Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Development and Validation of a Nomogram for Early Identification of ABO Hemolytic Disease of the Newborn Using Routinely Available Clinical and Laboratory Parameters: A Retrospective Study

Yike Li
Hunan Normal University
Jun Deng
2018050749@usc.edu.cn
University of South China

Research Article

Keywords: ABO hemolytic disease of the newborn, Hyperbilirubinemia, Three neonatal hemolysis tests, Nomogram, LASSO regression, Multiple imputation

Posted Date: August 26th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10639364/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Abstract

Purpose: To facilitate early identification of ABO hemolytic disease of the newborn (ABO–HDN) in neonates with hyperbilirubinemia, we developed and validated a nomogram based on routinely available clinical and laboratory parameters. The model is intended to assist clinicians particularly when hemolysis test results are inconclusive or unavailable.

Methods: This retrospective study included 350 neonates with hyperbilirubinemia who completed the three neonatal hemolysis tests (2023–2024). ABO–HDN was defined as a positive direct antiglobulin test (DAT) and/or antibody release test; isolated hyperbilirubinemia was defined as negative results for all three tests. Cases with positive free antibody test alone were excluded to ensure a gold–standard definition for model derivation. Demographic, perinatal, neonatal complications, maternal prenatal complications, and laboratory data (within 24 h of admission) were collected. Missing data were imputed using MICE. LASSO regression selected predictors, followed by multivariate logistic regression to build the nomogram. Model performance was assessed by ROC curves, calibration curves, Decision curve analysis (DCA), clinical impact curve (CIC), and Bootstrap internal validation (1000 resamples).

Results: A total of 350 neonates (154 ABO–HDN, 196 isolated hyperbilirubinemia) were included. LASSO and multivariate logistic regression identified five independent predictors: age at sampling, time of jaundice onset, red blood cell count (RBC), reticulocyte percentage (RET), and neonatal blood type (all $P<0.05$). The nomogram showed good discrimination (AUC=0.835, 95% CI: 0.794 – 0.876), excellent calibration (Hosmer–Lemeshow P=0.7454), and superior net benefit at thresholds 0.1–0.83. Bootstrap–corrected AUC was 0.839 (95% CI: 0.798–0.879).

Conclusion: This nomogram serves as a bedside-friendly decision-support tool for early identification of ABO–HDN in neonates with hyperbilirubinemia, particularly when hemolysis test results are inconclusive or delayed. By translating five routinely available parameters into an individualized probability, the model enables clinicians to expedite risk stratification and initiate timely interventions—such as intensified phototherapy or intravenous immunoglobulin therapy—during the critical therapeutic window, potentially reducing the need for exchange transfusion and preventing bilirubin encephalopathy. This practical, quantitative tool directly addresses an unmet need in routine neonatal care, offering actionable guidance without imposing additional testing costs or delays.

1 Introduction

ABO hemolytic disease of the newborn (ABO–HDN) is the most common alloimmune hemolytic disease in neonates, and one of the primary causes of neonatal hyperbilirubinemia and severe jaundice [1, 2]. ABO–HDN results from the destruction of fetal or neonatal red blood cells (RBCs) by maternal immunoglobulin G (IgG) antibodies due to maternal-fetal blood group incompatibility[3]. Without timely intervention, it may lead to bilirubin encephalopathy or even death[4, 5]. Clinical diagnosis of ABO–HDN typically relies on the three hemolysis tests (direct antiglobulin test, antibody release test, and free antibody test) [6, 7]. However, in clinical practice, discrepancies between the results of these tests and

clinical symptoms are frequently observed.For example, a positive free antibody test may occur while the antibody release test and direct antiglobulin test remain negative. This can lead to diagnostic challenges, delayed treatment, or unnecessary overtreatment.

The etiology of neonatal hyperbilirubinemia (NHB) is complex. It encompasses not only immune-mediated hemolysis due to ABO incompatibility but also multiple factors such as G6PD deficiency, infections, intracranial hematoma, congenital hypothyroidism, and insufficient breast milk intake[1, 8–11]. Treatment strategies vary significantly depending on the underlying cause. For severe hyperbilirubinemia caused by ABO hemolytic disease, early initiation of intravenous immunoglobulin (IVIG) therapy can effectively delay or avoid exchange transfusion[12, 13]. In contrast, conditions like G6PD deficiency and infections require targeted interventions, including enhanced phototherapy[9, 13] and antimicrobial therapy. Therefore, accurate etiological differentiation is critical for clinical decision-making. Consequently, etiological studies must thoroughly account for potential confounding factors such as maternal prenatal complications (e.g., gestational diabetes, hypertension) and neonatal complications (e.g., infections, G6PD deficiency).

At present, there are many early prediction models for hyperbilirubinemia [5, 14, 15]. However, early prediction studies on ABO hemolytic disease mostly focus on a single indicator, such as the titer of anti-A /B IgG at delivery or the positive rate of DAT [7, 16, 17]. Moreover, comprehensive prediction models combining multiple clinical and laboratory indicators are still lacking. A nomogram, as an intuitive individualized risk prediction tool, has been widely applied in various diseases[18–20].

This study aims to develop and validate a nomogram for early identification of ABO–HDN using easily accessible clinical and laboratory parameters obtained within 24 hours of admission. By applying strict case screening (excluding diagnostically uncertain cases), along with LASSO and multivariate logistic regression, we constructed a model that can assist clinicians in identifying ABO–HDN in neonates with hyperbilirubinemia, especially when hemolysis test results are inconclusive or delayed. Critically, this nomogram is designed to integrate seamlessly into existing clinical workflows—all predictors are routinely collected within the first 24 hours of admission, requiring no specialized assays or additional blood draws. In real-world practice, this allows attending neonatologists to quickly differentiate immune-mediated hemolytic jaundice from other etiologies at the point of care, facilitating earlier discussions with families regarding treatment pathways and optimizing the allocation of neonatal intensive care resources. Unlike complex biomarker panels restricted to tertiary centers, this pragmatic tool holds particular promise for community hospitals and resource-limited settings, where access to prompt hemolysis testing may be constrained.

2 Methods

2.1 Study design and subjects

This retrospective study was conducted at Changsha Maternal and Child Health Hospital, Hunan, China, from January 2023 to December 2024. Cases of infants diagnosed with hyperbilirubinemia who completed the three hemolytic tests were collected. The diagnosis of hyperbilirubinemia was based on the "Guidelines for Diagnosis and Treatment of Neonatal Hyperbilirubinemia (2014)"[21]. The study employed the following exclusion criteria: (1) No laboratory tests performed within 24 hours of admission, (e.g., complete blood count (CBC), liver function tests, kidney function tests); (2) Weakly positive results in the three hemolytic tests (e.g., direct antiglobulin test or antibody release test) with uncertain outcomes; (3) Positive only in the free antibody test (suspicious diagnosis)-these cases were excluded to avoid outcome misclassification and ensure a gold–standard definition for model derivation; (4) Unknown blood type of the mother or infant; (5) RhD–negative blood type in the mother or infant (to avoid confounding factors from Rh incompatibility); (6) Confirmed hemolytic disease due to other blood group incompatibilities. Cases were ultimately divided into two groups: the ABO hemolytic disease group (clearly positive for DAT and/or antibody release test) and the isolated neonatal hyperbilirubinemia (NHB) group (negative for all three hemolytic tests).

2.2 Data collection

The following five categories of data were extracted from the electronic medical record system:

1. Demographic data: gender, gestational age (days), birth weight (kg), age at sampling (days), neonatal blood type, jaundice onset (hours after birth), jaundice to admission interval (days), and transcutaneous bilirubin (TCB) median.
2. Perinatal data: maternal blood type, maternal age, delivery method, feeding method, amniotic fluid condition, premature rupture of membranes (PROM), cord entanglement, gravidity, and parity. The classification variables are categorized as follows: Delivery method: 1) cesarean section, 2) vaginal delivery, 3) assisted delivery; Feeding method: 1) exclusive breastfeeding, 2) formula feeding, 3) mixed feeding; Amniotic fluid grade: 1) clear, 2) grade I meconium-stained, 3) grade II meconium-stained, 4) grade III meconium-stained, 5) bloody; PROM: 1) no membrane rupture, 2) intraoperative rupture, 3) spontaneous rupture < 12 h, 4) 12–24 h, 5) ≥ 24 h; Cord entanglement: 1) no entanglement, 2) one loop, 3) two loops, 4) ≥three loops, 5) cord torsion.

