

Project BA2023

study protocol and statistical analysis

plan

Title: Analyses of Anemia Risk Factors and Prognosis in VLBW Infants.

Study center

Shenzhen Bao'an Women's and Children's Hospital (ShenzhenBMCHH)

Shenzhen Longhua Women's and Children's Hospital

Time frame

Study Start: March 1, 2023

Study Completion: March 24, 2024

1. Background

Preterm infants (accounts for 9.9% of newborn infants in 2020) are born with immature organs and immune system[1]. Especially, 4% of them are extremely preterm infants with a gestational age of less than 28 weeks[2]. In recent years, the treatment and management of extremely preterm infants has become the main task of NICU. Moreover, the morbidity rate for extremely premature infants around the world has been rising annually. These vulnerable patients are prone to various complications. Anemia is one of the most common complications that occur during hospitalization in these infants[3]. Therefore, According to a survey by the Canadian Neonatal Network (CNN), over 75% of extremely preterm infants receive at least one blood transfusion treatment during hospitalization[4], and WhyteRK et al.[5] even pointed out that over 90% of extremely preterm infants require blood transfusion treatment during hospitalization. Most of the recipients of blood transfusions are caused by anemia, indicating a very high incidence and severity of anemia in these immature infants. Anemia of prematurity (AOP) can cause a series of adverse consequences such as apnoea, prolonged mechanical ventilation, prolonged hospital stay, necrotizing enterocolitis (NEC) [6], retinopathy of prematurity (ROP) [7], infection, and extrauterine growth restriction (EUGR). It can be seen that anemia in premature infants, especially in extremely preterm infants, has a significant impact on their prognosis.

There is a lack of evidence regarding the relationship between blood hemoglobin level at birth and the neonatal morbidity and mortality of preterm infants despite the mode of delivery and prenatal infection. It remains unknown the impact of early anemia on the neonatal morbidity and mortality of extremely preterm infants[8]. With this study, We hope to demonstrate the relationship between anemia on admission to the neonatal intensive care unit (NICU) and the neonatal morbidity and mortality in extremely preterm infants.

2. Objective and hypotheses

Objective: To study the risk factors of anemia in different stages of extremely preterm infants and their impact on prognosis, which provide a certain evidence for improving the prognosis of these infants.

Hypothesis: Small gestational age and low birth weight are risk factors for anemia and receiving blood transfusion treatment in extremely preterm infants. The younger the gestational age and the lower the birth weight, the more severe the anemia, and the greater the likelihood of receiving blood transfusion treatment.

3. Study design

3.1. study subject

From January 1, 2020 to December 31, 2022, extremely preterm infants with a gestational age of less than 28 weeks were admitted to Bao'an Women's and Children's Hospital affiliated with Jinan University and Longhua District Women's and Children's Hospital.

3.2. The method of study

This was a retrospective study on the maternal and infant clinical and electronic records. We analyzed the maternal and obstetrical data, demographics, as well as the neonatal morbidity and mortality diagnosed during hospitalization. The study was approved by the Ethics Committee of our institution.

3.3. Inclusion criteria

Extremely preterm infants with admission age \leq 24 hours, hospital stay \geq 14 days, and gestational age less than 28 weeks were included.

Anemia of Premature (AOP):

- Within 2 weeks of birth, venous blood hemoglobin level (Hb) \leq 130g/L, capillary blood \leq 145g/L.
- Age 2 weeks to 1 month, Hb $<$ 110g/L.
- Age after one month, Hb $<$ 90g/L.

3.4. Exclusion criteria

- (1) Transfer or automatic discharge within 2 weeks.
- (2) Patients with combined neonatal hemorrhagic diseases.
- (3) Newborns with severe malformations (such as congenital diaphragmatic hernia, umbilical protrusion, multiple malformations, etc.) or chromosomal abnormalities.
- (4) Incomplete and inaccurate hospitalization data.

3.5. General information

Several variables on mother, pregnancy, delivery, resuscitation, hematologic parameters including the Hct and Hb at admission, PRBC transfusions, use of vasopressor agents within the first 72 h of life, data on invasive and noninvasive respiratory support, neonatal morbidity and mortality, and length of NICU stay were collected and analyzed.

Care practices included antenatal steroid use, the use of antenatal magnesium sulfate, cesarean section, and delivery room resuscitation.

