Risk factors for severe retinopathy of prematurity: A retrospective case-control study

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Abstract. The present study aimed to investigate the risk factors associated with severe retinopathy of prematurity (ROP) in preterm infants and to identify predictive biomarkers for early risk stratification. The present retrospective case-control study analyzed preterm infants who underwent ROP screening at Hebei General Hospital (Shijiazhuang, China) between September 2018 and July 2023. Participants were stratified into mild (n=42) and severe ROP (n=38) groups based on fundus examination results and treatment requirements. Demographic characteristics, perinatal complications, maternal third-trimester lipid profiles and serial hematological parameters (within 24 h of birth and at 1 week of age) were analyzed. Binary logistic regression analysis was performed to identify independent risk factors, and receiver operating characteristic (ROC) curves were constructed to evaluate predictive performance. Significant differences were observed between severe and mild ROP groups in gestational age, birth weight and 1-min Apgar score (all P<0.001). Perinatal complications significantly associated with severe ROP included neonatal bronchopulmonary dysplasia (P<0.001), neonatal respiratory distress syndrome (P=0.003), neonatal sepsis (P=0.009) and blood transfusion requirements (P<0.001). Among hematological parameters, lymphocyte-to-monocyte ratio (LMR) was significantly lower in the severe ROP group both within 24 h of birth (P=0.015) and at 1 week of age (P=0.01). Multivariate logistic regression identified gestational age as the sole independent risk factor for severe ROP (P<0.001). ROC curve analysis revealed that gestational age ≤30.5 weeks predicted severe ROP with 76.2% sensitivity and 71.1% specificity (P<0.001). Maternal third-trimester lipid parameters showed no significant associations with severe ROP development. In conclusion, gestational age represents the most significant independent predictor of severe ROP in preterm infants, with infants born at ≤30.5 weeks showing significantly increased risk. Additional risk factors include major perinatal complications and altered inflammatory profiles characterized by reduced LMR. These findings support enhanced surveillance protocols for preterm infants and suggest the potential utility of inflammatory biomarkers in ROP risk stratification strategies.

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Introduction

Retinopathy of prematurity (ROP) is a proliferative retinal vascular disorder that predominantly affects premature infants, representing one of the leading preventable causes of childhood blindness worldwide (1,2). ROP arises from abnormal retinal vascularization in preterm neonates, where the incompletely developed retinal vasculature at birth renders them particularly vulnerable to various pathophysiological insults (2,3).

The epidemiological relevance of ROP has intensified in parallel with advances in neonatal intensive care medicine. While marked improvements in perinatal and neonatal care have notably reduced mortality rates among premature infants, these same advances have led to increased survival rates of extremely low birth weight and very low gestational age infants, populations at the highest risk for ROP development (4,5); this has resulted in an increased prevalence of ROP. ROP remains a leading cause of preventable childhood blindness worldwide, and can lead to irreversible blindness if severe and untreated. China accounts for 4,000 of these cases, which represents 1.9% of the prevalence of blindness among children <5 years old (6).

The clinical spectrum of ROP encompasses a broad range of severity, from mild self-resolving forms to severe progressive disease requiring immediate intervention. Severe ROP, characterized by advanced retinal pathology including neovascularization, vitreoretinal proliferation and potential retinal detachment, poses particularly notable challenges for both neonatologists and ophthalmologists (7). These cases are associated with substantial treatment complexity, marked

healthcare costs and poor visual prognosis despite optimal therapeutic interventions. The irreversible nature of severe ROP-related vision loss underscores the critical importance of early identification, risk stratification and timely therapeutic intervention (7).

Current screening protocols for ROP rely heavily on established demographic risk factors, primarily gestational age (<32 weeks) and birth weight (<1,500 g), which serve as fundamental parameters for determining screening eligibility and frequency (8). However, the pathogenesis of ROP is multifactorial, involving complex interactions between genetic predisposition, environmental factors, systemic inflammatory responses and various perinatal and neonatal complications (9,10). Research has increasingly focused on identifying additional biomarkers and risk factors that may enhance the ability to predict severe ROP development beyond traditional parameters (11).

Understanding the comprehensive risk factor profile associated with severe ROP development is key for optimizing clinical management strategies, enabling more precise risk stratification and facilitating early intervention in high-risk populations (10,12). Such insights could lead to the development of more sophisticated predictive models that complement existing screening protocols and potentially improve long-term visual outcomes in severe ROP.

Therefore, the present retrospective case-control study aimed to systematically investigate the associations between multiple clinical parameters, including general demographic characteristics, perinatal complications, hematological indices, biochemical markers and maternal factors during pregnancy, and the development of severe ROP in premature infants. By identifying novel risk factors and validating known predictors, the present study aimed to provide new perspectives on the pathogenesis of severe ROP, and contribute to the development of more comprehensive risk assessment strategies for early identification and prevention of severe ROP in clinical practice.

