Retrospective case-control study of necrotizing enterocolitis and feeding intolerance in premature infants receiving packed red blood cell transfusions

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Short Title: Retrospective case-control study of NEC and FI in premature receiving PRBC transfusions

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Abstract

Background and Aims: The study objectives were to compare the risk factors and clinical characteristics of necrotizing enterocolitis (NEC) or feeding intolerance (FI) in premature infants after packed red blood cell (PRBC) transfusion and to explore the relationship between PRBC transfusion and NEC and FI.

Methods: A retrospective analysis was conducted of premature infants with gestational age < 32 weeks born at our hospital, between January 2018 and December 2019. The infants were divided into three groups according to the occurrence of NEC and FI within 48 hours following PRBC transfusion: transfusion associated necrotizing enterocolitis (TANEC) group, transfusion associated feeding intolerance (TAFI) group, and control group. The maternal factors of perinatal pregnancy, data on the infants' general condition, complications were reviewed, and PRBC transfusion data.

Results: (1) The gestational age and birth weight were lower in the TANEC and TAFI groups than in the control group, and the incidence of hsPDA was higher in the TANEC and TAFI groups than in the control group ($P < 0.05$). (2) The average age of infants in the TANEC and TAFI groups at the time of first PRBC transfusion was lower than that in the control group, and infants in the TANEC and TAFI groups received a greater number of PRBC transfusions than infants in the control group ($P < 0. 05$). (3) The birth weight was a protective factor for NEC after PRBC transfusion ($P=0.006$), and the age of the first time of PRBC transfusion is a protective factor for FI after PRBC transfusion ($P=0.033$), and hsPDA is a risk factor for FI after PRBC transfusion ($P=0.006$).

Conclusions: PRBC transfusion in premature infants can cause the occurrence of transfusion-associated NEC or FI, especially in small birth age and low birth weight.The younger the age at the first transfusion, the greater the possibility of transfusion-associated FI, and the combination of hsPDA can increase this risk.

Key words: premature infants; necrotizing enterocolitis; feeding intolerance; transfusion

1.Introduction

Premature infants are prone to anemia, due to their growth and development after birth, the short life span of red blood cells, insufficient iron reserves, and iatrogenic blood loss[1]. At present, packed red blood cell (PRBC) transfusion is one of the most useful methods for treating anemia in premature infants. PRBC transfusion can improve anemia, increase blood supply to important organs, improve cardiopulmonary function, and increase body weight[2]. However, multiple retrospective studies have reported that some premature infants will develop NEC after PRBC transfusion[3-7]. At present, the occurrence of NEC within 48 hours of PRBC transfusion is defined as transfusion-associated necrotizing enterocolitis (TANEC)[8-12]. The pathogenesis of TANEC is currently not well understood. TANEC may occur due to immune activation of intestinal endothelial cells following PRBC transfusion[13] or intestinal ischemia-reperfusion injuries[14] and inflammatory reactions[15], resulting in intestinal mucosal barrier damage and intestinal necrosis.

Feeding intolerance (FI) refers to dyspepsia caused by increased gastric residue, abdominal distension and vomiting[16]. FI is a common gastrointestinal diseases during enteral feeding of premature infants. It is reported that the feeding tolerance of premature infants is affected by gestational age, birth weight, formula feeding, patent ductus arteriosus and caffeine use[17, 18]. In 2020, Sahin et al.[19] found that continued enteral feeding during PRBC transfusion leads to feeding intolerance in some premature infants. At present, it is not clear whether PRBC transfusion is a risk factor for feeding intolerance in premature infants.

The purpose of this study was to explore the incidence of and risk factors for NEC and FI after PRBC transfusion using a retrospective case-control study. This analysis will improve understanding of adverse reactions to PRBC transfusion and could reduce the risk of transfusion-associated NEC or FI.