3. Neonatal Complications: According to the classification framework of Chap. 19 "Certain Conditions of Perinatal Origin" and Chap. 20 "Developmental Abnormalities" in the International Classification of Diseases, 11th Edition (ICD-11)[22], neonatal complications are categorized as follows: 1) Perinatal complications, including perinatal brain injury (Perinatal Brain Injury), scalp hematoma, retinal hemorrhage, etc. 2) Neonatal infections, including pneumonia, skin and soft tissue infections, conjunctivitis, etc. 3) Feeding or gastrointestinal issues, including inadequate feeding, feeding intolerance, lactose intolerance, neonatal diarrhea, etc. 4) Congenital defects, including ventricular septal defect, atrial septal defect, congenital biliary atresia, congenital polycystic kidney disease, chromosomal abnormalities, etc. Additionally, congenital hypothyroidism and G6PD deficiency were treated as independent entries in baseline analysis due to their well-defined etiology and exclusion from the aforementioned perinatal-related classifications, to maintain analytical specificity.
4. Maternal prenatal complications: gestational diabetes (Hyperglycemia), gestational hyperlipidemia (Hyperlipidemia), gestational infection (Infection), gestational hypertension (Hypertension).
5. Laboratory data: 1) Complete blood count: white blood cell count (WBC), red blood cell count (RBC), hemoglobin (HGB), platelet count (PLT), neutrophil count (NEUT), monocyte count (MONO), basophil count (BASO), eosinophil count (EOS), lymphocyte count (LYMPH), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width–coefficient of variation (RDW–CV), red cell distribution width–standard deviation (RDW–SD), platelet distribution width (PDW), mean platelet volume (MPV), plateletcrit (PCT), and reticulocyte percentage (RET). 2) Novel inflammatory biomarkers: neutrophil–to–lymphocyte ratio (NLR), platelet–to–lymphocyte ratio (PLR), monocyte–to–lymphocyte ratio (MLR), and eosinophil–to–lymphocyte ratio (ELR)[23]. 3) Liver function tests: alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TBIL), direct bilirubin (DBIL), indirect bilirubin (IBIL), total protein (TP), albumin (ALB), globulin (GLB), albumin–to–globulin ratio (A/G), and total bile acids (TBA). 4) Kidney function tests: creatinine (Cr) and uric acid (UA). 5) Cardiac enzymes: creatine kinase (CK), lactate dehydrogenase (LDH), and myoglobin (MYO). 6) Inflammatory marker: high–sensitivity C–reactive protein (hsCRP). All laboratory indicators were obtained from initial test results within 24 hours of hospital admission.

2.3 Statistical analysis

- Statistical analysis was performed using R software (version 4.4.2). Continuous variables were expressed as mean ± standard deviation, and inter-group comparisons were conducted using t-tests or Mann-Whitney U tests; Categorical variables were presented as frequencies (percentages), and intergroup comparisons were performed using χ^2 tests or Fisher's exact test. In univariate analysis, variables with $P < 0.1$ were included in binomial LASSO regression (10-fold cross-validation, λ value set at 1 standard error) to screen core predictors. Selected variables were incorporated into multivariate logistic regression to construct predictive models and generate nomograms. Model performance evaluation included: (1) Discrimination: Area under the receiver operating characteristic (ROC) curve (AUC); (2) Calibration: Calibration curve and Hosmer-Lemeshow test; (3)

Clinical utility: Decision curve analysis (DCA) and clinical impact curve (CIC); (4) Internal validation: The bootstrapping method (1000 replications) was used to calculate adjusted AUC; (5) Sensitivity analysis: To assess the impact of missing values on model stability, sensitivity analysis was performed on data with missing proportions > 3%. Other variables with lower missing proportions (≤ 3%) had limited impact on overall analysis and were therefore included only in imputed datasets.

3 Results

- A total of 592 neonatal cases were enrolled in this study. The following cases were sequentially excluded: 47 cases with no laboratory tests within 24 hours of admission; 89 cases with weakly positive antibody release test results and 2 cases with weakly positive direct antiglobulin test results in the three hemolysis tests; 61 cases with only positive free antibody test; 33 cases with unknown blood types; 9 cases with RhD-negative mothers and/or infants; and 1 case with Rh incompatibility. Ultimately, 350 cases were included, consisting of 154 cases (44.0%) in the ABO-HDN group and 196 cases (56.0%) in the isolated NHB group (control group) (Fig. 1).

- Partial variables exhibited missing values: TCB_Median, Maternal_Age, Gravidity, and Parity each had 4 missing cases (1.1%), RET had 30 missing cases (8.6%), Cr, UA, CK, LDH, and MYO each had 8 missing cases (2.3%), ROM_Classification had 5 missing cases (1.4%), Amniotic_Fluid_Grade had 1 missing case (0.3%), and Cord_Entanglement had 3 missing cases (0.9%). To minimize the bias caused by missing data, multiple imputation by chained equations (MICE) was performed with 5 imputations, and the pooled analysis results were obtained. The imputation model included all analytical variables.

3.1 Comparison of clinical characteristics and laboratory test results between the two groups

3.1.1 Demographic Characteristics

- Compared with the control group, infants in the ABO-HDN group had a younger age at sampling (2.18 ± 1.21 d vs. 3.82 ± 2.03 d, P < 0.001), a lower proportion of males (42.9% vs. 54.6%, P = 0.038), and a significantly higher proportion of type A blood (63.6% vs. 44.9%, P = 0.002). The time of jaundice onset (20.54 ± 7.80 h vs. 28.05 ± 10.41 h, P < 0.001) and the time from jaundice onset to hospital admission (1.25 ± 1.06 d vs. 2.59 ± 1.93 d, P < 0.001) were significantly shorter than those in the control group. There were no significant differences in birth weight, gestational age and TCB median between the two groups (P > 0.05) (Table 1).

3.1.2 Perinatal Factors

- The maternal blood group distribution revealed that all mothers in the ABO-HDN group were type O (100%), while 94.4% of the control group were type O, with a minority of type A (4.6%) or type B

(1.0%), showing a statistically significant difference (P = 0.012). No significant differences were observed between the two groups in maternal age, delivery method, feeding method, amniotic fluid grade, ROM classification, cord entanglement, gravidity, and parity (P > 0.05) (Table 2).

3.1.3 Neonatal Complications

- The comparison of neonatal complications between the two groups is presented in Table 1. The results showed no significant differences between the ABO-HDN group and the control group in terms of congenital hypothyroidism (11.0% vs. 8.7%, P = 0.576), G6PD deficiency (2.6% vs. 4.6%, P = 0.487), perinatal complications (3.2% vs. 7.1%, P = 0.174), neonatal infections (7.8% vs. 5.1%, P = 0.419), feeding or gastrointestinal issues (5.2% vs. 3.6%, P = 0.632), and congenital defects (2.6% vs. 2.6%, P = 1.000). These findings indicate that baseline comparability in neonatal complications between the two groups was achieved after excluding other definitive etiologies, thereby enhancing the specificity of subsequent analyses.

3.1.4 Maternal prenatal complications

- A comparison between the two groups regarding maternal prenatal complications is presented in Table 2. The results showed no statistically significant differences between the ABO-HDN group and the control group in terms of gestational hyperglycemia (22.1% vs. 24.5%, P = 0.688), gestational hyperlipidemia (48.1% vs. 48.0%, P = 1.000), gestational infection (11.7% vs. 19.4%, P = 0.071), and gestational hypertension (11.0% vs. 14.8%, P = 0.383). Notably, the control group exhibited a slightly higher incidence of gestational infection (P = 0.071), but this difference was not statistically significant. These findings indicate that maternal prenatal complications were comparable between the two groups and did not constitute a confounding factor. The predictors identified by the model better reflect the pathophysiological characteristics of ABO hemolytic disease itself rather than the confounding effects of other complications.

3.1.5 Laboratory Parameter Characteristics

- The ABO-HDN group exhibited typical hemolytic features: significantly decreased RBC (4.46 ± 0.63 vs. 4.87 ± 0.58, P < 0.001) and HGB (159.60 ± 20.78 vs. 170.76 ± 21.47, P < 0.001); significantly elevated RET (4.66 ± 2.41 vs. 2.90 ± 1.67, P < 0.001), LDH (604.38 ± 183.11 vs. 548.31 ± 175.21, P = 0.004), and CK (450.88 ± 255.85 vs. 344.44 ± 262.96, P < 0.001). Inflammatory markers showed significantly elevated WBC (14.17 ± 6.04 vs. 10.98 ± 3.65, P < 0.001), NEUT (9.25 ± 5.31 vs. 5.95 ± 3.21, P < 0.001), and NLR (2.93 ± 1.52 vs. 1.99 ± 1.41, P < 0.001). Kidney function indicators demonstrated significantly increased Cr (49.16 ± 17.06 vs. 35.13 ± 17.93, P < 0.001) and UA (265.18 ± 98.30 vs. 214.38 ± 93.06, P < 0.001). Red blood cell morphology parameters including MCV, MCH, RDW-CV, and RDW_SD were all significantly elevated (P < 0.001). Notably, the TBIL (252.75 ± 59.80 vs. 299.81 ± 67.02, P < 0.001) and IBIL (242.66 ± 60.31 vs. 287.88 ± 65.92, P < 0.001) in the ABO-HDN

group were lower than those in the control group, which may be attributed to earlier hospital admission and timely intervention in the ABO-HDN group (Table 3).

Table 1
Comparison of demographic characteristics and neonatal complications between the two groups

Variable	level	Control group (n = 196)	ABO-HD group (n = 154)	P-value
Gender,n(%)	Male	107 (54.6)	66 (42.9)	0.038
	Female	89 (45.4)	88 (57.1)	
Gestational age(days)	Mean ± SD	272.52 ± 7.79	273.15 ± 7.75	0.449
Birth weight(kg)	Mean ± SD	3.24 ± 0.38	3.27 ± 0.37	0.4
Age at Sampling(days)	Mean ± SD	3.82 ± 2.03	2.18 ± 1.21	< 0.001
Blood Type, n(%)	A	88 (44.9)	98 (63.6)	0.002
	B	102 (52.0)	56 (36.4)	
	O	5 (2.6)	0 (0.0)	
	AB	1 (0.5)	0 (0.0)	
Jaundice Onset(Hrs)	Mean ± SD	28.05 ± 10.41	20.54 ± 7.80	< 0.001
Jaundice to Admission Interval(days)	Mean ± SD	2.59 ± 1.93	1.25 ± 1.06	< 0.001
TCB Median(mg/dl)	Mean ± SD	7.90 ± 2.26	8.19 ± 2.52	0.25
Congenital Hypothyroidism, n(%)	No	179 (91.3)	137 (89.0)	0.576
	Yes	17 (8.7)	17 (11.0)	
G6PD Deficiency,n(%)	No	187 (95.4)	150 (97.4)	0.487
	Yes	9 (4.6)	4 (2.6)	
Perinatal complications, n(%)	No	182 (92.9)	149 (96.8)	0.174
	Yes	14 (7.1)	5 (3.2)	
Neonatal Infections,n(%)	No	186 (94.9)	142 (92.2)	0.419
	Yes	10 (5.1)	12 (7.8)	

Notes: SD, standard deviation; Hrs, hours; Perinatal complications: Perinatal Brain Injury,scalp hematoma, retinal hemorrhage; Neonatal Infections: pneumonia, skin and soft tissue infection, conjunctivitis; Feeding or Gastrointestinal Issues: inadequate feeding, lactose intolerance, feeding intolerance, neonatal diarrhea; Congenital Defect: ventricular septal defect, atrial septal defect, congenital biliary dilatation, congenital polycystic kidney disease, chromosomal abnormality.