Materials and methods

Study design and population. The present retrospective case-control study was conducted at Hebei General Hospital (Shijiazhuang, China), analyzing clinical data from preterm infants admitted to the Neonatal and Ophthalmology Departments between September 2018 and July 2023. All enrolled infants underwent comprehensive fundus examination for ROP screening according to established protocols (13).

The present study was conducted in accordance with The Declaration of Helsinki and approved by the Institutional Review Board of Hebei General Hospital [approval no. 115 (2024)]. Given the retrospective nature of the study utilizing existing medical records, informed consent requirements were waived by the ethics committee, with appropriate measures taken to ensure patient confidentiality and data protection.

Participants

Inclusion criteria. Case group (severe ROP): Preterm infants with birth weight <3,000 g, gestational age <37 weeks and diagnosis of severe ROP confirmed by fundus screening. Control group (mild ROP): Preterm infants with birth weight <3,000 g, gestational age <37 weeks and diagnosis of mild ROP confirmed by fundus screening.

Control infants were restricted to those who developed any stage of ROP that subsequently resolved without treatment; neonates who completed screening but never developed ROP were excluded. The present design intentionally compared two ends of the ROP spectrum (mild vs. severe) rather than 'ROP vs. no-ROP', allowing for the identification of factors that drive progression rather than initial disease occurrence. Consequently, the inclusion of relatively more mature neonates (32-36 weeks gestational age) in the mild ROP group may have attenuated the observed effect sizes of classical risk factors; this limitation is addressed in the discussion.

Exclusion criteria. Infants were excluded if they had: i) Gestational age ≥37 weeks or birth weight ≥3,000 g; ii) incomplete clinical data or medical records; iii) inability to ensure adequate follow-up until retinal vascularization reached zone III in both eyes without pre-threshold or threshold lesions; iv) systemic diseases markedly affecting hematological and biochemical parameters (such as hematological disorders, genetic and metabolic diseases); and v) concurrent ocular diseases or systemic conditions that could independently cause ocular pathology.

ROP diagnosis and classification. ROP diagnosis and classification were performed according to the International Classification of Retinopathy of Prematurity, Third Edition criteria (13). Based on fundus screening results and clinical course, participants were stratified into two groups: Mild ROP group included infants diagnosed with ROP during screening that did not meet treatment criteria and subsequently resolved spontaneously during follow-up; severe ROP group included infants requiring therapeutic intervention [retinal laser photocoagulation or intravitreal anti-vascular endothelial growth factor (VEGF) injection]. Severe ROP includes type I threshold lesions and rapidly progressive ROP, whereas other types of lesions were considered mild ROP (13).

Fundus examination. All examinations were performed by experienced ophthalmologists using the RetCam 3 digital imaging system (Clarity Medical Systems). Initial examination was conducted at 4-6 weeks postnatal age or 31-32 weeks corrected gestational age, whichever occurred later. Subsequent follow-up intervals were determined based on initial findings and disease progression, continuing until complete retinal vascularization to zone III or 45 weeks corrected gestational age without threshold lesions. Pupil dilation was achieved using compound tropicamide eye drops instilled bilaterally at 5-10 min intervals, repeated ≥4 times until maximum pupillary dilation was achieved; topical anesthesia was provided with proparacaine hydrochloride eye drops. Following appropriate infant restraint and head stabilization, sterile pediatric eyelid specula were used to maintain adequate eyelid separation. Gatifloxacin ophthalmic gel was applied to the eyelid margins, and comprehensive digital fundus photography was performed using RetCam 3 system to document retinal findings.

Overall, 94 of 102 eligible infants (92.2%) completed follow-up until zone III vascularisation or 45 weeks corrected age; 22 infants were excluded for insufficient documentation and 80 were included in the final analysis.

Table I. Comparison of general data in preterm infants with different severity of ROP.