2.Material and Methods

2.1. Participants

Ethics approval was obtained prior to any data collection. Premature infants born at Neonatal Intensive Care Unit(NICU), Tongji Hospital, affiliated with Huazhong University of Science and Technology between 2018 and 2019 were selected within 24 hours of birth. Inclusion criteria: 1) gestational age < 32 weeks; 2) received at least one PRBC transfusion during hospitalization. Exclusion criteria: 1) patients with congenital malformations of digestive tract and genetic metabolic diseases; 2) patients

with FI or NEC before PRBC transfusion; 3) patients with hospital stays less than 1 week; 4) patients with incomplete clinical data.

The infants were divided into three groups according to the occurrence of NEC and FI within 48 hours of PRBC transfusion: TANEC group (NEC occurred within 48 hours of PRBC transfusion), TAFI group (FI occurred within 48 hours of PRBC transfusion) and control group (infants received PRBC transfused but were not diagnosed with NEC or FI).

2.2. Measurements

2.2.1. Clinical data

The following clinical data wre recorded: gestational age (GA), birth weight (BW), whether the infant was small for gestational age (SGA), whether the infant was part of a multiple pregnancy, the mode of delivery (including vaginal delivery, cesarean section), initial feeding time, feeding method, use of antibiotics and recombinant human erythropoietin (rhEPO), PRBC transfusion data(age at the first transfusion, hemoglobin and hematocrit levels before transfusion), times of PRBC transfusions), hemoglobin and hematocrit levels before NEC or FI onset, age and corrected gestational age at NEC or FI onset, clinical manifestation, treatment and prognosis, and occurrence of periventricular-intraventricular hemorrhage (PIVH), neonatal respiratory distress syndrome (RDS), Hemodynamically significant patent ductus arteriosus(hsPDA), and neonatal septicemia.

2.2.2. Diagnosis

FI:1) gastric retention (stomach residue greater than 30% of the previous feeding volume), accompanied by vomiting and/or abdominal distension, 2) feeding plan failure, including reduced milk volume, milk without increasing for more than two days, or fasting, 3) stomach residue or vomit is a coffee-like fluid or a bile-like substance, fecal occult blood positive (imaging confirmed NEC cases were excluded)[16, 20, 21]. Cases were diagnosed as FI if at least one of the diagnostic criteria were satisfied. NEC: refer to the Bell staging criteria; cases above stage II were included in the present study[22]. hsPDA: diagnosed according to echocardiography; diastolic ductus arteriosus with left-to-right shunt and duct diameter > 1.5mm or left atrial diameter / ascending aortic diameter (LA/AO) ≥ 1.4. and clinical manifestations (including heart murmur, tachycardia, increased pulse pressure difference and hypotension, etc.)[23]. All received ligation.

2.2.3. Transfusion strategy

Blood transfusion indications: according to guidelines[24], PRBC transfusions were carried out according to the judgment of the clinician. All infants were transfused with concentrated red blood cells; PRBC transfusion amounts were 15-20mL/kg, and the transfusions were completed within three to four hours. All infants were enterally fed normally during PRBC transfusion.

2.3. Statistical methods

Continuous variables were analyzed by ANOVA; bivariate data were analyzed by Chi square or Fisher's exact test. Fisher's least significant difference (LSD) test was used for multiple comparisons among the three groups of independent samples. Unordered multiclass logistic regression model was used to analyze the risk factors of NEC or FI after red blood cell transfusion. The test level α=0.05, and P<0.05 indicates a statistically significant difference.

3.Results

3.1. General data

From January 2018 to December 2019, a total of 426 cases of premature infants with GA <32 weeks were hospitalized in our hospital NICU. A total of 185 cases received at least one PRBC transfusion during hospitalization. The PRBC transfusion rate was 43.42% (185/ 426). There were seven cases (7.29%, 7/96) in the TANEC group, 26 cases (27.08%, 26/96) in the TAFI group, and 63 cases (65.63%, 63/96) in the control group. (Figure 1)

The average GA and BW of the TANEC and TAFI groups were significantly lower than those in the control group (P<0.05), Figure 2. The incidence of hsPDA was 42.9% in the TANEC group, 69.2% in the TAFI group, and 36.5% in the control group. The incidence of hsPDA was significantly higher in the TANEC and TAFI groups than in the control group (P=0.019). There were no significant differences in SGA, multiple pregnancy, birth mode, Apgar score, time of first starting feeding, feeding mode, use of antibiotics, rhEPO, PIVH, NRDS and neonatal septicemia among the three groups (Table 1).