Variable	level	Control group (n = 196)	ABO-HD group (n = 154)	P-value
Feeding or Gastrointestinal Issues,n(%)	No	189 (96.4)	146 (94.8)	0.632
	Yes	7 (3.6)	8 (5.2)	
Congenital Defects,n(%)	No	191 (97.4)	150 (97.4)	1
	Yes	5 (2.6)	4 (2.6)	

Notes: SD, standard deviation; Hrs, hours; Perinatal complications: Perinatal Brain Injury,scalp hematoma, retinal hemorrhage; Neonatal Infections: pneumonia, skin and soft tissue infection, conjunctivitis; Feeding or Gastrointestinal Issues: inadequate feeding, lactose intolerance, feeding intolerance, neonatal diarrhea; Congenital Defect: ventricular septal defect, atrial septal defect, congenital biliary dilatation, congenital polycystic kidney disease, chromosomal abnormality.

Table 2
Comparison of perinatal factors and maternal prenatal complications between the two groups

Variable	level	Control group (n = 196)	ABO-HD group (n = 154)	P-value
Maternal Blood Type,n(%)	A	9 (4.6)	0 (0.0)	0.012
	B	2 (1.0)	0 (0.0)	
	O	185 (94.4)	154 (100.0)	
Maternal Age	Mean ± SD	30.16 ± 3.87	30.39 ± 4.12	0.598
Delivery method,n(%)	1	58 (29.6)	55 (35.7)	0.379
	2	128 (65.3)	94 (61.0)	
	3	10 (5.1)	5 (3.2)	
Feeding Method,n(%)	1	84 (42.9)	53 (34.4)	0.206
	2	21 (10.7)	15 (9.7)	
	3	91 (46.4)	86 (55.8)	
Amniotic Fluid Grade,n(%)	1	190 (96.9)	148 (96.1)	0.844
	2	2 (1.0)	2 (1.3)	
	3	2 (1.0)	3 (1.9)	
	4	1 (0.5)	1 (0.6)	
	5	1 (0.5)	0 (0.0)	
ROM Classification,n(%)	1	7 (3.6)	8 (5.2)	0.546
	2	39 (19.9)	37 (24.0)	
	3	108 (55.1)	85 (55.2)	
	4	34 (17.3)	18 (11.7)	
	5	8 (4.1)	6 (3.9)	
Cord Entanglement,n(%)	1	135 (68.9)	111 (72.1)	0.869
	2	49 (25.0)	35 (22.7)	

Notes: SD, standard deviation; ROM, Rupture of Membranes; Delivery method: 1,cesarean section; 2, vaginal delivery; 3, assisted delivery; Feeding method: 1, exclusive breastfeeding; 2, formula feeding; 3, mixed feeding; Amniotic Fluid Grade: 1, clear; 2, grade I meconium-stained; 3, grade II meconium-stained; 4, grade III meconium-stained; 5, bloody; ROM Classification: 1, no PROM; 2, intraoperative rupture; 3, spontaneous rupture < 12 h; 4, spontaneous rupture 12–24 h; 5, spontaneous rupture ≥ 24 h; Cord Entanglement: 1, no entanglement; 2, one loop; 3, two loops; 4, three or more loops; 5,cord torsion.

Variable	level	Control group (n = 196)	ABO-HD group (n = 154)	P-value
	3	7 (3.6)	3 (1.9)	
	4	2 (1.0)	2 (1.3)	
	5	3 (1.5)	3 (1.9)	
Gravidity	Mean ± SD	1.90 ± 1.25	2.06 ± 1.17	0.236
Parity	Mean ± SD	1.37 ± 0.56	1.44 ± 0.62	0.275
Hyperglycemia,n(%)	No	148 (75.5)	120 (77.9)	0.688
	Yes	48 (24.5)	34 (22.1)	
Hyperlipidemia,n(%)	No	102 (52.0)	80 (51.9)	1
	Yes	94 (48.0)	74 (48.1)	
Infection,n(%)	No	158 (80.6)	136 (88.3)	0.071
	Yes	38 (19.4)	18 (11.7)	
Hypertension,n(%)	No	167 (85.2)	137 (89.0)	0.383
	Yes	29 (14.8)	17 (11.0)	

Notes: SD, standard deviation; ROM, Rupture of Membranes; Delivery method: 1,cesarean section; 2, vaginal delivery; 3, assisted delivery; Feeding method: 1, exclusive breastfeeding; 2, formula feeding; 3, mixed feeding; Amniotic Fluid Grade: 1, clear; 2, grade I meconium-stained; 3, grade II meconium-stained; 4, grade III meconium-stained; 5, bloody; ROM Classification: 1, no PROM; 2, intraoperative rupture; 3, spontaneous rupture < 12 h; 4, spontaneous rupture 12–24 h; 5, spontaneous rupture ≥ 24 h; Cord Entanglement: 1, no entanglement; 2, one loop; 3, two loops; 4, three or more loops; 5,cord torsion.

Table 3
Comparison of laboratory indicators between the two groups

Variable	level	Control group (n = 196)	ABO-HD group (n = 154)	P-value
WBC	Mean ± SD	10.98 ± 3.65	14.17 ± 6.04	< 0.001
RBC	Mean ± SD	4.87 ± 0.58	4.46 ± 0.63	< 0.001
HGB	Mean ± SD	170.76 ± 21.47	159.60 ± 20.78	< 0.001
PLT	Mean ± SD	276.53 ± 75.74	291.41 ± 70.04	0.06
NEUT	Mean ± SD	5.95 ± 3.21	9.25 ± 5.31	< 0.001
MONO	Mean ± SD	1.15 ± 0.40	1.11 ± 0.42	0.403
BASO	Mean ± SD	0.01 ± 0.01	0.02 ± 0.01	< 0.001
EOS	Mean ± SD	0.45 ± 0.24	0.52 ± 0.31	0.016
LYMPH	Mean ± SD	3.43 ± 1.20	3.28 ± 1.06	0.207
NLR	Mean ± SD	1.99 ± 1.41	2.93 ± 1.52	< 0.001
PLR	Mean ± SD	88.41 ± 33.79	96.90 ± 35.88	0.024
MLR	Mean ± SD	0.36 ± 0.15	0.35 ± 0.13	0.683
ELR	Mean ± SD	0.14 ± 0.08	0.17 ± 0.10	0.002
HCT	Mean ± SD	0.50 ± 0.06	0.47 ± 0.06	< 0.001
MCV	Mean ± SD	101.77 ± 5.07	104.60 ± 5.19	< 0.001
MCH	Mean ± SD	35.06 ± 1.88	35.86 ± 1.66	< 0.001
MCHC	Mean ± SD	344.45 ± 6.91	342.92 ± 7.00	0.041
RDW_CV	Mean ± SD	14.96 ± 0.95	15.50 ± 1.09	< 0.001
RDW_SD	Mean ± SD	55.62 ± 4.35	58.96 ± 5.44	< 0.001
PDW	Mean ± SD	16.14 ± 1.31	16.25 ± 1.08	0.415

Notes: WBC, white blood cell; RBC, red blood cell; HGB, hemoglobin; PLT, platelet; NEUT, neutrophil; MONO, monocyte; BASO, basophil; EOS, eosinophil; LYMPH, lymphocyte; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; MLR, monocyte-to-lymphocyte ratio; ELR, eosinophil-to-lymphocyte ratio; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW-CV, red cell distribution width-coefficient of variation; RDW-SD, red cell distribution width-standard deviation; PDW, platelet distribution width; MPV, mean platelet volume; PCT, plateletcrit; RET, reticulocyte percentage; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBIL, total bilirubin; DBIL, direct bilirubin; IBIL, indirect bilirubin; TP, total protein; ALB, albumin; GLB, globulin; A/G, albumin-to-globulin ratio; TBA, total bile acids; Cr, creatinine; UA, uric acid; CK, creatine kinase; LDH, lactate dehydrogenase; MYO, myoglobin; hsCRP, high-sensitivity C-reactive protein.