Parameter	Mild ROP group, n=42	Severe ROP group, n=38	Statistical quantity	P-value
Sex (%)			χ^2=0.005	0.946
Male	24 (57.14)	22 (57.89)		
Female	18 (42.86)	16 (42.11)		
GA, weeks	32.00 (4.25)	29.00 (2.75)	Z=-4.886	<0.001
BW, g	1,645.00 (804.25)	1,137.50 (497.50)	Z=-4.972	<0.001
Mode of delivery			χ^2=2.759	0.097
Caesarean section (%)	26 (61.90)	30 (78.95)		
Natural birth,n (%)	16 (38.10)	8 (21.05)		
Gestational diabetes, n (%)	8 (19.05)	9 (23.68)	χ^2=0.346	0.556
Gestational hypertension, n (%)	9 (21.43)	13 (34.21)	χ^2=0.529	0.467
Lipid parameters in the third trimester of pregnancy				
TC, mmol/l	6.34 (1.17)	6.15 (1.07)	Z=-1.053	0.292
TG, mmol/l	3.28 (1.22)	3.09 (0.64)	Z=-0.416	0.678
LDL, mmol/l	3.37 (0.60)	3.60 (0.70)	Z=-1.517	0.129
ApoB, g/l	1.14 (0.13)	1.04 (0.27)	Z=-1.779	0.058
HDL, mmol/l	1.81 (0.33)	1.65 (0.31)	Z=-1.759	0.079
ApoA1, g/l	1.93 (0.32)	1.89 (0.47)	Z=-0.846	0.398
1-min Apgar score, points	10 (0)	9 (3)	Z=-3.512	<0.001
Neonatal pneumonia (%)	7 (16.67)	13 (34.21)	χ^2=1.671	0.196
NBPD, n (%)	11 (26.19)	32 (84.21)	χ^2=27.015	<0.001
Neonatal sepsis, n (%)	5 (11.90)	14 (36.84)	χ^2=6.851	0.009
NRDS, n (%)	15 (35.71)	26 (68.42)	χ^2=8.542	0.003
Blood transfusion, n (%)	18 (42.86)	37 (97.37)	χ^2=15.402	<0.001

ROP, retinopathy of prematurity; GA, gestational age; BW, birth weight; TC, total cholesterol; TG, triglycerides; LDL, low-density lipoprotein; Apo, apolipoprotein; HDL, high-density lipoprotein; NBPD, neonatal bronchopulmonary dysplasia; NRDS, neonatal respiratory distress syndrome.

Data collection. Comprehensive clinical data were systematically collected for each participant. Neonatal characteristics included sex, gestational age, birth weight, mode of delivery and 1-min Apgar score. Perinatal complications assessed included neonatal pneumonia, neonatal bronchopulmonary dysplasia (NBPD), neonatal sepsis, neonatal respiratory distress syndrome (NRDS) and requirement for blood transfusion.

Clinical laboratory data, including routine blood test results, were also collected. Hematological and biochemical analyses were performed at two time points: Within 24 h of birth and at 1-week postnatal age. Complete blood count included white blood cell count (WBC; x10^9/l), neutrophil count (N; x10^9/l), lymphocyte count (L; x10^9/l), monocyte count (M; x10^9/l), platelet count (PLT; x10^9/l), mean platelet volume (MPV; fl) and hemoglobin (Hb; g/l). Biochemical profile included total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), apolipoprotein (Apo)B, high-density lipoprotein (HDL) and ApoA1. Based on laboratory values, the following inflammatory indices were calculated: Neutrophil-to-lymphocyte ratio (NLR), lymphocyte-to-monocyte ratio (LMR), platelet-to-lymphocyte ratio (PLR) and systemic immune-inflammation index (SII=platelet count × Neutrophil count/Lymphocyte count).

Maternal clinical characteristics during the third trimester were assessed, including gestational diabetes mellitus, gestational hypertension and TC, TG, LDL, ApoB, HDL and ApoA1.

Statistical analysis. Statistical analyses were conducted using SPSS version 24.0 (IBM Corp.). Normally distributed data are presented as the mean ± SD and differences between two groups were analyzed using a two-tailed unpaired Student's t-test. Non-normally distributed data are presented as the median with interquartile range [M (Q_1, Q_3)] and were compared using the Mann-Whitney U test. Categorical data are presented as frequencies and percentages [n (%)] and were compared using χ^2 test. Comparison of neonatal sepsis and blood transfusion was performed with Fisher's exact test. Variables demonstrating statistical significance (P<0.05) in univariate analyses were included in multivariate binary logistic regression models to identify independent risk factors for severe ROP development. To avoid multicollinearity, pairwise Pearson correlation and variance inflation factor (VIF) were examined between continuous variables; when VIF ≥5 or r ≥0.7, the variable with weaker clinical utility was

Table II. Comparison of hematological parameters within 24 h of birth in preterm infants with ROP of different severities.

