Fukushima J. Med. Sci.,
Vol. 69, No. 2, 2023

[Original article]

Risk factors for anemia of prematurity among 30–35-week preterm infants

Hiroki Kitaoka[1)2)], Yoshihiko Shitara[2)], Kohei Kashima[2)], Shingo Ochiai[1)], Hayato Chikai[1)3)], Keiko Watanabe[1)], Hiroto Ida[1)], Tadayuki Kumagai[1)] and Naoto Takahashi[2)]

[1)]Department of Pediatrics, Yaizu City Hospital, Shizuoka, Japan, [2)]Department of Pediatrics, The University of Tokyo Hospital, Tokyo, Japan, [3)]Department of Neonatology, Tokyo Metropolitan Bokutoh Hospital, Tokyo, Japan

(Received May 23, 2022, accepted March 22, 2023)

Abstract

Background : The risk factors for anemia of prematurity (AOP) among late preterm infants are unelucidated. We identified risk factors for declining hemoglobin (Hb) concentration and triggering factors for AOP treatment in infants born at 30–35 gestational weeks.

Methods : From 2012 to 2020, we conducted a single-center retrospective study of infants born at 30–35 weeks of gestation without congenital anomalies or severe hemorrhage. The primary outcome was AOP development, defined by initiation of treatments including red blood cell transfusion, subcutaneous injections of erythropoietin, and iron supplementation. A multivariable logistic regression model was used to investigate potential risk factors for AOP.

Results : A total of 358 infants were included. Lower gestational age (odds ratio, 0.19 ; 95% confidence interval 0.11–0.32), small for gestational age (SGA ; 7.17, 2.15–23.9), low maternal Hb level before birth (0.66, 0.49–0.87), low Hb at birth (0.71, 0.57–0.89), and multiple large blood samplings (1.79 ; 1.40–2.29) showed significantly higher odds for AOP development.

Conclusions : Gestational age, SGA, low maternal Hb before birth, Hb at birth, and high number of large blood samplings were positively associated with AOP development in infants born at 30–35 gestational weeks.

Key words : anemia of prematurity, erythropoietin, iron deficiency, late preterm, phlebotomy

Introduction

Anemia of prematurity (AOP) is a common complication in preterm infants and is characterized by reduced erythropoietin (EPO) production, reduced red blood cell (RBC) lifespan, and a hypo-regenerative bone marrow[1,2)]. Almost all extremely low birth weight (ELBW) infants experience a pronounced decline in hemoglobin (Hb) concentration, and nearly 80% of ELBW infants receive RBC transfusion[2,3)]. On the other hand, very-, moderate-, and late-preterm infants, but not ELBW infants, also often experience a remarkable decline in Hb concentration, although it is less severe than that in ELBW infants. Recombinant human EPO and/or iron supplementation have been used as preventive or therapeutic measures for AOP, and the time of initiation of these procedures in ELBW infants has been discussed in previous reports[4)]. The effectiveness of these treatments for AOP among very low birth weight or low birth weight infants has also been shown in previous studies, and recombinant human EPO and/or iron supplementation has been initiated for AOP in infants other than those with ELBW[5,6)].

The pathophysiology and risk factors for AOP among ELBW infants have been studied[1)], and phlebotomy loss has been reported as a major contributor to AOP. Recently, a reduction in excessive loss of blood to prevent AOP has been discussed[7)]. Other findings can be used to evaluate hematopoiesis

Corresponding author : Hiroki Kitaoka E-mail : kitaoka.hir@gmail.com

and anemia, including Hb count, hematocrit (Ht), and reticulocyte count (Ret). However, the role of these factors in AOP among moderate- to late-preterm infants is unclear. Additionally, the influence of other perinatal conditions, such as small for gestational age (SGA) and breastfeeding status are not well elucidated. Because adequate initiation of treatment for AOP may lead to better neurodevelopmental outcomes even in non-ELBW preterm infants[8], identification of the risk factors of AOP in ELBW and larger preterm infants is important. If a higher risk of AOP can be predicted from the birth situation, neonate characteristics, or laboratory data at birth, earlier initiation of treatment and careful management to prevent anemia can be considered. For this reason, it is also important to understand the progress of interventions for AOP during admission. Therefore, we aimed to explore potential risk factors for declining Hb concentration and the triggering factors for AOP treatment among preterm infants born at a gestational age of 30-35 weeks.