In pairwise comparisons, the GA and BW in TANEC group and TAFI group were significantly lower than those in control group (P < 0.05). There were no significant differences in GA and BW between the TANEC group and the TAFI group (P > 0.05). The incidence of hsPDA in the TAFI group was significantly higher

than that in the control group (P<0.05). The incidence of hsPDA was higher in the TANEC group than in the control group; however, this difference was not statistically significant (P>0.05).

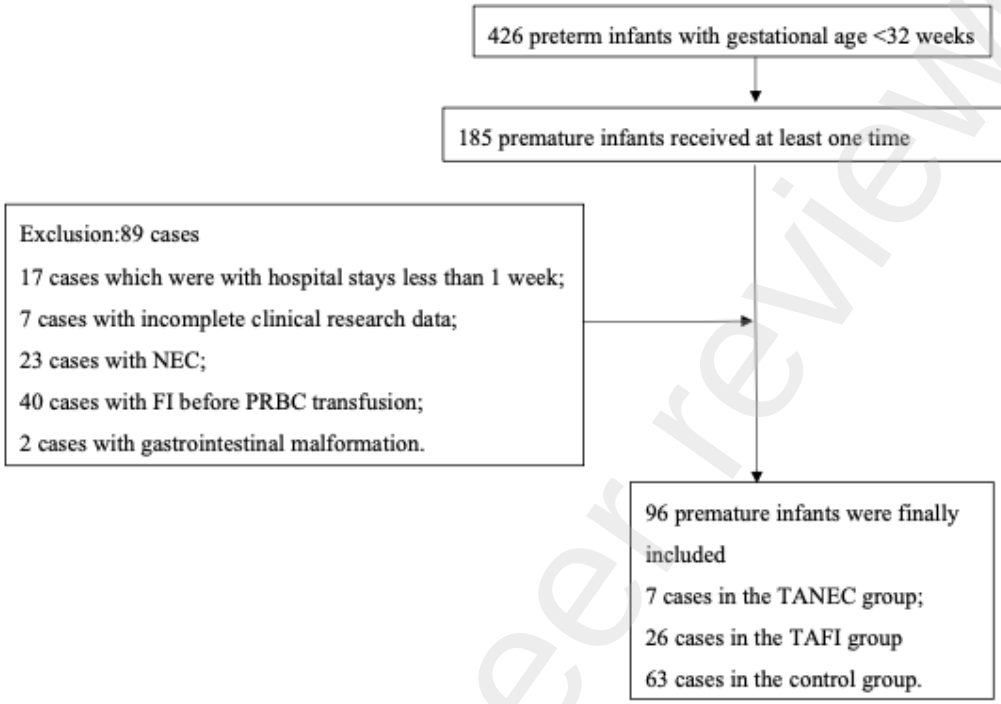

Figure 1 Patient and control selection flow chart

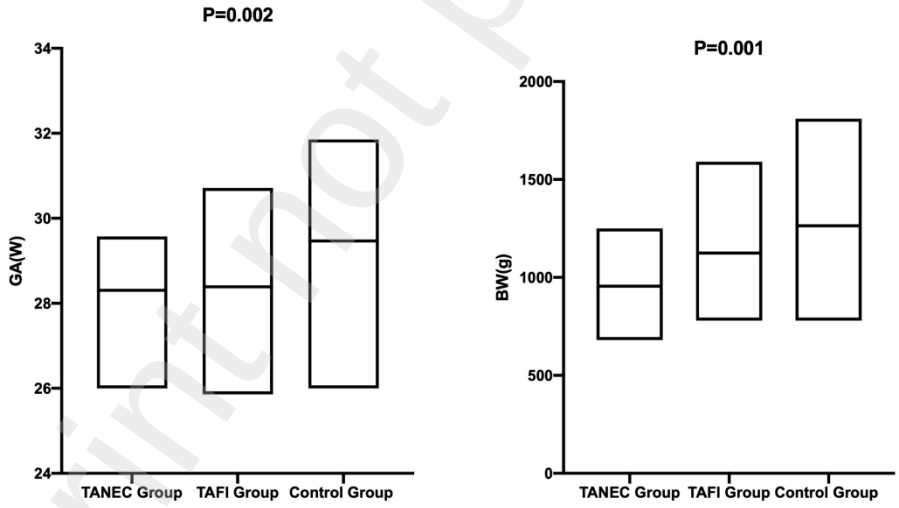

Figure 2 Comparison of GA and BW among the three groups

Table 1 General data among the three group

	TANEC group （n=7）	TAFI group (n=26)	Control group (n=63)	F/χ^2 value	P value
GA（w）	28.30±1.37	28.39±1.32	29.47±1.41	6.793	0.002
BW（g）	955.71±196.71	1124.2±200.40	1263.17±233.11	8.254	0.001

SGA[n（%）]	3（42.9%）	4（15.4%）	8（12.7%）	3.929	0.120
multiple pregnancy [n（%）]	2（28.6%）	10（38.5%）	26（41.3%）	0.419	0.891
mode of delivery [n（%）]				0.447	0.874
vaginal delivery	2（28.6%）	9（34.6%）	18（28.6%）		
cesarean section	5（71.4%）	17（65.4%）	45(71.4%)		
Apgar 1’	5.57±1.90	5.73±1.75	5.52±2.00	0.105	0.900
Apgar 5’	6.86±1.21	6.96±1.68	7.11±1.29	0.177	0.838
initial feeding time [M（P_{25}，P_{75}）,d]	3.0（3.0，3.0）	3.0（2.0，4.0）	3.0（2.0，3.0）	1.219	0.550