Blood parameter	Mild ROP group, n=42	Severe ROP group, n=38	Z statistic	P-value
WBC, x10^9/l	9.38 (5.71)	9.10 (5.97)	-8.029	0.407
N, x10^9/l	3.34 (3.67)	3.47 (6.66)	-1.060	0.916
L, x10^9/l	3.90 (2.83)	4.59 (2.98)	-2.144	0.032
PLT, x10^9/l	222.5 (83.00)	249.0 (79.80)	-1.595	0.110
MPV, fl	10.35 (1.125)	10.10 (1.125)	-1.124	0.260
Hb, g/l	167.50 (24.00)	164.50 (27.50)	-0.328	0.261
NLR	0.71 (0.76)	0.91 (2.78)	-1.098	0.272
LMR	8.21 (5.21)	6.17 (5.42)	-2.441	0.015
PLR	56.00±23.56	58.62 (47.44)	-1.252	0.210
SII	172.40 (181.34)	206.10 (519.10)	-0.723	0.470

ROP, retinopathy of prematurity; WBC, white blood cell count; N, neutrophil count; L, lymphocyte count; PLT, platelet count; MPV, mean platelet volume; HB, hemoglobin; NLR, neutrophil-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio; PLR, platelet-to-lymphocyte ratio; SII, systemic immune-inflammation index.Data are resented as median (interquartile range), except for PLR which is given as mean ± SD.

Table III. Comparison of hematological parameters at 1 week of life in preterm infants with ROP of different severities.

Blood parameter	Mild ROP group, n=42	Severe ROP group, n=38	Z statistic	P-value
WBC, x10^9/l	8.80 (4.81)	9.55 (4.73)	-1.147	0.252
N, x10^9/l	4.06 (2.78)	6.49 (5.66)	-1.325	0.185
L, x10^9/l	200.00 (159.25)	225.50 (120.25)	-1.233	0.217
PLT, x10^9/l	3.00 (1.92)	3.63 (1.83)	-1.113	0.266
MPV, fl)	11.87 (1.20)	11.46 (0.93)	-1.988	0.047
Hb, g/l	136.50 (29.45)	139.62 (28.75)	-0.892	0.373
NLR	1.26 (0.57)	1.78 (1.56)	-1.310	0.190
LMR	3.50 (1.34)	2.25 (2.10)	-2.582	0.010
PLR	66.61 (36.91)	62.76 (34.89)	-0.366	0.714
SII	292.74 (260.64)	344.22 (400.67)	-0.318	0.751
TC, mmol/l	3.00 (0.85)	2.99 (0.91)	-0.337	0.736
TG, mmol/l	0.68 (0.55)	0.70 (0.74)	-0.458	0.647
LDL, mmol/l	2.06 (0.59)	1.84 (0.96)	-0.790	0.429
ApoB, g/l	0.45 (0.12)	0.46 (0.22)	-0.762	0.446
HDL, mmol/l	0.84 (0.30)	0.84 (0.29)	-1.571	0.116
ApoA1, g/l	0.77 (0.22)	0.88 (0.31)	-1.688	0.091

ROP, retinopathy of prematurity; WBC, white blood cell count; N, neutrophil count; L, lymphocyte count; PLT, platelet count; MPV, mean platelet volume; HB, hemoglobin; NLR, neutrophil-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio; PLR, platelet-to-lymphocyte ratio; SII, systemic immune-inflammation index; TC, total cholesterol; TG, triglycerides; LDL, low-density lipoprotein; Apo, apolipoprotein; HDL, high-density lipoprotein. Data are median (interquartile range, IQR).

excluded before the final model was fitted. Receiver operating characteristic (ROC) curves were constructed for independent risk factors to evaluate their predictive capacity for severe ROP. The area under the curve (AUC) with 95% confidence intervals (CIs) was calculated. Optimal cut-off values were determined using Youden's index, with corresponding sensitivity and specificity values calculated. P<0.05 was considered to indicate a statistically significant difference.

Results

Study population characteristics. The present retrospective case-control study included 80 preterm infants who were admitted to the Neonatal and Ophthalmology Departments of Hebei General Hospital between September 2018 and July 2023, and underwent comprehensive fundus examination for ROP screening. Based on fundus screening results and clinical

Table IV. Univariate and multivariate logistic regression analysis of retinopathy of prematurity severity.