Materials and methods

All infants born at a gestational age of 30-35 weeks in Yaizu City Hospital from April 1, 2012, to April 30, 2020 were candidates for this single-center retrospective cohort exploratory study. Yaizu City Hospital is a regional perinatal medical center in Japan that mainly treats infants with a gestational age above 30 weeks and birth weight above 1,000 g. Infants with major chromosomal abnormalities, congenital hematologic diseases, or severe hemorrhage and those who were transferred to another hospital after birth due to the need for more intensive care or other reasons were excluded. From the medical records, we extracted data on the following variables : sex, gestational age, birth weight and height, delivery method, multiple pregnancies, presence of placental abruption, laboratory data at birth (Hb, Ht, mean corpuscular volume [MCV], mean corpuscular hemoglobin concentration [MCHC], and absolute Ret, treatment for jaundice, Hb at initiation of treatment, days of initiation of treatment, breastfeeding status, treatment intervention for anemia during admission, and number of blood samplings. These variables were selected on the basis of our clinical perspective and previous reports[1,2]. Although the decision to initiate treatment was made by the doctors, our institutional guidelines recommend starting RBC transfusion at an Hb level of 8 g/dL, and EPO and iron supplementation at an Hb level of 12 g/dL. EPO was injected subcutaneously at 200 U/kg/dose twice a week and iron supplementation was prescribed at 6 mg/kg/day.

All blood samples were collected using venipuncture or heel-prick methods. Although complete blood counts using blood from different sites are known to show differences in composition[9-11], we could not categorize these differences since this was a retrospective study. Hb, Ht, MCV, MCHC and Ret were measured using a Sysmex XE-2100 (Sysmex Corporation, Kobe, Japan). Infants who received ≥80% breast milk were considered to be in the condition of "high-intensity breastfeeding," according to a previous study[12]. Birth weight and height were converted into two categories : (i) appropriate for gestational age or (ii) SGA based on standard values reported by the Japan Pediatric Society[13]. Infants classified as light for gestational age were also included in the SGA category. Each blood sampling was classified as "small sampling" or "large sampling" based on the sampling amount. Acquisition of a single blood sample by one capillary tube was defined as a "small sampling," and sampling of higher blood volumes was defined as a "large sampling." Small and large samplings were counted separately, and these categories were classified by inspection items in medical records. The estimated amounts of blood loss in small and large samplings were <0.3 and ≥0.3 mL, respectively. The present study was approved by the institutional review board of Yaizu City Hospital (approval No. 233), and informed consent was obtained in the form of an opt-out on the website. Those who refused to provide consent were excluded.

Statistical analysis

Development of AOP was defined by the initiation of any treatment for AOP, including RBC transfusion, subcutaneous injections of EPO, and iron supplementation. First, univariable analysis was performed using the χ^2 (chi-square) test for categorical variables, the unpaired Student's t-test and Mann-Whitney U test for continuous variables. To examine associations between the development of AOP and variables, a multivariable logistic regression model was constructed while adjusting for potential confounding factors, including sex, gestational age, SGA status, cesarean delivery, twin birth, placental abruption, last maternal Hb before birth, laboratory data at birth, breastfeeding status, treatment for jaundice, and number of blood samplings. Multivariable logistic regression analysis was also used

to examine the associations between the initiation of subcutaneous EPO or iron supplementation and variables adjusted for the same potential confounders. Infants who received monotherapy with EPO or iron supplementation or who received combination therapy with EPO and iron supplementation were both included in the treated groups. To estimate the performance of the models, statistically significant variables were selected from the multivariable regression models, and a receiver operating characteristic (ROC) analysis of AOP development was performed. The cutoff point was obtained using Youden's index analysis. The sensitivity and specificity at the cutoff point were also calculated. Because sampling times could be strongly associated with gestational age, we evaluated interactions between gestational age and sampling times. No tested interaction was significant ; therefore, we did not use any interaction term in the multivariate model. In addition, a sensitivity analysis was performed to define the development of AOP, which was defined by the lowest Hb level during admission <12 g/dL during hospitalization. Associations between the development of AOP and daily weight gain or duration of parenteral nutrition were also investigated using the Mann–Whitney *U* test. The threshold for significance was set at $p < 0.05$. All statistical analyses were conducted using STATA/BE 17.0 (StataCorp, College Station, Texas, USA).

Results

Of the 407 infants enrolled in the present study, we excluded 39 infants (nine were transferred to other hospitals, three had unknown gestational ages, one died on day 0 due to severe respiratory distress syndrome, two had trisomy 21, one had hemophilia A, and 33 had missing information) ; the remaining 358 infants were included in the analysis (Figure 1). We performed a complete case analysis. The patient characteristics are shown in Table 1. The infants were delivered at a median gestation period of 34.4 weeks (interquartile range [IQR], 33.3–35.3 weeks), and their average birth weight was 1,993 ± 397 g. Moreover, 21% of the infants (n = 75/358) were categorized as showing an SGA status, 23% (n = 84/358) had a twin birth, and 75% (n = 269/358) were born via cesarean delivery. The Hb at birth was 16.9 ± 2.2 g/dL, and the Ret at birth was 25.1 ± 6.5 × 10^4/μL. Among the included children, 44% (n = 158/358) were treated for AOP. The Hb at treatment initiation was 12.6 ± 1.5 g/dL, and the corresponding values at the initiation of EPO and iron supplementation were 13.0 ± 1.3 g/dL and 12.7 ± 1.5 g/dL, respectively.