feeding method [n（%）]				1.441	0.467
Breastfeeding	5(71.4%)	17(65.4%)	34(54.0%)		
formula	2(28.6%)	9(34.6%)	29(46.0%		
Prophylactic antibiotics[n（%）]	6(85.7%)	21(80.8%)	57(90.5%)	1.955	0.392
EPO[n（%）]	1(14.3%)	9(34.6%)	21(33.3%)	0.955	0.641
PIVH	5(71.4%)	19（73.1%）	46（73.0%）	0.157	1.000
RDS	6(85.7%)	20（76.9%）	42（66.7%）	1.462	0.503
hsPDA	3(42.9%)	18（69.2%)	23（36.5%）	7.933	0.019
neonatal septicemia	3(42.9%)	4（15.4%）	8（12.7%）	3.929	0.120

3.2. Comparison of the age at time of first PRBC transfusion and the number of PRBC transfusions

The average ages at time of first PRBC transfusion were 18.43±11.63 days in the TANEC group, 17.92±11.97 days in the TAFI group, and 26.54±14.08 days in the control group. The average number of PRBC transfusions during hospitalization were 3.0 (2.0, 5.0) for the TANEC group, 2.5 (2.0, 4.0) for the TAFI group, and 1.0 (1.0, 2.0) for the control group. The age at the time of first PRBC transfusion was lower in the

TANEC and TAFI groups than in the control group, and the TANEC and TAFI groups received a greater number of PRBC transfusions during hospitalization than the control group (P<0.05). There were no significant differences between the three groups in the average hemoglobin and the average hematocrit levels before the first PRBC transfusion (P>0.05) (Table 2).