		Univariate analysis		Multivariate analysis					
Variable	Category/unit	OR (95% CI)	P-value	Regression coefficient	Standard error	Wald	df	OR (95% CI)	P-value
Gestational age	Per 1 week increase	0.52 (0.40-0.68)	<0.001	-0.545	0.131	17.264	1	0.580 (0.4-0.7)	<0.001
Birth weight	Per 100 g increase	5.60 (3.10-10.2)	<0.001	-	-	-	-	-	-
1-min Apgar score	Per 1-point increase	3.42 (1.40-8.37)	<0.001	-	-	-	-	-	-
Sex	Male vs. female	0.97 (0.41-2.29)	0.946	-	-	-	-	-	-
Mode of delivery	Cesarean vs. vaginal	0.43 (0.16-1.17)	0.097	-	-	-	-	-	-
Maternal gestational diabetes	Yes vs. no	1.05 (0.37-3.00)	0.556	-	-	-	-	-	-
Maternal gestational hypertension	Yes vs. no	1.48 (0.56-3.91)	0.467	-	-	-	-	-	-
Neonatal pneumonia	Yes vs. no	2.32 (0.83-6.48)	0.196	-	-	-	-	-	-
NBPD	Yes vs. no	14.5 (4.8-43.7)	<0.001	-	-	-	-	-	-
NRDS	Yes vs. no	3.90 (1.60–9.50)	0.003	-	-	-	-	-	-
Neonatal sepsis	Yes vs. no	4.30 (1.40-13.5)	0.009	-	-	-	-	-	-
Blood transfusion	Yes vs. no	33.1 (4.3-256)	<0.001	-	-	-	-	-	-
LMR within 24 h	Per 1-unit increase	4.29 (2.02-9.12)	0.015	-	-	-	-	-	-
LMR at 1 week	Per 1-unit increase	4.30 (2.02-9.15)	0.010	-	-	-	-	-	-
MPV at 1 week	Per 1 fl increase	0.49 (0.25-0.96)	0.047	-	-	-	-	-	-

Reference categories: Female (sex), vaginal delivery (mode of delivery), no (all binary morbidities); continuous variables are per-unit increase as specified. - indicates that the variable was not retained in the final multivariate model owing to P≥0.05 in the univariate screen or multicollinearity (birth weight: variance inflation factor=8.9 vs. gestational age). Other variables were significant in univariate analysis but were excluded from the multivariate model after adjustment for gestational age. LMR, lymphocyte-to-monocyte ratio; MPV, mean platelet volume; NBPD, neonatal bronchopulmonary dysplasia; NRDS, neonatal respiratory distress syndrome; OR, odds ratio; CI, confidence interval; df, degrees of freedom.

Table V. Early predictive value of gestational age in severe retinopathy of prematurity.

Indicator	AUC	Sensitivity	Specificity	Optimal threshold, weeks	95% CI	P-value
Gestational age	0.815	0.762	0.711	30.5	0.725-0.906	<0.001

AUC, area under the curve; CI, confidence interval.

course, participants were stratified into two groups: 42 infants in the mild ROP group and 38 infants in the severe ROP group.

Demographic and clinical characteristics. Comparative analysis of demographic and clinical parameters between the mild and severe ROP groups revealed several significant differences (Table I). Infants with severe ROP demonstrated significantly lower gestational age (median 29.00 vs. 32.00 weeks; Z=-4.886; P<0.001) and birth weight (median 1,137.5 vs. 1,645.0 g; Z=-4.972; P<0.001) compared with those with mild ROP, and the 1-min Apgar score was significantly lower in the severe ROP group (median 9 vs. 10; Z=-3.512; P<0.001).

Regarding perinatal complications, significant differences were observed between groups; NBPD was significantly more prevalent in the severe ROP group compared with in the mild ROP group (84.21 vs. 26.19%; χ^2=27.015; P<0.001). Similarly, NRDS occurred more frequently in the severe ROP group (68.42 vs. 35.71%; χ^2=8.542; P=0.003), as did neonatal sepsis (36.84 vs. 11.90%; χ^2=6.851; P=0.009). Blood transfusion requirements were also significantly higher in the severe ROP group (97.37 vs. 42.86%; χ^2=15.402; P<0.001) compared with in the mild ROP group.

No statistically significant differences were observed between groups regarding sex distribution, mode of delivery, maternal gestational diabetes, maternal gestational hypertension or neonatal pneumonia incidence.

Maternal third-trimester lipid parameters. Analysis of maternal lipid profiles during the third trimester revealed no significant differences between groups for any measured parameters, including TC, TG, LDL, ApoB, HDL and ApoA1 (all P>0.05; Table I).

Hematological parameters within 24 h of birth. Comparison of hematological parameters obtained within 24 h of birth demonstrated selective differences between groups (Table II). For example, the L was significantly higher in the severe ROP group compared with that in the mild ROP group (median 4.59 vs. 3.90x10^9/l; Z=-2.144; P=0.032). In addition, the LMR was significantly lower in the severe ROP group (median 6.17 vs. 8.21; Z=-2.441; P=0.015). Other hematological parameters including WBC, N, PLT, MPV, Hb, NLR, PLR and SII showed no statistically significant differences between groups at this time point (all P>0.05).

Hematological parameters at 1 week of age. Analysis of hematological parameters obtained at 1 week postnatal age revealed additional significant differences (Table III); the MPV was significantly lower in the severe ROP group compared with that in the mild ROP group (median 11.46 vs. 11.87 fl; Z=-1.988; P=0.047) and the LMR remained significantly lower in the severe ROP group (median 2.25 vs. 3.50; Z=-2.582; P=0.010).