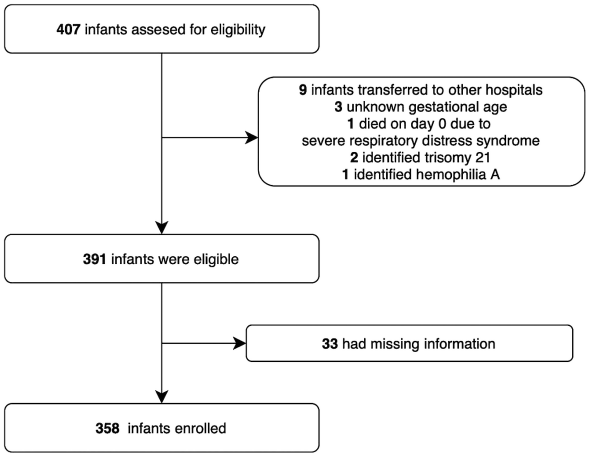

Fig. 1. Infant selection flow chart

Results of the univariable analysis and multivariable logistic regression model of the primary outcome and treatment of AOP are shown in Table 2. In the univariable analysis, gestational age (p <0.001), SGA status (p = 0.004), cesarean delivery (p <0.001), maternal Hb before birth (p = 0.005), high-intensity breastfeeding (p <0.001), number of large samplings (p <0.001), and number of small samplings (p <0.001) were significantly associated with the development of AOP. Laboratory data at birth, such as Hb (p = 0.03), MCV (p <0.001), MCHC (p = 0.03), and Ret (p = 0.007) were significantly associated with AOP treatment. Ht was excluded from the multivariable analysis because of its high multicollinearity (variance inflation factor > 10). In multivariable analysis, SGA infants required significantly higher administration of agents for AOP treatment (odds ratio [OR], 7.17 ; 95% confidence interval [CI], 2.15–23.9). Lower maternal Hb before birth (OR, 0.66 ; 95% CI, 0.49–0.87), infants with low Hb at birth (OR, 0.71 ; 95% CI, 0.57–0.89), and a higher number of large samplings (OR, 1.79 ; 95% CI, 1.40–2.29) also had significantly higher odds of receiving treatment for AOP. Cesarean delivery, treatment for jaundice, number of small samplings, MCV, MCHC, and Ret at birth were not significantly associated with the treatment of AOP. Results of the sensitivity analysis are shown in Table 3. Similar to the findings for the primary outcome, SGA, Hb at birth, and number of large samplings were significantly correlated with higher odds of minimum Hb <12 g/dL during hospitalization. On the other hand, maternal Hb before birth was not significantly correlated with higher odds of the outcome.

Table 1. Baseline characteristics of participants

	All participants (n = 358)
Sex, male/female (n)	193/165
Gestational age (weeks)	34.4 (33.3–35.3)
Birth weight (g)	1,993 ± 397
Birth height (cm)	43.8 ± 2.8
SGA, n (%)	75 (21)
Caesarian section, n (%)	269 (75)
Twin birth, n (%)	84 (23)
Placental abruption, n (%)	14 (3.9)
Maternal Hb before birth (g/dL), mean (SD)	10.9 ± 1.4
Laboratory data at birth	
Hb (g/dL)	16.9 ± 2.2
Ht (%)	49.4 ± 6.4
MCV (fL)	108.6 ± 6.1
MCHC (%)	34.2 ± 1.0
Ret ($\times 10^4$/μL)	25.1 ± 6.5
High intensity breastfeeding, n (%)	103 (29)
Treatment for jaundice, n (%)	220 (61)
No. of blood samplings	
Large sampling (blood volume ≥ 0.3 mL)	5 (3–7)
Small sampling (blood volume < 0.3 mL)	12 (8–15)

Continuous data are expressed as median (interquartile range) for gestational age, large sampling, and small sampling or mean ± SD for other variables. SGA, small for gestational age ; Hb, hemoglobin ; Ht, hematocrit ; MCV, mean corpuscular volume ; MCHC, mean corpuscular volume ; Ret, absolute reticulocyte count