In pairwise comparisons, age at the time of first PRBC transfusion in the TAFI group was significantly lower than that in the control group (P<0.05), and there was no significant difference in age at time of first PRBC transfusion between the TANEC group and the control group (P>0.05). The TANEC and TAFI groups received more PRBC transfusions during hospitalization than the control group (P<0.05), and there was not a significant difference in the number of PRBC transfusions received between the TANEC and TAFI groups (P>0.05).

Table2 Transfusion data among three groups

	TANEC group(n=7)	TAFI group(n=63)	Control group(n=63)	F value	P value
The age of first PRBC transfusion（d）	18.43±11.63	17.92±11.97	26.54±14.08	4.227	0.017
The average hemoglobin before first PRBC transfusion（g/L）	89.92±8.60	94.92±16.03	92.82±16.68	0.31	0.734
The average hematocrit before first PRBC transfusion（%）	26.84±3.09	28.63±4.99	27.95±5.20	0.388	0.69
The numbers of transfusion [M（P_{25}，P_{75}）]	3.0（2.0，5.0）	2.5（2.0，4.0）	1.0（1.0，2.0）	27.545	<0.001

3.3. PRBC transfusion data before NEC or FI in TANEC group and TAFI groups

The TANEC group received an average of 1.57±0.787 PRBC transfusions before the occurrence of NEC, and the TAFI group received an average of 1.88±1.03 before the occurrence of FI. NEC after first PRBC transfusion accounted for 57.1%(4 out of

7) of NEC cases, and FI after the first PRBC transfusion accounted for 46.2%(12 out of 26) of FI cases. The average hemoglobin before NEC or FI was 86.44±7.91g/L or 91.11±8.64g/L, respectively, and the average hematocrit before NEC or FI was 26.21±3.12% or 27.27±3.02%, respectively (Table 3).

Table 3 PRBC transfusion data before NEC or FI in TANEC group and TAFI groups

	TANEC group (n=7)	TAFI group (n=26)
The average number of PRBC transfusion before onset	1.57±0.787	1.88±1.03
Onset after the first PRBC transfusion[n（%）]	4（57.1%）	12（46.2%）
Onset after the second PRBC transfusion [n（%）]	2（28.6%）	9（34.6%）
Onset after the third PRBC transfusion [n（%）]	1（14.3%）	3（11.5%）
Onset after the 4th PRBC transfusion [n（%）]	0（0%）	2（7.7%）
the average hemoglobin before onset（g/L）	86.44±7.91	91.11±8.64
the average hematocrit before onset（%）	26.21±3.12	27.27±3.02

3.4. Multivariate analysis of influencing NEC or FI after PRBC transfusion

The indicators found to be statistically significant in univariate analysis, including GA, BW, hsPDA, and age at the time of the first PRBC transfusion, were included in an unordered multivariate logistic regression analysis model for multivariate analysis. The variables "whether NEC or FI occurred after PRBC transfusion " were used as the dependent variable, and GA, BW, hsPDA, and age at the time of the first PRBC transfusion were used as independent variables. (Figure 3, Figure 4)