Variable	level	Control group (n = 196)	ABO-HD group (n = 154)	P-value
MPV	Mean ± SD	9.55 ± 0.78	9.27 ± 0.82	0.002
PCT	Mean ± SD	0.26 ± 0.07	0.26 ± 0.05	0.979
RET	Mean ± SD	2.90 ± 1.67	4.66 ± 2.41	< 0.001
ALT	Mean ± SD	9.29 ± 5.16	8.96 ± 5.71	0.577
AST	Mean ± SD	48.67 ± 26.60	52.34 ± 24.67	0.187
TBIL	Mean ± SD	299.81 ± 67.02	252.75 ± 59.80	< 0.001
DBIL	Mean ± SD	11.94 ± 2.81	11.43 ± 2.46	0.076
IBIL	Mean ± SD	287.88 ± 65.92	242.66 ± 60.31	< 0.001
TP	Mean ± SD	52.80 ± 4.52	52.41 ± 4.25	0.415
ALB	Mean ± SD	35.81 ± 2.98	35.89 ± 3.01	0.806
GLB	Mean ± SD	16.99 ± 3.27	16.52 ± 3.19	0.177
A/G	Mean ± SD	2.20 ± 0.53	2.26 ± 0.50	0.269
TBA	Mean ± SD	17.68 ± 11.17	13.35 ± 8.29	< 0.001
Cr	Mean ± SD	35.13 ± 17.93	49.16 ± 17.06	< 0.001
UA	Mean ± SD	214.38 ± 93.06	265.18 ± 98.30	< 0.001
CK	Mean ± SD	344.44 ± 262.96	450.88 ± 255.85	< 0.001
LDH	Mean ± SD	548.31 ± 175.21	604.38 ± 183.11	0.004
MYO	Mean ± SD	55.83 ± 43.57	75.20 ± 55.33	< 0.001
hsCRP	Mean ± SD	3.85 ± 5.56	5.74 ± 11.88	0.05

Notes: WBC, white blood cell; RBC, red blood cell; HGB, hemoglobin; PLT, platelet; NEUT, neutrophil; MONO, monocyte; BASO, basophil; EOS, eosinophil; LYMPH, lymphocyte; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; MLR, monocyte-to-lymphocyte ratio; ELR, eosinophil-to-lymphocyte ratio; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW-CV, red cell distribution width-coefficient of variation; RDW-SD, red cell distribution width-standard deviation; PDW, platelet distribution width; MPV, mean platelet volume; PCT, plateletcrit; RET, reticulocyte percentage; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBIL, total bilirubin; DBIL, direct bilirubin; IBIL, indirect bilirubin; TP, total protein; ALB, albumin; GLB, globulin; A/G, albumin-to-globulin ratio; TBA, total bile acids; Cr, creatinine; UA, uric acid; CK, creatine kinase; LDH, lactate dehydrogenase; MYO, myoglobin; hsCRP, high-sensitivity C-reactive protein.

3.2 Variable Screening: Univariate Analysis and Lasso Regression Analysis

A univariate analysis was performed on the 66 variables included in the analysis (Table S1), identifying 35 candidate variables with $P < 0.1$. The results are presented in the form of a forest plot (Fig. 2). In the plot, protective factors (odds ratio [OR] < 1) are indicated in blue, while risk factors (OR > 1) are shown in red; the size of the points reflects statistical significance (-log10(P-value)). For instance, older age at sampling (OR = 0.470), later onset of jaundice (OR = 0.907), and higher RBC (OR = 0.312) were each associated with reduced risk of ABO hemolytic disease, whereas elevated RET (OR = 1.540) and NEUT (OR = 1.221) were associated with increased risk. Notably, BASO ($OR \approx 2.54\times10^{13}$) and HCT ($OR \approx 0$) were truncated from the plot for clarity due to their extreme values, yet their effect directions remain clearly identifiable.

All 35 candidate variables were subjected to LASSO regression to identify the most concise and predictive subset. The ten-fold cross-validation deviation plot for LASSO regression (Fig. 3) demonstrates that the optimal regularization parameter λ was determined by minimizing the binomial deviance. According to the "one-standard-error rule," the maximum allowable λ value within the range of the minimum cross-validation deviation plus one standard error (i.e., λ.1se = 0.0578, indicated by the blue dashed line in the figure) was selected as the final penalty parameter. This strategy achieved the simplest variable set while maintaining predictive performance. In comparison, although λ.min (indicated by the red dashed line in the figure) corresponded to the minimum deviance, it involved a larger number of variables. Ultimately, seven variables with non-zero coefficients were selected at λ.1se = 0.0578: age at sampling, time of jaundice onset, RBC, NEUT, RET, Cr, and neonatal blood type. These selected variables represent the most influential factors in predicting the risk of hemolytic disease due to ABO blood group incompatibility, providing a solid foundation for subsequent nomogram construction. To further assess multicollinearity, the variance inflation factors (VIF) for the seven predictive variables were calculated. All of them were less than 5, confirming the absence of multicollinearity.

3.3 Multivariate Logistic Regression and Model Simplification

The seven variables identified by LASSO regression were incorporated into a multivariate logistic regression model, with ABO hemolytic disease as the outcome variable(Table S2). The results demonstrated that age at sampling (OR = 0.734, 95% CI: 0.558–0.967, P = 0.028), time of jaundice onset (OR = 0.952, 95% CI: 0.919–0.987, P = 0.007), RBC (OR = 0.441, 95% CI: 0.275–0.708, P < 0.001), RET (OR = 1.206, 95% CI: 1.037–1.404, P = 0.015), and neonatal blood type (OR = 0.341, 95% CI: 0.203–0.573, P < 0.001) were all independent predictors of ABO hemolytic disease ($P < 0.05$). The NEUT (OR = 1.023, 95% CI: 0.941–1.113, P = 0.590) and Cr (OR = 1.010, 95% CI: 0.992–1.029, P = 0.292) showed no statistically significant association in the multivariate analysis.

To obtain a more concise model, we compared the model incorporating all seven variables with the five-variable model excluding NEUT and Cr. The results showed that the AUC values for the two models were 0.837 (95% CI: 0.797–0.878) and 0.835 (95% CI: 0.794–0.876), respectively. The difference in AUC was

minimal, with highly overlapping 95% confidence intervals, indicating no clinically significant difference in discriminative power between the two models. The P-values for the Hosmer-Lemeshow test were 0.4423 and 0.7454, both > 0.05. Given their comparable predictive performance and the greater simplicity of the five-variable model, the five-variable model (including age at sampling, time of jaundice onset, RBC, RET, and neonatal blood type) was ultimately selected to construct the nomogram prediction model. The multivariate logistic regression results for the five-variable model are detailed in Table 4.

Table 4
The multivariate logistic regression results for the five-variable model

Variable	β	SE	Z	P_value	OR (95% CI)
Age_at_Sampling	-0.419	0.117	-3.586	0.0003	0.658(0.523–0.827)
Jaundice_Onset_Hrs	-0.053	0.018	-2.935	0.0033	0.948(0.915–0.982)
RBC	-0.818	0.237	-3.447	0.0006	0.441(0.277–0.703)
RET	0.188	0.076	2.464	0.0137	1.207(1.039–1.402)
Blood_Type	-1.053	0.26	-4.054	0.0001	0.349(0.21–0.58)

Notes: OR, odds ratio; CI, confidence interval; The blood types were referenced as type A (coded as 1), while types B, O, and AB were coded as 2, 3, and 4, respectively. An OR < 1 indicates a reduced risk as the code value increases.

3.4 Evaluation and Internal Validation of the Nomogram Model Performance

A nomogram was constructed based on the five variables (Fig. 4). Each variable corresponds to a specific score, and the total score reflects the risk of ABO hemolytic disease. The model demonstrated excellent discrimination power, with an area under the ROC curve (AUC) of 0.835 (95% CI: 0.794–0.876; Fig. 5A). The calibration curve showed good consistency between predicted and actual probabilities (Hosmer-Lemeshow test, P = 0.7454; Fig. 5B).

The DCA (Fig. 5C) demonstrates that across a broad range of threshold probabilities from 0.1 to 0.83, the net benefits derived from decisions based on this model are significantly higher than those of the "full treatment" and "full no-treatment" strategies. This extensive range of benefits indicates that the model delivers superior clinical net benefits compared to conventional decision-making, regardless of whether clinicians adopt a relatively aggressive or conservative treatment approach. The CIC (Fig. 5D) further visually illustrates the potential clinical outcomes of the model under different thresholds. As the threshold increases from 0 to 1, the number of neonates classified as "high-risk" (i.e., with a predicted probability ≥ the threshold) gradually decreases, as does the number of cases actually diagnosed with ABO hemolytic disease. However, the positive predictive value progressively rises. It visually represents the intervention burden and potential benefit of clinical decisions, thereby helping clinicians select an appropriate intervention threshold based on their risk preferences. By integrating the DCA with the CIC,

the nomogram model developed in this study demonstrates excellent clinical practicality and translational application potential.

Internal validation using Bootstrap (1,000 resampling iterations) demonstrated that the model's original AUC was 0.835 (95% CI: 0.794–0.876), while the corrected AUC was 0.839 (95% CI: 0.798–0.879), with an optimism score of − 0.004. The corrected AUC closely matched the original AUC, indicating robust model stability and no significant overfitting. This confirms the model's robustness and generalizability to similar patient populations.

3.5 Sensitivity Analysis (RET Missing Value Handling)

Given that RET demonstrated substantial predictive value in the model yet exhibited a relatively high missing rate (8.6%), we performed a complete-case analysis by excluding the 30 observations with missing RET values to assess the potential impact of missing data on model estimates and performance. Subsequently, we re-fitted the five-variable model. The results showed that the OR for RET in the complete-case dataset was 1.186 (95% CI: 1.011–1.390, P = 0.0359), while the OR for the full dataset following multiple imputation was 1.207 (95% CI: 1.039–1.402, P = 0.0137). The corresponding AUC values were 0.838 (95% CI: 0.796–0.881) and 0.835 (95% CI: 0.794–0.876), respectively. The 95% CIs for the ORs in both groups exhibited substantial overlap, and no statistically significant differences were observed in either AUC or its confidence interval. Overall, these findings indicate that the missingness in RET did not introduce significant bias in model estimates or impair discriminative performance, supporting the validity and robustness of the multiple imputation strategy.