Table 2. Patients' primary outcomes

	Treated (n = 158)	Not treated (n = 200)	P-value	Adjusted OR (95% CI)	Adjusted P-value
Male, n (%)	78 (49)	115 (58)	0.13	0.93 (0.43–2.01)	0.86
Gestational age (weeks), median (IQR)	33.2 (31.9–34.0)	35.1 (34.6–35.6)	<0.001	0.19 (0.11–0.32)	<0.001
SGA, n (%)	44 (28)	31 (16)	0.004	7.17 (2.15–23.9)	0.001
Caesarian section, n (%)	133 (84)	136 (68)	<0.001	1.52 (0.54–4.22)	0.43
Twin birth, n (%)	41 (26)	43 (22)	0.32	1.52 (0.61–3.80)	0.37
Placental abruption	9 (5.7)	5 (2.5)	0.12	5.27 (0.75–36.8)	0.09
Maternal Hb before birth (g/dL), mean (SD)	10.7 (1.5)	11.1 (1.3)	0.005	0.66 (0.49–0.87)	0.004
Laboratory data at birth					
Hb (g/dL), mean (SD)	16.7 (2.3)	17.1 (2.1)	0.03	0.71 (0.57–0.89)	0.003
MCV (fL), mean (SD)	110 (6.8)	107.6 (5.2)	<0.001	0.94 (0.88–1.01)	0.12
MCHC (%), mean (SD)	34.3 (1.2)	34.3 (0.92)	0.03	0.93 (0.64–1.35)	0.69
Ret ($\times 10^4$/μL), mean (SD)	26.1 (7.3)	24.4 (5.7)	0.007	0.94 (0.88–1.01)	0.10
High intensity breastfeeding, n (%)	67 (42)	36 (18)	<0.001	0.49 (0.21–1.14)	0.10
Treatment for jaundice, n (%)	126 (80)	94 (47)	<0.001	0.80 (0.30–2.10)	0.65
Number of blood samplings, median (IQR)					
Large sampling	7 (6–9)	3 (1–5)	<0.001	1.79 (1.40–2.29)	<0.001
Small sampling	13 (10–21)	9 (6–12)	<0.001	0.98 (0.91–1.06)	0.58
Hb at the initiation of treatment (g/dL), mean (SD)	12.6 (1.5)				

SGA, small for gestational age ; Hb, hemoglobin ; MCHC, mean corpuscular hemoglobin concentration ; MCV, mean corpuscular volume ; Ret, absolute reticulocyte count

Table 3. Sensitivity analysis

	Lowest Hb during hospitalization <12 g/dL (n = 132)	Lowest Hb during hospitalization ≥12 g/dL (n = 226)	P–value	Adjusted OR (95% CI)	Adjusted P–value
Male, n (%)	64 (48)	129 (57)	0.12	0.92 (0.48–1.78)	0.82
Gestational age (weeks), median (IQR)	33.4 (31.7–34.2)	35.0 (34.1–35.4)	<0.001	0.48 (0.33–0.70)	<0.001
SGA, n (%)	36 (27)	39 (13)	0.03	4.24 (1.60–11.2)	0.004
Caesarian section, n (%)	112 (85)	157 (69)	<0.001	1.03 (0.43–2.47)	0.95
Twin birth, n (%)	31 (23)	53 (23)	0.99	0.88 (0.40–1.95)	0.76
Placental abruption	6 (4.5)	8 (3.5)	0.64	1.52 (0.35–6.66)	0.58
Maternal Hb before birth (g/dL), mean (SD)	10.8 (1.5)	11.0 (1.3)	0.14	0.92 (0.72–1.17)	0.52
Laboratory data at birth					
Hb (g/dL), mean (SD)	16.2 (2.3)	17.3 (2.0)	<0.001	0.52 (0.42–0.65)	<0.001
MCV (fL), mean (SD)	110 (6.7)	108 (5.6)	<0.001	1.01 (0.95–1.07)	0.77
MCHC (%), mean (SD)	34.1 (1.1)	34.3 (1.0)	0.009	1.00 (0.95–1.06)	0.86
Ret ($\times 10^4$/μL), mean (SD)	26.2 (7.2)	24.6 (6.0)	0.01	1.01 (0.95–1.06)	0.86
High intensity breastfeeding, n (%)	53 (40)	50 (22)	<0.001	0.84 (0.42–1.70)	0.63
Treatment for jaundice, n (%)	102 (77)	118 (52)	<0.001	0.86 (0.36–2.04)	0.73
Number of blood samplings, median (IQR)					
Large sampling	7.5 (6–10)	3 (2–5)	<0.001	1.35 (1.14–1.59)	<0.001
Small sampling	13 (10–21)	9 (7–13)	<0.001	1.09 (1.02–1.16)	0.01

SGA, small for gestational age ; Hb, hemoglobin ; MCHC, mean corpuscular hemoglobin concentration ; MCV, mean corpuscular volume ; Ret, absolute reticulocyte count

The results of multivariable logistic models for EPO administration and iron supplementation are shown in Table 4 and 5. Infants with an SGA status (OR, 3.67 ; 95% CI, 1.35–9.99) and a higher number of large samplings (OR, 1.42 ; 95% CI, 1.17–1.73) had significantly higher odds for administration of EPO. Infants with an SGA status (OR, 3.67 ; 95% CI, 1.51–16.1), low maternal Hb before birth (OR, 0.66 ; 95% CI 0.50–0.88), lower Hb at birth (OR, 0.75 ; 95% CI, 0.60–0.93), and a high number of large samplings (OR, 1.70 ; 95% CI, 1.34–2.16) showed significantly higher odds of iron supplementation. Thus, we selected gestational age, SGA status, maternal Hb before birth, Hb at birth, and number of large samplings as variables significantly associated with the primary outcome, and we used these five variables for the ROC analysis. The ROC analysis for the primary outcome is shown in Figure 2. The area under the curve was 0.953 for the development of AOP. The sensitivity and specificity at the cutoff point were 0.91 and 0.88, respectively. Results of the analysis of associations between development of AOP and duration of parenteral nutrition and daily weight gain are shown in Table 6. No significant association was observed among those factors.