Biochemical parameters measured at 1 week, including lipid profiles (TC, TG, LDL, ApoB, HDL, ApoA1), did not demonstrate significant differences between groups (all P>0.05). Similarly, other hematological parameters and inflammatory indices (WBC, N, PLT, Hb, NLR, PLR and SII) showed no significant differences at this time point (all P>0.05).

Multivariate analysis of risk factors. To identify independent risk factors for severe ROP development, multivariate binary logistic regression analysis was performed using all variables that demonstrated statistical significance (P<0.05) in univariate analyses as independent variables, with ROP severity as the dependent variable (Table IV). Neonatal pneumonia was also included due to its clinical relevance, despite being non-significant in the univariate analysis.

The analysis included the following variables: Gestational age, birth weight, 1-min Apgar score, neonatal pneumonia, NBPD, neonatal sepsis, NRDS, blood transfusion requirements,24 h LMR, 1 week MPV and 1 week LMR.

Although NBPD, NRDS, sepsis, blood transfusion, LMR and MPV were significant in univariate analysis, they lost independent significance after adjustment for gestational age and were therefore not retained in the final model. Univariate logistic regression was first performed for each potential risk factor; variables with P<0.05 were entered into the multivariate model. For dichotomous exposures, the reference category was always the group with the presumed lowest risk (such as female for sex, vaginal for delivery mode, no for morbidities). Continuous variables were analyzed per-unit increase. Multivariate logistic regression analysis identified gestational age as the sole independent risk factor for severe ROP development (β=-0.545; Wald=17.264; P<0.001; odds ratio=0.580; 95% CI: 0.4-0.7; Table IV). The OR indicates that for each additional week of gestational age, the risk of developing severe ROP decreased by ~42%. Due to high collinearity between gestational age and birth weight (r=0.78, VIF=8.9), birth weight was excluded from the final model; consequently, gestational age remained the only independent predictor.

Predictive performance of gestational age. To assess the early predictive value of gestational age for severe ROP development, ROC curve analysis was performed (Table V; Fig. 1) and the AUC was 0.815 (95% CI: 0.725-0.906; P<0.001), indicating good discriminatory ability. Using Youden's index to determine the optimal threshold, a gestational age of 30.5 weeks

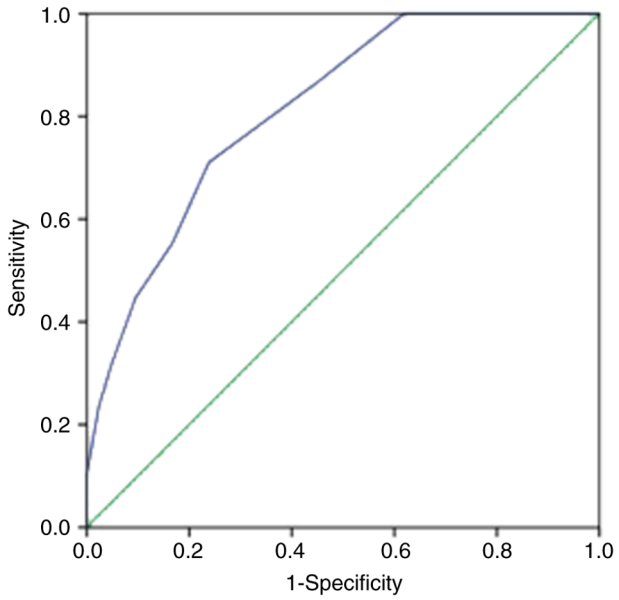

Figure 1. Receiver operating characteristic curve for prediction of severe retinopathy of prematurity according to gestational age (AUC=0.815).

was identified as the cut-off value, yielding a sensitivity of 76.2% and specificity of 71.1% for predicting severe ROP development. This threshold suggested that infants born at or before 30.5 weeks of gestational age have a significantly increased risk for developing severe ROP requiring therapeutic intervention.

Discussion

The present retrospective case-control study provided comprehensive insights into the risk factors associated with severe ROP in preterm infants. The present findings corroborated established knowledge while unveiling novel associations that may enhance the understanding of severe ROP pathogenesis and improve clinical risk stratification strategies.

Gestational age and birth weight are universally recognized as the most notable risk factors for ROP development and progression (4,13). The present study reinforces this relationship, demonstrating that infants with severe ROP had significantly lower gestational age and birth weight compared with those with mild ROP; these findings align with extensive literature documenting the inverse association between gestational age/birth weight and ROP severity (14,15). The pathophysiological basis for this association lies in the incomplete retinal vascularization at birth in extremely preterm infants, whose immature organ systems render them particularly vulnerable to oxidative stress and inflammatory insults that disrupt normal retinal angiogenesis (16).