4 Discussion

This study developed and validated a nomogram for early identification of ABO–HDN in neonates with hyperbilirubinemia using five readily available variables: age at sampling, time of jaundice onset, RBC, RET, and neonatal blood type. Through rigorous case screening (excluding diagnostically uncertain cases) and systematic analysis of neonatal and maternal complications, the model demonstrated strong discrimination (AUC = 0.835), excellent calibration (Hosmer–Lemeshow P = 0.7454), and great clinical utility. In clinical practice, when hemolysis test results are inconclusive (e.g., positive free antibody test with negative DAT and antibody release test) or unavailable, clinicians can use this nomogram as an adjunctive tool to estimate the probability of ABO–HDN.

Multivariate analysis results demonstrated that blood type is an independent risk factor for ABO hemolytic disease. Children with type A blood exhibit a significantly higher risk than those with type B blood, which is consistent with multiple previous studies[24–26]. However, it is noteworthy that a recent large - scale cohort study based on the Chinese population revealed that both the overall incidence and severity rate of ABO hemolytic disease were higher in children with type B blood compared to those with type A blood [27]. This finding appears to contrast with our result that type A accounted for a higher proportion of confirmed cases (blood type OR = 0.341, 95% CI: 0.203–0.573, P < 0.001). We attribute this

discrepancy primarily to differences in study populations: the cohort study assessed the incidence of severe cases in the general neonatal population, whereas our study enrolled only infants who developed hyperbilirubinemia and had either positive or completely negative hemolysis test results (isolated free–antibody–positive cases were excluded), thereby estimating the diagnostic probability among jaundiced infants. Type A infants tend to present with more typical clinical features (e.g., earlier onset and more prominent hemolytic evidence), making them more likely to meet the positive hemolysis criteria and be confirmed in our study. In contrast, the cohort study may have captured more severe cases associated with type B blood. Thus, the two findings are not contradictory but rather reflect different perspectives of disease assessment.

In addition to blood type, this model incorporates other clinically significant predictors. A younger age at sampling (OR = 0.658) and earlier onset of jaundice (OR = 0.948) both reflect an earlier disease onset, and serve as important markers of hemolytic jaundice, which is associated with increased bilirubin production due to erythrocyte destruction[28]. Reduced RBC (OR = 0.441) and increased RET (OR = 1.207) directly indicate ongoing hemolysis and the bone marrow's compensatory response. Mechanistically, maternal anti-A or anti-B IgG antibodies crossing the placenta bind to fetal erythrocyte surface antigens, leading to opsonization and subsequent phagocytosis by splenic and hepatic macrophages, a process primarily mediated by Fcγ receptors. This extravascular hemolysis results in a net reduction in circulating RBC mass and hemoglobin levels. Concurrently, the destruction of erythrocytes triggers a hypoxia-driven upregulation of erythropoietin (EPO) production, which stimulates the bone marrow to accelerate erythroid progenitor proliferation. The significant elevation in RET observed in our ABO-HDN group reflects the premature release of immature reticulocytes into the peripheral circulation—a hallmark of "stress erythropoiesis." Importantly, RET elevation may serve as an earlier indicator of hemolytic activity than the decline in hemoglobin, as the bone marrow's compensatory reserve in neonates is limited by their relatively brief erythrocyte lifespan (approximately 70–90 days) and rapidly expanding blood volume during the early postnatal period. This pathophysiological cascade underscores why RET, in conjunction with absolute RBC count, provides a more dynamic real-time assessment of hemolytic severity than static bilirubin levels alone, which may be confounded by sampling timing[29, 30].

Although NEUT and Cr were initially retained in LASSO regression, their P-values in multivariate analysis were both > 0.05 (0.590 and 0.292). Exclusion of these variables did not significantly impair model performance (AUC decreased from 0.837 to 0.835, and the Hosmer-Lemeshow test P-value improved from 0.443 to 0.745), indicating limited independent predictive value for ABO hemolytic disease. This may stem from the collinearity between NEUT and inflammatory markers such as WBC, while elevated Cr may be associated with transient renal impairment caused by hemolysis; however, its contribution was attenuated after controlling for other variables. The final five-variable model strikes a balance between model simplicity and predictive performance.

Notably, in this study, the TBIL level in the hemolytic disease group was actually lower than that in the control group, which is contrary to conventional assumptions. The possible reasons are as follows:

children in the hemolytic disease group developed early-onset jaundice and were hospitalized promptly, receiving laboratory results before bilirubin levels reached their peak. In contrast, those in the control group with physiological or breast milk-related jaundice were admitted later and already presented with elevated bilirubin levels at admission. This finding suggests that hemolytic disease cannot be diagnosed based solely on bilirubin levels, and comprehensive evaluation incorporating other biomarkers is essential.

Additionally, although maternal blood type is a prerequisite for ABO hemolytic disease, maternal blood type showed minimal variation in this study (100% of ABO hemolytic disease cases were type O, compared to 94.4% in the control group), and LASSO regression did not include maternal blood type in the model. In clinical practice, after confirming maternal-fetal blood type incompatibility, physicians should use the other biomarkers in this model to effectively predict the risk of ABO hemolytic disease in infants with hyperbilirubinemia.

This study offers the following advantages. First, unlike previous studies that mainly focused on single indicators such as maternal anti - A/B IgG titers or DAT positivity rates, this study systematically integrates routine parameters obtainable within 24 hours of hospital admission, (including age at sampling, onset time of jaundice, complete blood count, and liver/kidney function). This eliminates additional testing costs and makes the model suitable for resource-limited settings. Second, the nomogram model is visually intuitive. Clinicians can rapidly obtain individualized prediction probabilities by inputting parameter values, which provides a quantitative evaluation tool for diagnostically uncertain cases. It effectively addresses the limitation of "experience-based decision-making" in current clinical practice and demonstrates clear clinical translational value. Third, rigorous variable selection was performed using LASSO regression and multivariate logistic regression analysis, which effectively controls overfitting and enhances model simplicity. Additionally, the performance of the regression model was comprehensively evaluated using the discrimination index (AUC), calibration (Hosmer-Lemeshow test), and clinical utility measures (DCA and CIC). Internal validation was conducted via Bootstrap resampling, confirming the model's robustness and generalizability. Fourth, for the 30 cases (8.6%) with RET deletion, multiple imputation (MICE) was applied. Sensitivity analysis demonstrated that the between-group differences in RET expression, OR values, and model AUC remained largely consistent before and after imputation. This confirms that the missing data had no substantial impact on the final conclusions and thereby enhances the credibility of the results. Fifth, a comprehensive collection and analysis of maternal prenatal complications and neonatal complications confirmed that the two groups were balanced and comparable for these factors. This effectively eliminates interference from other etiological factors and significantly enhances the model's specificity.

This study still has certain limitations. First, as a single-center retrospective study, it may be affected by selection bias and lacks validation with an external independent dataset. Although bootstrap internal validation (1,000 resamples) demonstrated robust stability with a corrected AUC of 0.839, we fully acknowledge that external validation is essential to confirm generalizability. To address this, we have initiated a multi-center external validation protocol in collaboration with two affiliated tertiary hospitals in

our region (Changsha Central Hospital and The Second Hospital of Changsha), with a planned enrollment of approximately 300 additional neonates over the next 12 months. This prospective validation cohort will not only assess the discriminative performance of the nomogram but also evaluate its clinical utility in real-time decision-making through pre-post intervention comparison studies. Furthermore, we plan to release a user-friendly web-based nomogram calculator after external validation, facilitating broader adoption in routine neonatal practice. We consider this external validation an indispensable next step prior to any formal implementation in clinical guidelines. Second, some subgroups (e.g., infants with AB blood type) had small sample sizes, which led to limited precision in OR estimates. Further validation with expanded sample sizes is required. Third, due to the constraints of retrospective data collection, novel biomarkers including maternal IgG antibody titer and subtype, and UGT1A1 gene variants were not included in the analysis. These markers have been confirmed to be associated with neonatal hyperbilirubinemia[5, 26], so future prospective studies can incorporate them to improve the predictive performance of the model. Fourth, the model is currently only applicable to infants with maternal-fetal ABO blood type incompatibility, and its clinical value is limited for other types of blood group incompatibility. Fifth, the nomogram was developed exclusively using cases with definitive diagnoses. Cases with inconclusive hemolysis test patterns (isolated positive free antibody test) were excluded from model derivation to prevent outcome misclassification. While this approach guaranteed a reliable gold standard, it also means that the performance of the model in such inconclusive cases has not been directly evaluated. Future prospective studies should specifically enroll neonates with inconclusive test results and validate the nomogram using clinically meaningful outcomes (e.g., need for IVIG or exchange transfusion).

5 Conclusion

In summary, this study addresses the need for early identification of ABO–HDN in neonates with hyperbilirubinemia. By rigorously screening cases, systematically analyzing baseline comparability of maternal and neonatal complications, and employing multiple imputation for missing data, we developed and validated a nomogram based on age at sampling, time of jaundice onset, red blood cell count, reticulocyte percentage, and neonatal blood type. The model demonstrates good discrimination, calibration, and clinical utility, enabling clinicians to identify ABO–HDN early in neonates with hyperbilirubinemia, particularly when diagnostic uncertainty exists. It provides a quantitative reference for initiating appropriate treatment while awaiting confirmatory testing.

Declarations

Data availability statement

The original contributions to the study can be found in the article/supplementary material , further inquiries can be directed to the corresponding author.

Ethics approval and consent to participate

The study was approved by the Internal Review Board (IRB) of the Clinical Research Ethics Committee of Changsha Hospital for Maternal and Child Health Care (Changsha Maternal and Child Health Care and Family Planning Service Center) (Approval No. EC-20260604-32). Written informed consent was waived by the ethics committee because this was a retrospective analysis of data collected from electronic medical records, with privacy protection measures applied, in accordance with the Declaration of Helsinki.