- Antenatal steroids: A full cycle of antenatal steroids was considered when at least 12 h had elapsed after the last dose of dexamethasone (four doses of 6 mg of dexamethasone given intramuscularly 12 h apart) administered to the mother [9].
- Antenatal magnesium sulfate was defined as the intravenous administration of magnesium sulfate to the mother during pregnancy at any time before delivery.
- Gestational age (GA) was determined according to the first day of the last menstrual period, an early prenatal ultrasound examination, and a physical examination of the infant after birth [10].
- Maternal chorioamnionitis was diagnosed clinically during pregnancy, labor, or delivery.
- Small for gestational age (SGA) was defined as a birth weight below the 10th percentile, based on the Fenton Growth Chart [11].
- Neonatal sepsis was diagnosed in the presence of clinical signs (fever; hypothermia; lethargy; tachypnea; apnea/bradycardia; cyanosis; and hypoglycemia not explained by other diagnosis), associated to at least two laboratory markers (thrombocytopenia; leukocytosis or leucopenia; C-reactive protein elevation; PCT; I/T) according to our National Program of Infection Control Guidelines [12]. Early-onset sepsis and late-onset sepsis were considered if diagnosed before and after the first 72 h of life, respectively.
- Intraventricular hemorrhage (IVH) was classified according to Volpe [13].

- Bronchopulmonary dysplasia (BPD) was defined according to the National Institute of Health [14]. For the analysis of this study, we considered BPD as the dependency for oxygen until 36 weeks of corrected age.
- Parenteral nutrition was used as soon as there was clinical stability, preferably since day one of life, starting with a volume of 70 ml/kg/day, with daily increments until 150 mg/kg/day by day seven of life. Incubator humidity is usually 70% during the first week.
- The modified criteria of Bell were used for the diagnosis and staging of necrotizing enterocolitis (NEC) [15].
- Staging of retinopathy of prematurity (ROP) was done according to the International Classification [16,17].

3.6. Study outcomes

The study outcomes and endpoints are listed in Table 1.

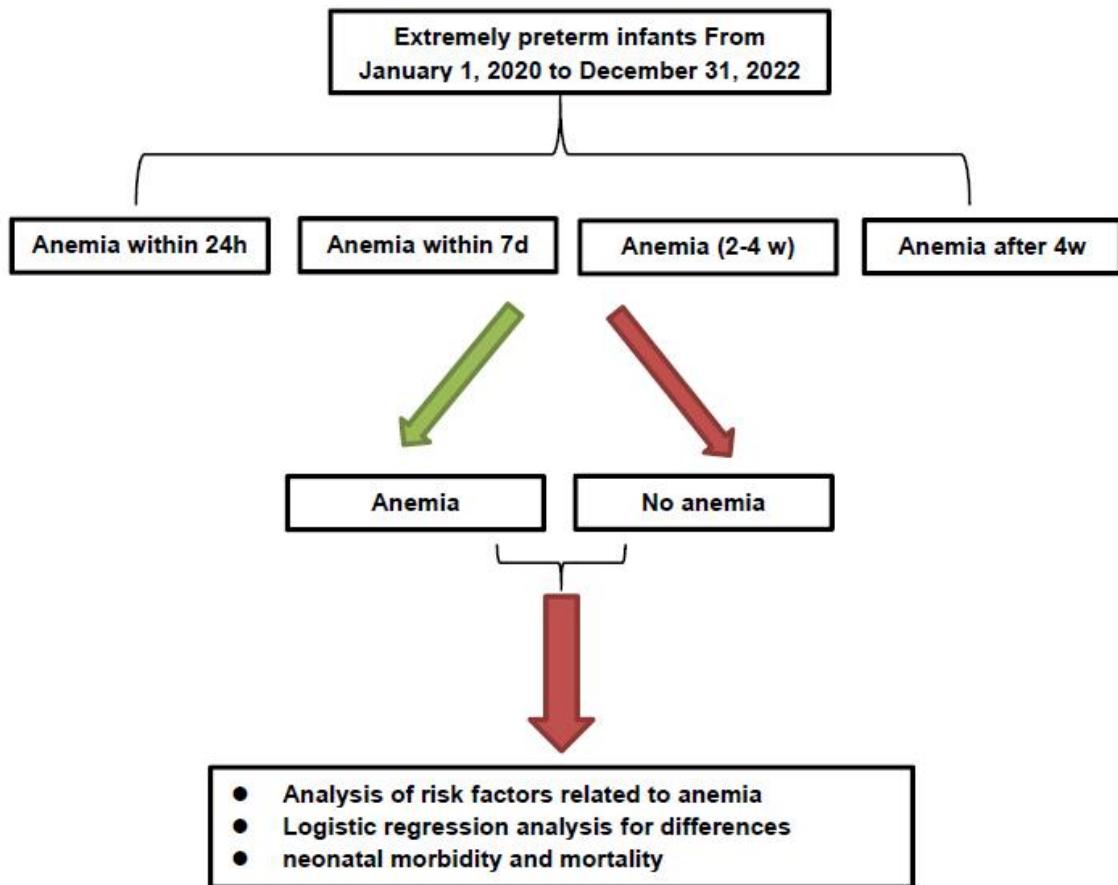
Table 1. List of study outcomes and endpoints.