Multivariate logistic regression analysis identified gestational age as an independent risk factor for severe ROP, with an optimal threshold of 30.5 weeks demonstrating 76.2% sensitivity and 71.1% specificity for predicting severe ROP development. This threshold aligns closely with current screening guidelines that emphasize heightened fundus examinations for infants born <32 weeks gestation (8). A recent large-scale prospective cohort study by Mhina *et al* (17) involving 193 Tanzanian preterm infants similarly identified gestational age <32 weeks as a critical threshold, with affected infants demonstrating a 6.8-fold increased risk for severe ROP. The consistency of these findings across diverse populations underscores the robust predictive value of gestational age in severe ROP risk assessment.

The present study identified several perinatal complications significantly associated with severe ROP development, including NBPD, NRDS and neonatal sepsis. These associations highlight the multifactorial nature of ROP pathogenesis, where systemic inflammatory responses and prolonged oxygen exposure contribute synergistically to abnormal retinal vascularization (10,16,18).

The association between NBPD and severe ROP observed in the present study is particularly notable, as it reflects the complex interplay between pulmonary and ocular vascular development in preterm infants. NBPD pathogenesis involves dysregulated expression of multiple angiogenic factors, including VEGF, insulin-like growth factor-1 (IGF-1) and transforming growth factor-β, which may directly influence retinal neovascularization (19,20). Furthermore, infants with NBPD typically require prolonged oxygen supplementation, exposing them to fluctuating oxygen tensions that promote oxidative stress and subsequent ROP development (21).

The significant association between neonatal sepsis and severe ROP in the present cohort aligns with emerging evidence implicating systemic inflammation in ROP pathogenesis (22). During sepsis, elevated levels of pro-inflammatory cytokines such as IL-6, IL-8 and tumor necrosis factor-α (TNF-α) can directly damage retinal vascular endothelium and induce retinal hypoxia, thereby stimulating pathological VEGF release and neovascularization (23,24). Meta-analysis evidence by Wang *et al* (25) demonstrated that sepsis increases ROP risk by ~2.5-fold, with early severe systemic inflammatory responses particularly disruptive to normal retinal vascular development.

The present investigation of hematological parameters revealed several novel associations that warrant further exploration. The LMR emerged as a potentially protective biomarker, with significantly higher values observed in the mild ROP group both within 24 h of birth and at 1 week of age. This finding suggests that a balanced inflammatory response, characterized by preserved lymphocyte populations relative to activated monocytes, may confer protection against severe ROP development (26).

The LMR is regarded as a reliable inflammatory biomarker across various diseases due to its stability, cost-effectiveness, and ease of calculation compared with traditional inflammatory mediators (27). In the context of ROP, elevated LMR may reflect a more controlled inflammatory response that minimizes collateral damage to developing retinal vasculature. A recent study by Xu *et al* (28) similarly identified LMR as a prognostic indicator in patients with sepsis, suggesting its broader applicability as an inflammatory biomarker.

The simultaneous increase in absolute L and the fall in LMR are not contradictory. LMR is a ratio; in the severe ROP cohort the median L value rose from 3.90 to 4.59x10^9/l at 24 h, whereas the concomitant median M value increased from 0.48 to 0.77x10^9/l (data not shown); since the relative expansion of monocytes exceeded that of lymphocytes, LMR declined from 8.21 to 6.17. This pattern, absolute lymphocytosis coupled

with a proportionally larger monocyte mobilization, mirrored an enhanced systemic inflammatory response, as circulating monocytes are primary sources of TNF-α, IL-6 and VEGF that drive retinal neovascularization (20). Consequently, the lower LMR reflected not lymphocyte depletion, but an over-proportionate increase in monocytes, supporting the hypothesis that dysregulated inflammation contributes to severe ROP development.

Notably, the present study also identified MPV as significantly lower in the severe ROP group at 1 week of age. This association may reflect enhanced platelet activation and altered hemostatic function in infants at risk of developing severe ROP (29). Platelets contain α granules storing diverse angiogenic regulatory factors, including both pro-angiogenic agents (VEGF, IGF-1) and anti-angiogenic factors (endostatin), which are released in response to various stimuli to modulate retinal neovascularization (30). The observed increase in MPV may indicate heightened platelet activation and degranulation, potentially contributing to the dysregulated angiogenic environment characteristic of severe ROP (31).

The 1-min Apgar score demonstrated a significant association with ROP severity in the present study, corroborating previous findings by Ali *et al* (32) and Marinov *et al* (33), which identified low Apgar scores as predictors of severe ROP development. The Apgar score provides a rapid assessment of neonatal adaptation immediately after birth, with lower scores reflecting compromised physiological status that may predispose infants to subsequent complications (34), including severe ROP. However, in the present multivariate analysis, Apgar score did not emerge as an independent predictor, suggesting that its influence on ROP development may be mediated through other factors such as increased susceptibility to sepsis, prolonged ventilatory support or hemodynamic instability (35).