Discussion

The aim of this study was to investigate the predictive factors of AOP treatment in premature infants born at 30–35 weeks. We adjusted the factors assessed during admission to evaluate the predictive factors for AOP treatment. Our findings showed that a lower Hb at birth was significantly correlated with a higher incidence of AOP treatment. Other factors, such as SGA status, maternal Hb before birth, and number of large samplings, were also associated with a higher incidence of AOP treatment. The major strength of this study is that it investigated AOP treatment in preterm infants born at 30–35 weeks' gestation, which has not been well addressed previously, and revealed an association between laboratory data at birth and the need for AOP treatment among these preterm infants.

To the best of our knowledge, this is the first study to investigate the association between laboratory data at birth and AOP treatment in infants born at 30–35 weeks' gestation. Although almost all ELBW infants need treatment for AOP[1], other preterm infants do not always require treatment for AOP. Since routine use of EPO is not recommended[14], initiation of adequate treatment of AOP in high–risk preterm infants is important. Thus, if the need for AOP treatment in these preterm infants

Table 4. Patients' secondary outcomes for administration of erythropoietin

	Treated (n = 110)	Not treated (n = 248)	P-value	Adjusted OR (95% CI)	Adjusted P-value
Male, *n* (%)	54 (49)	139 (56)	0.22	1.22 (0.59–2.53)	0.59
Gestational age (weeks), median (IQR)	32.8 (31.6–33.7)	35.0 (34.1–35.4)	<0.001	0.28 (0.18–0.42)	<0.001
SGA, *n* (%)	32 (29)	43 (17)	0.01	3.67 (1.35–9.99)	0.01
Caesarian section, *n* (%)	96 (87)	173 (70)	<0.001	2.36 (0.89–6.27)	0.09
Twin birth, *n* (%)	28 (25)	56 (23)	0.55	1.73 (0.74–4.07)	0.21
Placental abruption, *n* (%)	5 (4.5)	9 (3.6)	0.68	1.75 (0.34–8.95)	0.50
Maternal Hb before birth (g/dL), mean (SD)	10.8 (1.6)	11.0 (1.3)	0.07	0.79 (0.62–1.02)	0.07
Laboratory data at birth					
Hb (g/dL), mean (SD)	16.7 (2.2)	17.0 (2.2)	0.20	0.90 (0.74–1.08)	0.26
MCV (fL), mean (SD)	110 (6.7)	108 (5.7)	0.01	0.92 (0.86–0.98)	0.01
MCHC (%), mean (SD)	34.2 (1.1)	34.2 (1.0)	0.28	1.12 (0.80–1.56)	0.50
Ret ($\times 10^4/\mu L$), mean (SD)	26.4 (7.8)	24.6 (5.7)	0.007	0.95 (0.90–1.01)	0.12
High intensity breastfeeding, *n* (%)	47 (43)	56 (23)	<0.001	0.65 (0.31–1.35)	0.25
Treatment for jaundice, *n* (%)	93 (85)	127 (51)	<0.001	1.08 (0.41–2.84)	0.88
Number of blood samplings, median (IQR)					
Large sampling	8 (6–10)	3 (2–5)	<0.001	1.42 (1.17–1.73)	<0.001
Small sampling	14 (10–21)	9 (7–13)	<0.001	0.96 (0.90–1.02)	0.23
Days at the initiation of treatment (days), mean (SD)	17.6 (6.8)				
Hb at the initiation of treatment (g/dL), mean (SD)	13.0 (1.3)				

SGA, small for gestational age ; Hb, hemoglobin ; MCHC, mean corpuscular hemoglobin concentration ; MCV, mean corpuscular volume ; Ret, absolute reticulocyte count