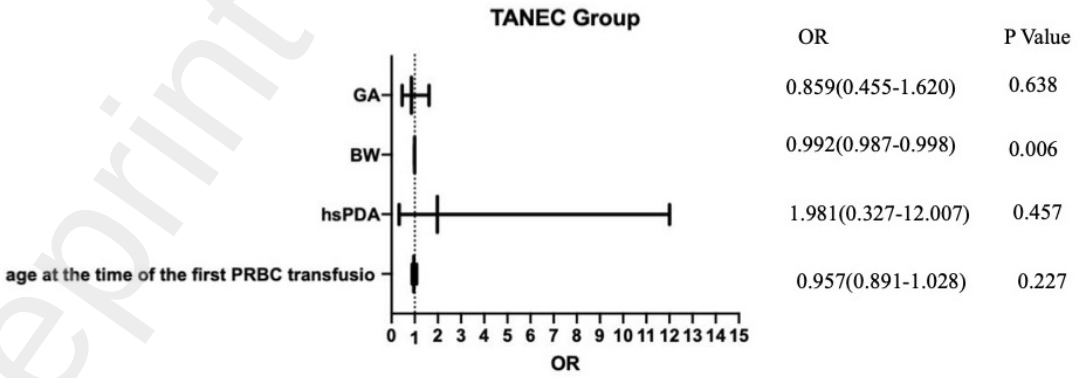

Figure 3 The forest illustration of TANEC Group

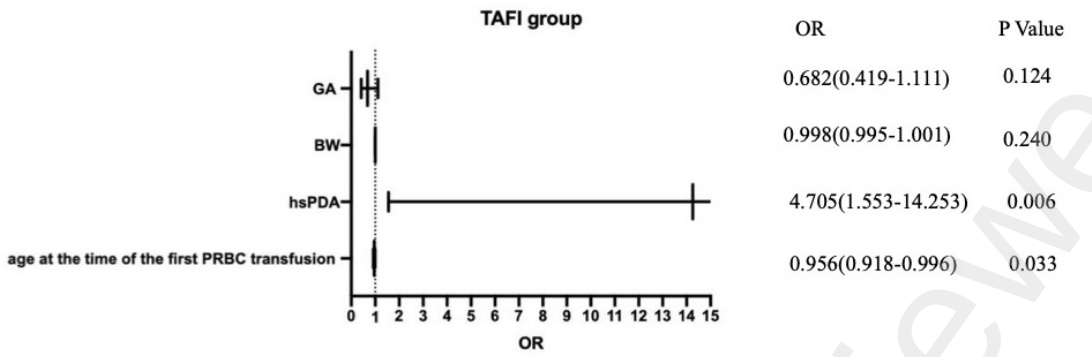

Figure 4 The forest illustration of TAFI Group

4. Discussion

Premature infants are prone to anemia due to their rapid growth and development, the short life span of red blood cells, and iatrogenic blood loss. Hypoxia can easily occur in the early postnatal period due to immature cardiopulmonary function and lack of alveolar surfactants; therefore, it is necessary to maintain high hemoglobin levels. For this reason, premature infants are the primary PRBC transfusion population in the NICU[1, 25, 26]. Valieva et al.[27] found that 78% of extremely low birth weight infants received PRBC transfusions while hospitalized. An Australian population-based study found that 60% of premature infants with GA less than 32 weeks received transfusions during hospitalization[28]. In the present study, 43.42% of the premature infants with gestational age less than 32 weeks in our hospital received PRBC transfusions. PRBC transfusion can increase hemoglobin content and improve tissue oxygenation and cardiopulmonary function[29], but PRBC transfusion in premature infants also carries risks such as metabolic disorders, hemolysis, allergic reactions, infections, and adverse outcomes[30].

In 1987, McGrady first reported in retrospective case studies that PRBC transfusion can increase the risk of NEC (OR=15.1, 95% CI=2.95-92.51)[3]. In 1998, it was reported that the incidence of NEC in NICUs with high PRBC transfusion rates (OR=1.1, 95% CI = 0.5-2.2) was significantly higher than the incidence in NICUs with low PRBC transfusion rates (OR=0.3, 95%CI=0.1-0.8)[4]. Over the past decade, there has been a substantial focus on premature infants, and a number of studies have reported on the relationship between NEC and PRBC transfusion[31-35]; however, there are few studies examining the relationship between PRBC transfusion and FI. Therefore, the present study compared clinical data from the TANEC, TAFI, and control groups to explore the relationship between PRBC transfusion and NEC or FI. The present study

found that the incidence of TANEC after PRBC transfusion was 7.29% (7/96), and the incidence of FI after PRBC transfusion was 27.08% (26/96). Lower gestational age, lower birth weight, and younger age at time of first blood transfusion were associated with greater risk of NEC or FI after transfusion. The cooccurrence of hsPDA further increased the risk of NEC or FI after PRBC transfusion.