Clinical trial number

Not applicable.

Author contributions

YL: Data curation, Writing original draft, review & editing; JD: Methodology, Validation, Visualization, Writing original draft, review & editing.

Funding

The authors declare that no financial support was received for the research and/or publication of this article.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Xu C, Bao Y, He Y, Wu M, Zhu J. Risk factors for readmission for hyperbilirubinemia in neonates with ABO hemolytic disease: a single-center retrospective cohort study. J Maternal-Fetal Neonatal Med Taylor Francis. 2023;36:2238106. https://doi.org/10.1080/14767058.2023.2238106.
2. Pan J, Zhan C, Yuan T, Chen X, Ni Y, Shen Y, et al. Intravenous immunoglobulin G in the treatment of ABO hemolytic disease of the newborn during the early neonatal period at a tertiary academic hospital: a retrospective study. J Perinatol. 2021;41:1397–402. https://doi.org/10.1038/s41372-021-00963-5.
3. Hall V, Vadakekut ES, Maines J, Avulakunta ID. Hemolytic Disease of the Fetus and Newborn. StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2026 [cited 2026 Mar 15]. http://www.ncbi.nlm.nih.gov/books/NBK557423/. Accessed 15 Mar 2026.
4. Rennie JM, Beer J, Upton M. Learning from claims: hyperbilirubinaemia and kernicterus. Arch Dis Child Fetal Neonatal Ed. 2019;104:F202–4. https://doi.org/10.1136/archdischild-2017-314622.
5. Wang X, Xiao T, Wang J, Wu B, Wang H, Lu Y, et al. Clinical and genetic risk factors associated with neonatal severe hyperbilirubinemia: a case–control study based on the China Neonatal Genomes

Project. Front Genet. 2024;14:1292921. https://doi.org/10.3389/fgene.2023.1292921.

6. Li Y, Deng J. The Diagnostic Potential of the L Score for ABO Hemolytic Disease of the Newborn: Insights from a Cross-Sectional Study. Indian J Hematol Blood Transfus. 2024;40:469–78. https://doi.org/10.1007/s12288-023-01723-5.
7. Song J, Yang T, Zhou X-F, Yang Y-Z, Peng D-L, Zhang J-B. [The Correlation Analysis between the Titer of IgG Anti-A/B Erythrocyte Antibody In Vivo of the Neonate and the Severity of Hemolytic Disease of Newborn]. Zhongguo Shi Yan Xue Ye Xue Za Zhi. 2022;30:547–51. https://doi.org/10.19746/j.cnki.issn.1009-2137.2022.02.038.
8. Xu J-X, Lin F, Wu Y-H, Chen Z-K, Ma Y-B, Yang L-Y. Etiology analysis for term newborns with severe hyperbilirubinemia in eastern Guangdong of China. World J Clin Cases. 2023;11:2443–51. https://doi.org/10.12998/wjcc.v11.i11.2443.
9. Lee HY, Ithnin A, Azma RZ, Othman A, Salvador A, Cheah FC. Glucose-6-Phosphate Dehydrogenase Deficiency and Neonatal Hyperbilirubinemia: Insights on Pathophysiology, Diagnosis, and Gene Variants in Disease Heterogeneity. Front Pediatr. 2022;10:875877. https://doi.org/10.3389/fped.2022.875877.
10. Rastogi MV, LaFranchi SH. Congenital hypothyroidism. Orphanet J Rare Dis. 2010;5:17. https://doi.org/10.1186/1750-1172-5-17.
11. Risk Factors for Severe Hyperbilirubinemia in Neonates. | Pediatric Research [Internet]. [cited 2026 Mar 15]. https://www.nature.com/articles/pr2004574. Accessed 15 Mar 2026.
12. de Winter DP, Verweij EJT (Joanne), Debeer A, Devlieger R, Lewi L, Verbeeck S Variations and Opportunities in Postnatal Management of Hemolytic Disease of the Fetus and Newborn, et al. editors. JAMA Netw Open. 2025;8:e2454330. https://doi.org/10.1001/jamanetworkopen.2024.54330
13. Bhutani VK, Wong RJ, Turkewitz D, Rauch DA, Mowitz ME, Barfield WD et al. Phototherapy to Prevent Severe Neonatal Hyperbilirubinemia in the Newborn Infant 35 or More Weeks of Gestation: Technical Report. Pediatrics. 2024;154:e2024068026. https://doi.org/10.1542/peds.2024-068026
14. Cui Z, Shen W, Sun X, Li Y, Liu Y, Sun Z. Developing and evaluating a predictive model for neonatal hyperbilirubinemia based on UGT1A1 gene polymorphism and clinical risk factors. Front Pediatr. 2024;12:1345602. https://doi.org/10.3389/fped.2024.1345602.
15. Diala UM, Usman F, Appiah D, Hassan L, Ogundele T, Abdullahi F, et al. Global Prevalence of Severe Neonatal Jaundice among Hospital Admissions: A Systematic Review and Meta-Analysis. J Clin Med. 2023;12:3738. https://doi.org/10.3390/jcm12113738.
16. Talwar M, Jain A, Sharma RR, Kumar P, Saha SC, Singh L. The spectrum of ABO haemolytic disease of the fetus and newborn in neonates born to group O mothers. Vox Sang. 2022;117:1112–20. https://doi.org/10.1111/vox.13327.
17. Krog GR, Donneborg ML, Hansen BM, Lorenzen H, Clausen FB, Jensen KV, Kjærbye-Thygesen A, Albertsen P, Ebbesen F, Bergholt T, Smed MK, Dziegiel MH. Prediction of ABO hemolytic disease of

the newborn using pre- and perinatal quantification of maternal anti-A/anti-B IgG titer. Pediatr Res. 2021;90(1):74–81. 10.1038/s41390-020-01232-5. . Epub 2020 Nov 10. PMID: 33173174.

18. Luo T, Li H, Chen A, Ouyang T, Wu M, Yang M, et al. A population-based nomogram for prognostic assessment in advanced lung cancer following progression with immune checkpoint inhibitor. J Thorac Dis. 2025;17:5078–94. https://doi.org/10.21037/jtd-2025-165.
19. Lian D, Lin C, Dong X, Wei J, Huang X, Jiang H, et al. Development and validation of a CD4+/CD8 + ratio-based nomogram to predict plastic bronchitis in pediatric Mycoplasma pneumoniae pneumonia. Front Pediatr. 2025;13:1625206. https://doi.org/10.3389/fped.2025.1625206.
20. Fan X, Zhao J, Luo Y, Li X, Tan W, Liu S. Development of a clinical nomogram for predicting sarcopenia in patients with chronic obstructive pulmonary disease based on NHANES data. Front Med (Lausanne). 2025;12:1612403. https://doi.org/10.3389/fmed.2025.1612403.
21. Subspecialty Group of Neonatology, the Society of Pediatrics, Chinese Medical Association, Board E. Chinese Journal of Pediatrics. [Guidelines on the clinical management of neonatal hyperbilirubinemia (2014)]. MEDLINE ISTIC PKU CSCD, 2014, 52(10):745–748. 10.3760/cma.j.issn.0578-1310.2014.10.006
22. World Health Organization. International Classification of Diseases, 11th Revision (ICD-11). Geneva: WHO. 2019. Available at: https://icd.who.int/browse11
23. Cai C, Zeng W, Wang H, Ren S. Neutrophil-to-Lymphocyte Ratio (NLR), Platelet-to-Lymphocyte Ratio (PLR) and Monocyte-to-Lymphocyte Ratio (MLR) as Biomarkers in Diagnosis Evaluation of Acute Exacerbation of Chronic Obstructive Pulmonary Disease: A Retrospective, Observational Study. International Journal of Chronic Obstructive Pulmonary Disease.
24. Yin MW, Chen XJ, Zeng Z, Xu J, Chen YY, Ma JH. Factors affecting the diagnosis rate of hemolytic disease of the newborn in infants with hyperbilirubinemia due to maternal–fetal ABO blood group incompatibility. Int J Lab Med. 2020;41:663–6.
25. Dong QQ, Ye GY. Blood type and multivariate analysis of neonatal ABO hemolytic disease. J Clin Hematol. 2022;35:410–3. 10.13201/j.issn.1004-2806.2022.06.005.
26. Chen Y, Zhang XD, Ye RT, Wu WX, Liu CY. Distribution characteristics of blood group IgG antibody titers and subtypes in children with ABO-HDN. Clin Transfus Lab Med. 2023;25:753–7.
27. Wu Y, Guo G, Wu Y, Xiu L, Ji Y, Li M, Sun M, Wang X, Ren X, Zhang L, Li J, Wu S, Wen M, Zeng J, Yuan Q, Xie Z, Yang Y. Effects of mother-infant ABO incompatibility on neonates: a cohort study in the Chinese population. Arch Dis Child Fetal Neonatal Ed. 2025 Oct;24:fetalneonatal–2024. 10.1136/archdischild-2024-328079. . Epub ahead of print. PMID: 41093439.
28. Li XM, Xing KH, Zhuang TP, Chen CH. Comparison of clinical characteristics of term neonatal hyperbilirubinemia caused by ABO hemolytic disease and G6PD deficiency. Hainan Med J. 2016;27:1060–2.
29. Parastatidou S, Tsantes AG, Emmanouil C-C, Konstantinidi A, Kapetanaki A, Sokou R. Neonatal Hemolytic Jaundice: Causes, Diagnostic Approach, and Management. Child (Basel). 2025;12:666. https://doi.org/10.3390/children12060666.