Table 5. Patients' secondary outcomes for administration of iron supplementation

	Treated (n = 152)	Not treated (n = 206)	P-value	adjusted OR (95% CI)	adjusted P-value
Male, n (%)	75 (49)	118 (57)	0.14	0.85 (0.39–1.85)	0.68
Gestational age (weeks), median (IQR)	33.0 (31.9–34.0)	35.1 (34.6–35.6)	<0.001	0.21 (0.12–0.34)	<0.001
SGA, *n* (%)	42 (28)	33 (16)	0.008	4.92 (1.51–16.1)	0.008
Caesarian section, *n* (%)	128 (84)	141 (68)	0.001	1.13 (0.40–3.19)	0.82
Twin birth, *n* (%)	40 (26)	44 (21)	0.55	1.88 (0.74–4.79)	0.19
Placental abruption	9 (5.9)	5 (2.4)	0.09	6.03 (0.89–40.8)	0.07
Maternal Hb before birth (g/dL), mean (SD)	10.7 (1.5)	11.1 (1.3)	0.010	0.66 (0.50–0.88)	0.005
Laboratory data at birth					
Hb (g/dL), mean (SD)	16.7 (2.3)	17.0 (2.1)	0.05	0.75 (0.60–0.93)	0.01
MCV (fL), mean (SD)	110 (6.5)	107 (5.4)	<0.001	0.99 (0.92–1.06)	0.80
MCHC (%), mean (SD)	34.1 (1.1)	34.3 (0.93)	0.007	0.81 (0.55–1.19)	0.29
Ret ($\times 10^4/\mu L$), mean (SD)	26.2 (7.3)	24.3 (5.7)	0.003	0.95 (0.89–1.01)	0.12
High intensity breastfeeding, *n* (%)	65 (43)	38 (18)	<0.001	0.48 (0.21–1.11)	0.09
Treatment for jaundice, *n* (%)	123 (81)	97 (47)	<0.001	0.80 (0.30–2.11)	0.65
Number of blood samplings, median (IQR)					
Large sampling	7 (6–9)	3 (1–5)	<0.001	1.70 (1.34–2.16)	<0.001
Small sampling	13.5 (10–21)	9 (6–12)	<0.001	1.00 (0.93–1.08)	0.95
Days at the initiation of treatment (days), mean (SD)	24.7 (7.3)				
Hb at the initiation of treatment (g/dL), mean (SD)	12.7 (1.5)				

SGA, small for gestational age ; Hb, hemoglobin ; MCHC, mean corpuscular hemoglobin concentration ; MCV, mean corpuscular volume ; Ret, absolute reticulocyte count

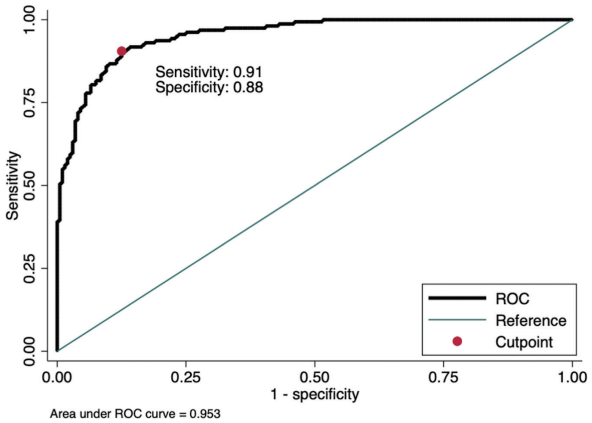

Fig. 2. Receiver operating characteristic curve of the multivariable logistic regression model using statistically significant variables to detect the development of anemia of prematurity.
We selected gestational age, hemoglobin level at birth, small for gestational age or light for gestational age, last maternal hemoglobin level before birth, and number of large blood samplings as the variables. The cutoff point was investigated using Youden's index test. The sensitivity and specificity at the cutoff point are shown.

can be predicted, initiation of treatment or measures to reduce unnecessary medical interventions that cause AOP development in high-risk infants can be initiated promptly. Considering the treatment threshold for AOP among infants born at 30–35 weeks' gestation, assessments based on the data at birth are important for preventing unnecessary Hb decline.

Because neonates experience a rapid decline in Hb due to erythrocyte destruction, a higher Hb is generally considered to indicate an advantage in preventing AOP[1]. Although early preterm infants have low Hb and iron stores at birth[15,16], the correlation between Hb at birth and the need for AOP treatment is unknown. This study demonstrated that a lower Hb at birth correlated with a higher incidence of AOP treatment, with the analysis adjusted for gestational age. Interestingly, no association was observed between Hb at birth or maternal Hb before birth and the initiation of EPO treatment, although low Hb at birth and maternal Hb before birth were significantly correlated with the initiation of iron supplementation. The vast majority of iron stores in neonates are formed by transplacental transport from the mother during the third trimester of pregnancy[1]. Rapid Hb decline in early infancy is understood to be a result of the transition from fetal to mature hemoglobin and the lack of erythropoiesis[3]. Thus, high Hb at birth may reflect higher iron storage in neonates, and may not mean sufficient production of EPO in neonates.

Another factor, Ret, is generally used as a parameter of anemia and erythropoiesis ; however, the role of Ret in premature infants has not been well elucidated. Although reticulocyte hemoglobin equivalent has been reported to be a useful marker of iron and erythropoiesis in pediatric dialysis[17], this marker is not routinely measured in neonatal intensive care unit settings. Although Ret is known to be a marker of hematopoiesis and is more prevalent than reticulocyte hemoglobin content[18], the usefulness of Ret at birth to predict AOP treatment has not been clarified. The correlation between Ret at birth and the development of AOP was not proven in this study. The reticulocyte hemoglobin equivalent was reported to be a more relevant marker of iron status in pediatric end-stage kidney disease[17]. In addition, reticulocyte hemoglobin content has been reported to be an attractive marker for detecting iron deficiency[19,20]. In this study, of the reticulocyte-related markers, we only evaluated Ret at birth. Further studies on the other markers are needed.