The requirement for blood transfusion was significantly more common in the severe ROP group, reflecting both the overall medical complexity of these infants and potential direct effects of transfusion on ROP development. Blood transfusions in preterm infants have been associated with increased oxidative stress due to iron overload and altered oxygen-carrying capacity, potentially contributing to retinal vascular damage (36). Additionally, frequent transfusions often indicate underlying medical instability, prolonged hospitalization and exposure to multiple risk factors that collectively increase severe ROP risk (37).

Notably, the present study did not identify significant associations between maternal third-trimester lipid parameters and severe ROP development, despite theoretical considerations suggesting potential relationships. Maternal lipid metabolism during pregnancy influences fetal development, including retinal vascularization, through effects on placental function and nutrient transfer (10). However, the complex interplay between maternal metabolic factors and neonatal ROP development may be overshadowed by more potent postnatal risk factors in extremely preterm infants. Future studies with larger sample sizes and more detailed maternal metabolic profiling may be needed to detect subtle associations between maternal factors and severe ROP risk.

The identification of multiple independent risk factors for severe ROP development has important clinical implications for risk stratification and screening protocols. While current guidelines primarily rely on gestational age and birth weight criteria, the present findings suggested that incorporating additional parameters such as systemic inflammatory markers, specific perinatal complications and early hematological indices could potentially enhance predictive accuracy (36). The development of composite risk scores incorporating multiple biomarkers may enable more precise identification of high-risk infants requiring intensive surveillance and early intervention.

Furthermore, the recognition of modifiable risk factors such as sepsis prevention, optimal oxygen management and inflammatory response modulation provides opportunities for targeted interventions that may reduce severe ROP incidence (11). Enhanced infection control measures, use of oxygen therapy and consideration of anti-inflammatory strategies in high-risk infants represent potential areas for clinical improvement.

The present control group comprised only infants who developed any stage of ROP that subsequently regressed, which introduces selection bias inherent to the case-control design; infants who never developed ROP were excluded. This approach intentionally compared two points on the same disease spectrum (mild vs. severe) rather than comparing 'disease' with 'no disease'. The advantage is that constitutional determinants of prematurity *per se* are partially neutralized, allowing the research to focus on factors that drive progression from mild to severe ROP. However, this has limitations: First, effect sizes of classical risk factors (gestational age and birth weight) may be diluted because numerous controls were still relatively mature (median gestational age, 32 weeks); second, the baseline risk of developing ROP cannot be quantified, only the risk of escalating within the ROP phenotype. Consequently, the ORs reported here are specific to 'progression' rather than 'incidence' and extrapolation to the broader <37-week population should be made cautiously. Future studies limited to infants born at ≤30 weeks gestation and explicitly including an ROP-free cohort, will be needed to re-estimate both incidence and progression effects without this spectrum bias.

The present study has several other limitations that should be acknowledged: The retrospective design limits the ability to establish causal relationships and may introduce selection bias; the relatively small sample size may have limited the power to detect subtle associations, particularly regarding maternal factors. Additionally, the single-center design may limit the generalizability of the present findings to other populations with different demographic characteristics or clinical practices.

In conclusion, the present study identified gestational age ≤30.5 weeks as the strongest predictor of severe ROP, whereas a persistently low LMR emerged as a potential novel biomarker for early risk stratification. Enhanced surveillance and timely intervention targeting high-risk infants, particularly those with very low gestational age, systemic complications and abnormal inflammatory profiles, are essential for improving clinical outcomes and preserving vision in this vulnerable population.

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Availability of data and materials

The data generated in the present study are not publicly available due to restrictions imposed by the institutional review ethics committee concerning participant confidentiality and privacy, but may be requested from the corresponding author.

Authors' contributions

QM and XL were involved in the study conception and design, data acquisition, analysis and interpretation, and drafting of the manuscript. JW performed the statistical analysis, prepared the figures and provided critical revision for intellectual content. KL and WT were involved in patient recruitment, data collection and technical support. QM and XL confirm the authenticity of all the raw data. All authors read and approved the final manuscript, and agree to be accountable for all aspects of the work.

Ethics approval and consent to participate

The present study protocol was reviewed and approved by the Medical Ethics Committee of Hebei General Hospital [approval no. 115 (2024)]. Given the retrospective nature of the study utilizing existing medical records, informed consent requirements were waived by the ethics committee, with appropriate measures taken to ensure patient confidentiality and data protection.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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