Outcomes	Endpoints
General information	<ul style="list-style-type: none"> ● Sex (male or female) ● Pregnancy mode (In vitro fertilization or not) ● Chorioamnionitis (Yes or not) ● GBS colonization (Yes or not) ● Early onset sepsis (Yes or not) ● Antenatal steroids (Yes or not) ● Delivery mode (natural delivery or cesarean section) ● Multiple births (Yes or not) ● Gestational age (weeks) ● Birth weight (grams) ● Mother's hemoglobin level before delivery ● Mother's HCT level before delivery
Neonatal morbidity and mortality	<ul style="list-style-type: none"> ● BPD ● ROP ● NEC ● Early onset sepsis ● Late onset sepsis ● EUGR ● IVH ● Death

4. Statistical analysis plan

Categorical variables were expressed as proportions n/N (%) and compared using the Chi-square (χ^2) test. Continuous variables were expressed as (mean \pm SD) or median and interquartile range (P25, P75), and compared using Student's t-test or a nonparametric test, as appropriate. Two-tailed p-values < 0.05 were considered statistically significant. A multivariate analysis by logistic regression was performed to evaluate predictors of neonatal morbidities. Statistical analyses were performed using IBM SPSS Statistics, version 20.

Appendix:



References

1. Ohuma EO, Moller AB, Bradley E, et al. National, regional, and global estimates of preterm birth in 2020, with trends from 2010: a systematic analysis. *Lancet*. 2023;402(10409):1261-1271.
2. Venkatesh KK , Lynch CD , Costantine MM ,et al. Trends in active treatment of live-born neonates between 22 weeks 0 days and 25 weeks 6 days by gestational age and maternal race and ethnicity in the US, 2014 to 2020[J]. *JAMA*, 2022,328(7):652-662.
3. Lin HJ, Du LZ, Ma XL, et al. Mortality and morbidity of extremely low birth weight infants in the mainland of China: a multi-center study. *Chin Med J (Engl)*. 2015;128(20):2743-2750.
4. Keir A K, Yang J, Harrison A, et al. Temporal changes in blood product usage in preterm neonates born at less than 30 weeks' gestation in Canada[J]. *Transfusion*, 2015, 55(6):1340-1346.
5. Whyte R, Kirpalani H. Low versus high haemoglobin concentration threshold for blood transfusion for preventing morbidity and mortality in very low birth weight infants[J]. *Cochrane Database of Systematic Reviews*, 2011 (11).
6. Patel R M, Knezevic A, Shenvi N, et al. Association of red blood cell transfusion, anemia, and necrotizing enterocolitis in very low-birth-weight infants[J]. *JAMA*, 2016, 315(9): 889-897.
7. Alajbegovic-Halimic J, Zvizdic D, Alimanovic-Halilovic E, et al. Risk factors for retinopathy of prematurity in premature born children[J]. *Medical Archives*, 2015, 69(6): 409.
8. Ghavam S, Batra D, Mercer J, et al. Effects of placental transfusion in extremely low birthweight infants: meta-analysis of long- and short-term outcomes. *Transfusion*. 2014;54(4):1192-1198.
9. NIH Consensus Development Panel on the Effect of Corticosteroids for Fetal Maturation on Perinatal Outcomes. Effect of corticosteroids for fetal maturation on perinatal outcomes. *JAMA*. 1995;273(5):413-418.
10. MacDonald H. American Academy of Pediatrics, Committee on Fetus and Newborn. Perinatal care at 6 G. ROCHA ET AL. the threshold of viability. *Pediatrics*. 2002;110(5):1024-1027.
11. Fenton TR, Kim JH. A systematic review and meta-analysis to revise the Fenton growth chart for preterm infants. *BMC Pediatr*. 2013;13:59.
12. Subspecialty Group of Neonatology Pediatric Society Chinese Medical Association; Editorial Board Chinese Journal of Pediatrics. [Protocol for diagnosis and treatment of neonatal septicemia]. *Zhonghua Er Ke Za Zhi*. 2003;41(12):897-899.
13. Volpe JJ. *Neurology of the newborn*. 4th ed. Philadelphia (PA): WB Saunders; 2001. p. 428-493.
14. Jobe AH, Bancalari E. Bronchopulmonary dysplasia. *Am J Respir Crit Care Med*. 2001;163(7):1723-1729.
15. Walsh MC, Kliegman RM. Necrotizing enterocolitis: treatment based on staging criteria. *Pediatr Clin North Am*. 1986;33(1):179-201.
16. An international classification of retinopathy of prematurity. *Pediatrics*. 1984;74(1):127-133.
17. International Committee for the Classification of Retinopathy of Prematurity. The International Classification of Retinopathy of Prematurity revisited. *Arch Ophthalmol*. 2005;123(7):991-999.