A number of retrospective studies have found that TANEC often occurs in premature infants with a low GA and low BW[6, 7, 12, 13]. This is believed to be due to hypoplasia of the intestinal tissue and the immature blood flow regulation function in preterm infants. Studies have shown that compared with full-term infants, preterm infants have reduced nitric oxide synthesis, delayed development of vasodilation capacity, and decreased intestinal oxygen storage, all of which can lead to decreased intestinal oxygen saturation when the arterial pressure is reduced and result in intestinal ischemia and hypoxia damage[36]. Under normal circumstances, eating can cause vasodilation, increase intestinal blood flow and oxygen transport, and satisfy needs for intestinal digestion and absorption. However, Krimmel et al.[37] found that PRBC transfusion in premature infants whose birth weight is less than 1250g can inhibit the increase in mesenteric blood flow after a meal. Similarly, Marin et al.[38] used near-infrared spectroscopy and found that premature infants who received enteral feeding during PRBC transfusion had postprandial intestinal tissue oxygen saturation within 15 hours after the completion of the transfusion and saturation levels decreases. These findings show that due to the delayed development of intestinal motor function and the imperfect development of enteric nerve regulation in premature infants, the time of intestinal congestion after PRBC transfusion. This delay makes the intestine unable to effectively decompose and absorb food after eating, thereby causing gastric emptying delays, prolonged intestinal transit, abdominal distension, and related symptoms. Studies have shown that if nutrients remain in the intestinal lumen for too long, these undigested contents can cause neutrophil chemotaxis, produce toxic substances, and allow invasive bacteria to bypass the intestinal mucosal barrier, thereby increasing the risk of NEC or FI[39-41]. The present study shows that there is a risk of NEC and FI after PRBC transfusion. The gestational age and birth weight of the TANEC and the TAFI groups were significantly lower than those of the control group.

Ledo et al.[42] showed that in premature infants with hsPDA, the left-to-right shunt of blood through the arterial catheter can lead to increased pulmonary blood flow and

insufficient systemic blood perfusion, resulting in decreased mesenteric arterial blood flow. To meet and support the high nutritional requirements for growth and development, there is a high demand for oxygen in the intestinal tract of premature infants. However, due to the presence of hsPDA, the blood flow of the systemic circulation is reduced, and the intestinal perfusion pressure is insufficient. Consequently, there is insufficient oxygen flow to the intestine. When the needs of normal intestinal metabolism cannot be met in premature infants, intestinal feeding cannot be carried out smoothly, and this increases the risk of NEC or FI[36]. Studies by Gupta et al. showed that the superior mesenteric artery blood flow of premature infants with hsPDA was significantly reduced four hours after transfusion[43]. Some studies have found that the incidence of PDA is higher in infants who develop NEC after PRBC transfusion than in those who do not develop NEC[6, 8], and the rate of PDA ligation is also higher in infants who develop NEC[44]. In the present study, the incidence of hsPDA in the TANEC and TAFI groups was significantly higher than that in the control group, which is consistent with existing literature.

In the present study, infants in the TANEC and TAFI groups received more PRBC transfusions during hospitalization than infants in the control group ($P<0.05$). In the TANEC group, infants who had received multiple PRBC transfusions accounted for 42.9% (3/7) of cases, and in the TAFI group, infants who had received multiple PRBC transfusions accounted for 53.8% (14/26) of cases. Teisrskas et al.[45] reported that the occurrence of NEC was related to the number of PRBC transfusions before the onset (OR=1.5, 95%CI=1.0-2.2, P=0.04). A retrospective study conducted by Bak et al. found that the risk of NEC increased with increased frequency of PRBC transfusions prior to NEC diagnosis (OR=1.63, 95%CI=1.145-2.305, P=0.007)[46]. One possible explanation for the increased risk of NEC from blood transfusions is an inflammatory reaction caused by damage of stored red blood cells during transfusion. Studies have shown that the plasticity and deformability of red blood cells is reduced during storage, which makes it difficult for red blood cells to pass through capillaries and splenic sinus, where they are then swallowed by macrophages and cleared[47, 48]. Moreover, red blood cells with poor deformability may take longer to transport oxygen into the microcirculation, resulting in an inability to effectively supply oxygen to the tissues. This prolonged time of oxygen transport may also increase blood viscosity, leading to intestinal microcirculation disorders[47]. Ho et al.[49] found that after PRBC transfusion in preterm