SGA infants showed a higher incidence of AOP development and a higher incidence of iron supplementation and EPO administration. Although the relationship between the need for AOP treatment and SGA status has not been well elucidated, rapid body growth after birth is considered to affect AOP development[21]. In addition, a correlation between maternal anemia during pregnancy and SGA status has been reported[22]. This study showed a correlation between development of AOP and maternal Hb before birth. Although we could not investigate iron levels at birth due to lack of data, previous reports and the present study suggest that SGA infants could have low iron storage due to low supply

Table 6. Association between treatment for anemia of prematurity and duration of parenteral nutrition or daily weight gain

	Treated (n = 158)	Not treated (n = 200)	P-value
Duration of parental nutrition, median (IQR)	9 (7–11)	7 (5–10)	0.10
Daily weight gain during admission, median (IQR)	32.9 (29.0–37.9)	32.3 (27.0–37.2)	0.27

from their mothers during pregnancy, and they may tend to need AOP treatment. Consequently, early initiation of EPO and iron supplementation is recommended for SGA infants.

Since phlebotomy loss is regarded as a major contributor to AOP, reduction of the volume of blood collected for laboratory testing should be considered, especially in ELBW infants[1]. For example, the use of an in-line blood gas and chemistry monitor in ELBW infants was shown to reduce the number and volume of RBC transfusions[23]. Additionally, blood sampling stewardship for reducing phlebotomy loss in ELBW infants reduced the rate of multiple RBC transfusions[7]. However, the effect of phlebotomy reduction strategies among moderate-to-late preterm infants has not been clarified. This study revealed that a larger volume of blood loss, such as that caused by multiple large samplings, was likely to result in the development of AOP even in infants born at 30-35 weeks' gestation. The in-line blood sampling method is usually not used in moderate-to-late preterm infants because they show less severe conditions than ELBW infants and are not likely to be placed on arterial catheters for continuous invasive monitoring. Consequently, efforts to reduce the amount of phlebotomy in preterm infants other than ELBW infants are still warranted. Therefore, unnecessary large sampling should not be performed even in moderate to late preterm infants.

This study had some limitations. The first major limitation of this study is that it was a retrospective study, and information obtained from medical records, such as iron status at birth, was limited. Second, the indications for treatment were based on the physician's decision ; therefore, uniformity of treatment could not be secured. To address this point, we used the institutional guidelines for the threshold for treatment of AOP and performed sensitivity analysis using the minimum Hb during admission, and the results were similar to those of the primary analysis. Despite these limitations, however, our results newly revealed potential risk factors for the development of AOP in moderate- to late-preterm infants. Finally, we could not evaluate the effects of "delayed cord clamping"[24] or "umbilical cord milking"[25] on anemia, because of data insufficiency. Delayed cord clamping significantly reduces hospital mortality and increases the Hct value without major adverse events[1,24]. Umbilical cord milking may be effective in reducing RBC transfusion, and its benefits are under discussion[24,26]. Further prospective studies are needed to reveal the correlation between these factors and AOP in moderate-to-late preterm infants.

In conclusion, gestational age, SGA status, maternal Hb before birth, low Hb at birth, and a high number of large blood samplings were positively associated with the development of AOP among infants born at 30-35 weeks' gestation. These variables can be possible risk factors for the development of AOP in infants born at 30-35 weeks' gestation. Further studies are needed to evaluate the effect of AOP development on these variables.

Acknowledgements

The authors are grateful to Mayuka Fujimoto, MD ; Atsushi Nishioka, MD ; Yuya Asai, MD ; Joonho Shin, MD ; Tomoki Yaguchi, MD ; and Chino Yatomi, MD (Department of Pediatrics, Yaizu City Hospital) for contributing to the data acquisition.

Conflict of interest disclosure

The authors declare no conflict of interest.

References

1. Saito-Benz M, Flanagan P, Berry MJ. Management of anaemia in pre-term infants. Br J Haematol, **188** : 354-366, 2020.
2. Alan S, Arsan S. Prevention of the anaemia of prematurity. Int J Pediatr Adolesc Med, **2** : 99-106, 2015.
3. Strauss RG. Anaemia of prematurity : Pathophysiology and treatment. Blood Rev, **24** : 221-225, 2010.
4. Aher SM, Ohlsson A. Early versus late erythropoietin for preventing red blood cell transfusion in preterm and/or low birth weight infants. Cochrane Database Syst Rev, **2** : CD004865, 2020.
5. Ohlsson A, Aher SM. Early erythropoiesis-stimulating agents in preterm or low birth weight infants. Cochrane Database Syst Rev, **2** : CD004863, 2020.
6. Aher SM, Ohlsson A. Late erythropoiesis-stimulating agents to prevent red blood cell transfusion in preterm or low birth weight infants. Cochrane Database Syst Rev, **1** : CD004868, 2020.
7. Balasubramanian H, Atyalgade M, Garg B, Srinivasan L, Kabra NS, Khapekar S. Effects of blood sampling stewardship and erythropoietin administration in extremely low birth weight infants-a quality improvement non-controlled before-and-after retrospective study. Eur J Pediatr, **180** : 1617-1626, 2021.

