2015.140.

[41] Raffaeli G, Manzoni F, Cortesi V, Cavallaro G, Mosca F, Ghirardello S. Iron Homeostasis Disruption and Oxidative Stress in Preterm Newborns. Nutrients. 2020; 12: 1554. https://doi.org/10.3390/nu12061554.

[42] Wang Y, Wu Y, Li T, Wang X, Zhu C. Iron Metabolism and Brain Development in Premature Infants. Frontiers in Physiology. 2019; 10: 463. https://doi.org/10.3389/fphys.2019.00463.

[43] Franz AR, Engel C, Bassler D, Rüdiger M, Thome UH, Maier RF, *et al*. Effects of Liberal vs Restrictive Transfusion Thresholds on Survival and Neurocognitive Outcomes in Extremely Low-Birth-Weight Infants: The ETTNO Randomized Clinical Trial. JAMA. 2020; 324: 560–570. https://doi.org/10.1001/jama.2020.10690.

[44] Kirpalani H, Bell EF, Hintz SR, Tan S, Schmidt B, Chaudhary AS, *et al*. Higher or Lower Hemoglobin Transfusion Thresholds for Preterm Infants. The New England Journal of Medicine. 2020; 383: 2639–2651. https://doi.org/10.1056/NEJMoa2020248.

[45] Lee EY, Kim SS, Park GY, Lee SH. Effect of red blood cell transfusion on short-term outcomes in very low birth weight infants. Clinical and Experimental Pediatrics. 2020; 63: 56–62. https://doi.org/10.3345/kjp.2019.00990.

[46] Harijith AK, Bhandari V. Hyperoxia in the pathogenesis of bronchopulmonary dysplasia. In Bhandari V (ed.) Bronchopulmonary dysplasia. Respiratory medicine (pp. 3–26). Humana Press: Cham. 2016. https://doi.org/10.1007/978-3-319-28486-6_1.

[47] Collard KJ. Is there a causal relationship between the receipt of blood transfusions and the development of chronic lung disease of prematurity? Medical Hypotheses. 2006; 66: 355–364. https://doi.org/10.1016/j.mehy.2005.04.046.

[48] Costescu OC, Boia ER, Boia M, Cioboata DM, Doandes FM, Lungu N, *et al*. The Role of Erythropoietin in Preventing Anemia in the Premature Neonate. Children (Basel, Switzerland). 2023; 10: 1843. https://doi.org/10.3390/children10121843.

[49] Bui KCT, Kim R, Abbasi A, Nguyen M, Villosis MF, Chen Q. Erythropoietin treatment is associated with a reduction in moderate to severe bronchopulmonary dysplasia in preterm infants. A regional retrospective study. Early Human Development. 2019; 137: 104831. https://doi.org/10.1016/j.earlhumdev.2019.104831.

[50] Kimáková P, Solár P, Solárová Z, Komel R, Debeljak N. Erythropoietin and Its Angiogenic Activity. International Journal of Molecular Sciences. 2017; 18: 1519. https://doi.org/10.3390/ijms18071519.

[51] Luan Y, Zhang L, Chao S, Liu X, Li K, Wang Y, *et al*. Mesenchymal stem cells in combination with erythropoietin repair hyperoxia-induced alveoli dysplasia injury in neonatal mice via inhibition of TGF-β1 signaling. Oncotarget. 2016; 7: 47082–47094. https://doi.org/10.18632/oncotarget.9314.

[52] Villar J, Cavoretto PI, Barros FC, Romero R, Papageorghiou AT, Kennedy SH. Etiologically Based Functional Taxonomy of the Preterm Birth Syndrome. Clinics in Perinatology. 2024; 51: 475–495. https://doi.org/10.1016/j.clp.2024.02.014.