infants, the levels of fecal calprotectin, derived from activated neutrophils, increased in the intestinal mucosa and lumen. That study indicated that PRBC transfusion It can cause inflammation of the neonatal intestinal mucosa. In very small preterm infants, the excessive mucosal response may be clinically manifested as NEC. A number of studies have also reported that blood transfusion can cause transfusion-related immunomodulation, which can trigger endothelial cell activation and cause related immune activation, leading to the release of related pro-inflammatory factors and causing intestinal tissue damage[50, 51].

Josephson et al.[6] reported that PRBC transfusion can increase the risk of late-onset NEC (onset age ≥ 4 weeks) (OR=14.5; 95%CI=4.8-43.6; P<0.0001), and the a younger gestational age is associated with a greater likelihood of late-onset NEC. Blau et al.[31] found that the average age at onset of infants with TANEC at onset was 30±5 days, and the average corrected gestational age at onset was 30±1weeks. In the present study, the average age at onset and the average corrected gestational age of infants in the TANEC and TAFI groups were older than in other studies. During the development and maturation of premature infants, hypoxia and lengthening of the intestine can promote the expression of vascular endothelial growth factor-A, thereby promoting intestinal angiogenesis. The newly formed blood vessels are immature, and once they encounter risk factors such as increased blood viscosity, decreased red blood cell deformability, and hemodynamic changes, these newly formed blood vessels are easily damaged. This could thereby increase the susceptibility of premature infants to NEC or FI[13, 52].

5. Conclusion

In summary, premature infants in the TANEC and TAFI groups had a low gestational age and low birth weight, indicating that the premature infants were still immature at the onset of NEC and FI within 48 hours following PRBC transfusion. The age at the time of first PRBC transfusion in the TANEC and TAFI groups was younger than in the control group. The proportion of infants who received multiple PRBC transfusions was higher in the TANEC and TAFI groups than in the control group, indicating that infants may have cardiopulmonary dysfunction, high PRBC transfusion needs. At the same time, multiple PRBC transfusions may cause inflammation, thereby damaging the intestinal mucosa and increasing the incidence of NEC or FI. Premature infants with hsPDA had an elevated risk of NEC or FI following PRBC transfusion. But the present study is a single-center study with a small sample size. The storage

method on red blood cell suspensions, preservation solution and storage time were not grouped and analyzed. Future studies should include relevant data and expand sample sizes for more robust conclusions.

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Authors' contributions
All authors have read and approved the manuscript.
Zhu Yanhong: Collected the data, analyzed and interpreted the data and drafted the manuscript.
Chen Lin: Designed the study, revised and approved the manuscript.

Competing interests
The authors declare that the have no competing interests

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Abbreviations
PRBC: packed red blood cell
NEC: necrotizing enterocolitis
FI: feeding intolerance
hsPDA: hemodynamically significant patent ductus arteriosus
NICU: neonatal intensive care unit
GA: gestational age
BW: birth weight
SGA: small for gestational age
rhEPO: recombinant human erythropoietin
PIVH: periventricular-intraventricular hemorrhage
NRDS: neonatal respiratory distress syndrome

Ethics approval and consent to participate

Institutional review board of Tongji Hospital approved this retrospective study (IRB number, TJ-IRB20210345) and the requirement for obtaining informed consent was waived. All methods were performed in accordance with the relevant guidelines and regulations.

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