30. Wagle S. Hemolytic Disease of the Newborn Workup. Medscape. 2024 Jun 3 [cited 2026 Mar 22]. https://emedicine.medscape.com/article/974349-workup?form=fpf

Figures

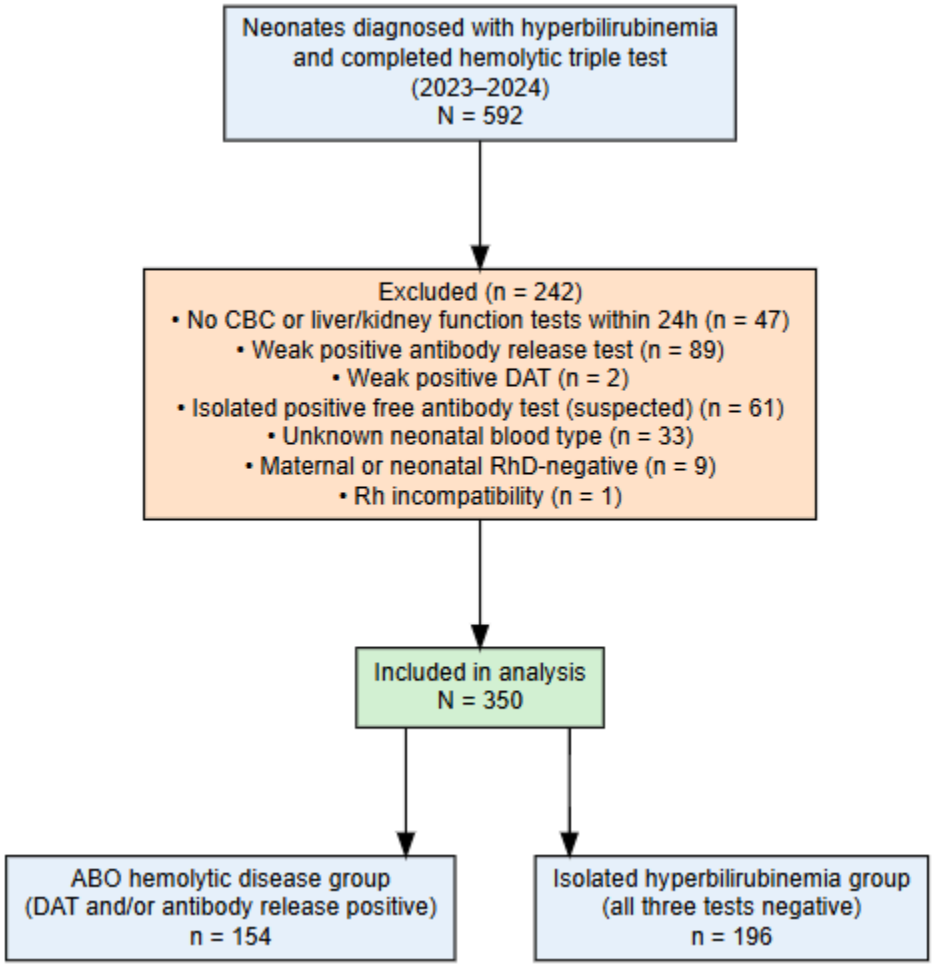


Figure 1

Flowchart of the neonatal cases inclusion the study.

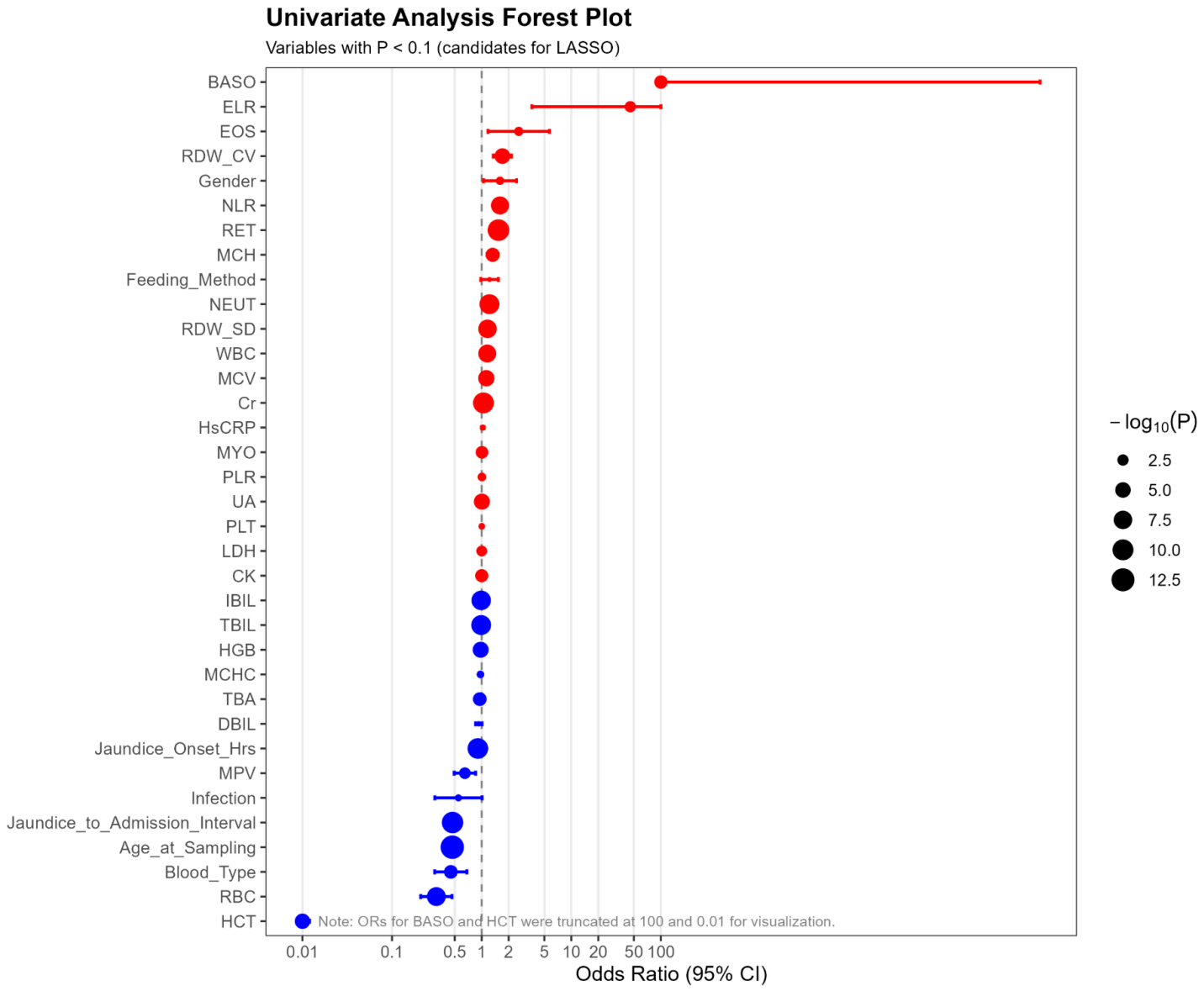


Figure 2

Univariate analysis forest plot The horizontal axis represents the odds ratio (OR), and the vertical axis represents the candidate variables (only variables with P<0.1 are shown). The OR and 95% CI are presented on a log10 scale. Points are colored according to direction (blue: OR<1, protective; red: OR>1, risk factor). Point size is proportional to -log10(P value). The ORs for BASO and HCT were truncated at 100 and 0.01, respectively, for visualization.

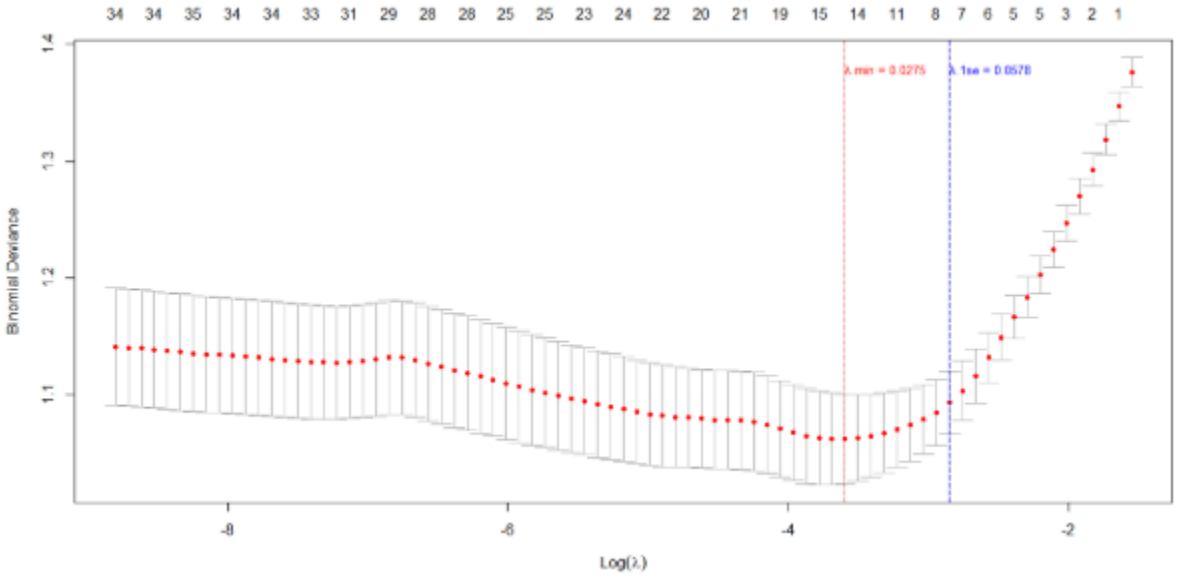


Figure 3

LASSO regression cross-validation bias plot The horizontal axis represents the logarithm of the penalty parameter λ, while the vertical axis shows the binomial deviance. The curve depicts the average deviation from 10-fold cross-validation, with error bars indicating ±1 standard error. The red dashed line marks λ.min (the λ value corresponding to the minimum deviation), and the blue dashed line marks λ.1se (the λ value for the simplest model with deviation within one standard error). In this study, λ.1se was identified as the optimal λ value, selecting seven non-zero coefficient variables. The specific numerical values of λ.min and λ.1se are labeled on the plot.