8. Fischer HS, Reibel NJ, Bührer C, Dame C. Prophylactic early erythropoietin for neuroprotection in preterm infants : A meta–analysis. Pediatrics, **139** : e20164317, 2017.
9. Proytcheva MA. Issues in neonatal cellular analysis. Am J Clin Pathol, **131** : 560–573, 2009.
10. Shohat M. Preterm blood counts vary with sampling site. Arch Dis Child, **62** : 74–75, 1987.
11. Kayiran SM, Ozbek N, Turan M, Gürakan B. Significant differences between capillary and venous complete blood counts in the neonatal period. Clin Lab Haematol, **25** : 9–16, 2003.
12. Yasuhi I, Yamashita H, Maeda K, Nomiyama M, Mizunoe T, Tada K, *et al.* High–intensity breastfeeding improves insulin sensitivity during early post–partum period in obese women with gestational diabetes. Diabetes Metab Res Rev, **35** : e3127, 2019.
13. Itabashi K, Fujimura M, Kusuda S, Tamura M, Hayashi T, Takahashi T, *et al.* Committee of Neonatal Medicine of Japan Pediatric Society. Introduction of new gestational age–specific standards for birth size. J Jpn Pediatr Soc, **114** : 1271–1293, 2010. [In Japanese]
14. Venkatesh V, Khan R, Curley A, New H, Stanworth S. How we decide when a neonate needs a transfusion. Br J Haematol, **160** : 421–433, 2013.
15. Ochiai M, Matsushita Y, Inoue H, Kusuda T, Kang D, Ichihara K, *et al.* Blood reference intervals for preterm low–birth–weight infants : A multicenter cohort study in Japan. PLoS One, **11** : e0161439, 2016.
16. Colombatti R, Sainati L, Trevisanuto D. Anemia and transfusion in the neonate. Semin Fetal Neonatal Med, **21** : 2–9, 2016.
17. Davidkova S, Prestidge TD, Reed PW, Kara T, Wong W, Prestidge C. Comparison of reticulocyte hemoglobin equivalent with traditional markers of iron and erythropoiesis in pediatric dialysis. Pediatr Nephrol, **31** : 819–826, 2016.
18. Piva E, Brugnara C, Spolaore F, Plebani M. Clinical Utility of Reticulocyte Parameters. Clin Lab Med, **35** : 133–163, 2015.
19. Lorenz L, Peter A, Arand J, Springer F, Poets CF, Franz AR. Reticulocyte Haemoglobin Content Declines More Markedly in Preterm than in Term Infants in the First Days after Birth. Neonatology, **112** : 246–250, 2017.
20. Lorenz L, Arand J, Büchner K, Wacker–Gussmann A, Peter A, Poets CF, *et al.* Reticulocyte haemoglobin content as a marker of iron deficiency. Arch Dis Child Fetal Neonatal Ed, **100** : F198–202, 2015.
21. Von Kohorn I, Ehrenkranz RA. Anemia in the Preterm Infant : Erythropoietin Versus Erythrocyte Transfusion–It's not that Simple. Clin Perinatol **36** : 111–123, 2009.
22. Badfar G, Shohani M, Soleymani A, Azami M. Maternal anemia during pregnancy and small for gestational age : a systematic review and meta–analysis. J Matern Neonatal Med, **32** : 1728–1734, 2019.
23. Widness JA, Madan A, Grindeanu LA, Zimmerman MB, Wong DK, Stevenson DK. Reduction in red blood cell transfusions among preterm infants : Results of a randomized trial with an in–line blood gas and chemistry monitor. Pediatrics, **115** : 1299–1306, 2005.
24. Rabe H, Gyte GM, Díaz–Rossello JL, Duley L. Effect of timing of umbilical cord clamping and other strategies to influence placental transfusion at preterm birth on maternal and infant outcomes. Cochrane Database Syst Rev, **9** : CD003248, 2019.
25. Katheria A, Reister F, Essers J, Mendler M, Hummler H, Subramaniam A, *et al.* Association of umbilical cord milking vs delayed umbilical cord clamping with death or severe intraventricular hemorrhage among preterm infants. JAMA, **322** : 1877–1886, 2019.
26. Purisch SE, Ananth CV, Arditi B, Mauney L, Ajemian B, Heiderich A, *et al.* Effect of delayed vs immediate umbilical cord clamping on maternal blood loss in term cesarean delivery : A randomized clinical trial. JAMA, **322** : 1869–1876, 2019.