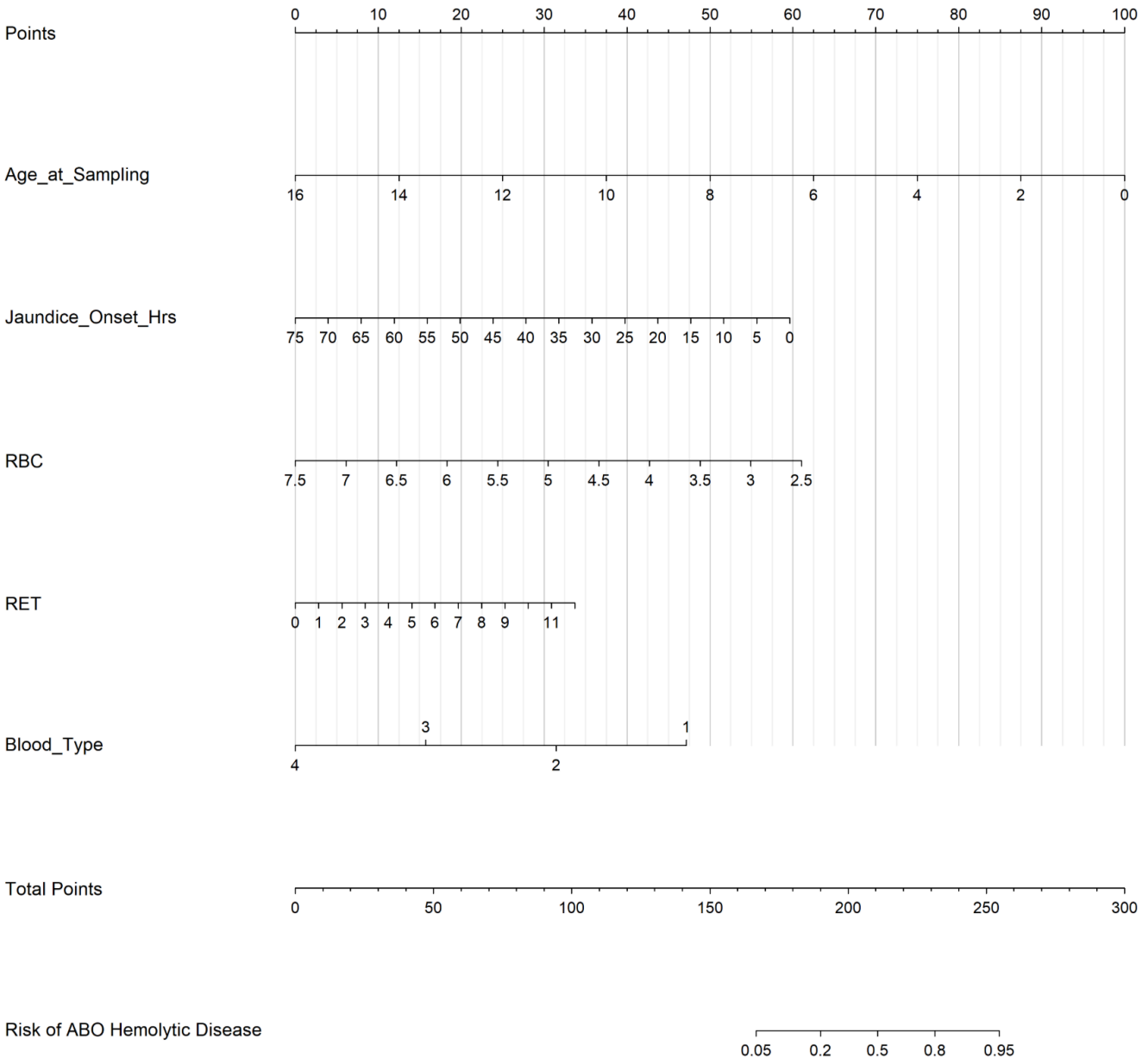


Figure 4

Nomogram to predict ABO-HDN risk in neonates with hyperbilirubinemia The nomogram is used to predict the risk of ABO blood group incompatibility-induced hemolytic disease of the newborn (ABO-HDN) in neonates with hyperbilirubinemia. This nomogram is based on five independent predictive factors identified through multivariate logistic regression analysis: age at sampling, time of jaundice onset, red blood cell count (RBC), reticulocyte percentage (RET), and neonatal blood type. When using this nomogram, the numerical values of each predictive factor must be plotted on the corresponding axis. Vertical lines are drawn to the top "Points" axis to obtain the score for each predictive factor. The scores of all predictive factors are aggregated, and the total score is plotted on the "Total Points" scale. The total score is then vertically projected onto the "risk" axis to determine the ABO-HDN risk level (0.05–0.95).

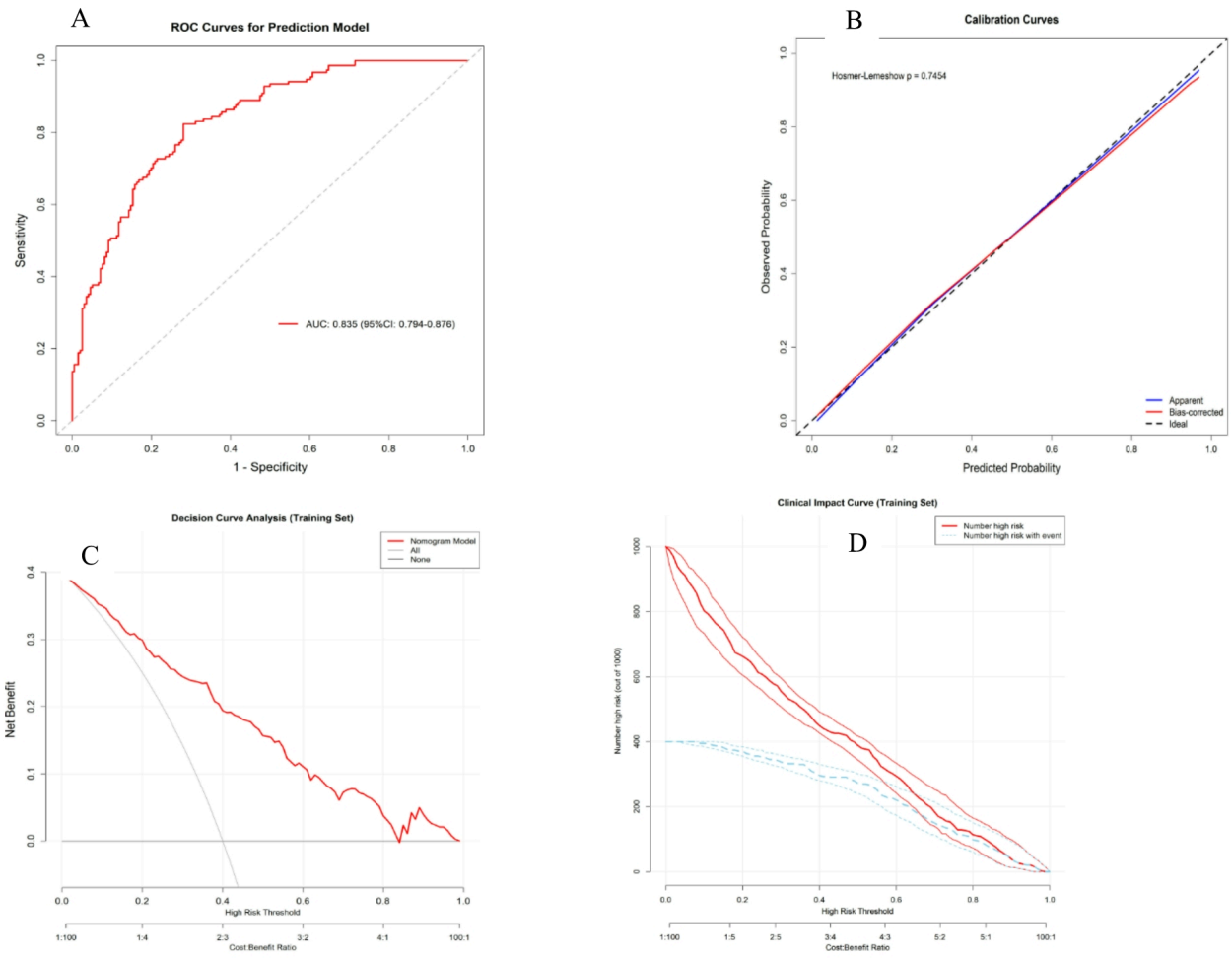


Figure 5

Performance evaluation of the nomogram for predicting the risk of ABO-HDN in neonates with hyperbilirubinemia. (A) Receiver operating characteristic (ROC) curve; area under the curve (AUC) = 0.835 (95% CI: 0.794–0.876). (B) Calibration curve; the Hosmer-Lemeshow test yielded a p-value of 0.7454 (P> 0.05). (C) Decision curve analysis (DCA); the nomogram shows higher net benefit than “treat all” and “treat none” strategies across risk thresholds of 0.1 to 0.83. (D) Clinical impact curve (CIC); the curve quantifies the intervention burden and diagnostic benefit at different decision breakpoints for clinical decision-making.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableS1andS2.docx