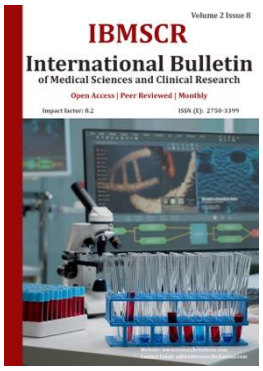

EARLY PREDICTION OF HEMOLYTIC DISEASE OF THE NEWBORN BASED ON CLINICAL INDICATORS

Xakimova Diyora Rahimjon qizi
Master's degree student in pediatrics and neonatology at Andijan state medical institute
Atadjanova Shoira Xalilovna
Associate professor, PhD, Department of pediatrics and neonatology, Andijan state medical institute.
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Abstract. Hemolytic disease of the newborn (HDN) is an immune-mediated condition caused by maternal alloantibodies against fetal red blood cell antigens, most commonly involving the Rh and ABO blood group systems. Early identification of neonates at risk is crucial to prevent severe anemia, hyperbilirubinemia, kernicterus, and perinatal mortality. This article reviews and synthesizes current evidence on early clinical and laboratory indicators predictive of HDN, including maternal blood group status, direct antiglobulin test (DAT) positivity, reticulocyte percentage (RET%), lactate dehydrogenase (LDH), γ-glutamyltransferase (γ-GT), hemoglobin levels, and early-onset jaundice. Evidence suggests that combined laboratory predictors improve diagnostic accuracy compared to single markers. Early screening strategies integrating maternal risk assessment and neonatal biochemical indicators can significantly enhance timely diagnosis and intervention.

Keywords: Hemolytic disease of the newborn, ABO incompatibility, Rh incompatibility, early prediction, direct antiglobulin test, reticulocyte count, neonatal hyperbilirubinemia, LDH, γ-GT

Introduction. Hemolytic disease of the newborn (HDN), also known as erythroblastosis fetalis, is caused by transplacental passage of maternal IgG antibodies that target fetal red blood cell (RBC) antigens, leading to immune-mediated hemolysis. Historically, Rh incompatibility was the leading cause of severe HDN; however, with the widespread use of anti-D immunoglobulin prophylaxis, ABO incompatibility and irregular antibodies have become increasingly significant contributors (Ajmani, 2020; Mutiawati, 2018).

Although ABO incompatibility occurs in approximately 14-20% of pregnancies, only a subset develop clinically significant HDN (Rao et al., 2012; Mutiawati, 2018). Early detection is essential because severe hemolysis may lead to anemia, hyperbilirubinemia, hydrops fetalis, or kernicterus.

Hemolytic disease of the newborn (HDN) represents a significant immunohematological condition in perinatal medicine, resulting from maternal alloimmunization against fetal erythrocyte antigens inherited from the father. The pathogenesis involves transplacental transfer of maternal IgG antibodies, which bind to fetal red blood cells and trigger hemolysis via the fetal reticuloendothelial system. The most clinically important antigen systems implicated in HDN are Rh (particularly anti-D) and ABO, although other irregular antibodies such as Kell, Duffy, Kidd, and MNS may also cause severe disease (Ajmani, 2020; Wu et al., 2024). While the incidence of Rh-mediated HDN has markedly decreased due to anti-D immunoglobulin prophylaxis, cases associated with ABO incompatibility and irregular antibodies remain clinically relevant.

ABO incompatibility occurs in approximately 14-20% of pregnancies, particularly when a mother with blood group O carries a fetus with blood group A or B (Rao et al., 2012; Mutiawati, 2018). However, only a proportion of these cases progress to clinically significant hemolysis. The variability in disease expression is influenced by factors such as antibody subclass (IgG1 and IgG3 being more hemolytic), antigen density on fetal erythrocytes, and maternal antibody titers. Clinical manifestations range from mild neonatal jaundice to severe anemia and, in extreme cases, hydrops fetalis or kernicterus. Therefore, identifying high-risk neonates before severe hyperbilirubinemia develops is essential to prevent long-term neurological complications.

Recent advances emphasize the importance of combining maternal risk assessment with early neonatal laboratory parameters to improve predictive accuracy. Traditional diagnostic tools such as blood grouping and the direct antiglobulin test (DAT) remain foundational; however, their sensitivity is limited when used alone (Rao et al., 2012). Emerging evidence highlights the diagnostic value of hemolysis-related biomarkers – including reticulocyte percentage (RET%), lactate dehydrogenase (LDH), and γ-glutamyltransferase (γ-GT) – which reflect active erythrocyte destruction and compensatory erythropoiesis (Liu et al., 2023). Integrating these indicators into early screening frameworks may allow clinicians to stratify risk more effectively and initiate timely therapeutic interventions.

This article aims to examine early clinical and laboratory indicators that enable prediction of HDN in neonates and to propose a structured predictive approach based on available evidence.

Methods. This article is a structured narrative review based on recent clinical and laboratory studies evaluating predictive markers of HDN. Key studies were identified from peer-reviewed sources focusing on:

- Maternal and neonatal blood group incompatibility
- Direct antiglobulin test (DAT) positivity
- Reticulocyte percentage (RET%)
- Lactate dehydrogenase (LDH)
- γ-glutamyltransferase (γ-GT)
- Early-onset hyperbilirubinemia

Findings were synthesized to identify consistent early predictors and assess their diagnostic value.

To enhance methodological rigor, studies were further evaluated for sample size, study duration, and clarity of diagnostic criteria for HDN. Particular attention was given to investigations that clearly defined hemolysis using objective laboratory thresholds such as declining hemoglobin levels, rising indirect bilirubin (>0.5–1 mg/dL/hour), reticulocytosis (>7%), and positive DAT results. Studies comparing hemolytic and non-hemolytic jaundice groups were prioritized, as they allowed better discrimination of predictive indicators.

Risk stratification parameters were categorized into **prenatal** and **postnatal** predictors. Prenatal predictors included maternal blood group O or Rh-negative status, detection of irregular antibodies during antenatal screening, and documented history of alloimmunization. Postnatal predictors included cord blood DAT testing, complete blood count, reticulocyte count, serum LDH, γ-GT levels, and serial bilirubin measurements within the first 24 – 72 hours of life.

This classification allowed structured comparison of early risk identification before and after birth.

For laboratory-based predictors, emphasis was placed on multivariate statistical approaches reported in the selected studies. Logistic regression analyses were examined to determine independent risk factors for hemolysis, while receiver operating characteristic (ROC) curve analyses were reviewed to assess diagnostic performance. In particular, the combined biomarker model incorporating RET%, LDH, and γ-GT was evaluated for its area under the curve (AUC) performance compared to single-marker testing (Liu et al., 2023). Such analytical methods provide stronger evidence for predictive accuracy and clinical applicability.

Finally, outcome-based validation was considered a critical methodological component. Indicators were assessed according to their association with clinically meaningful endpoints, including requirement for phototherapy, blood transfusion, exchange transfusion, length of hospital stay, and severity of anemia. Studies that demonstrated statistically significant associations ($p < 0.05$) between early indicators and adverse neonatal outcomes were considered stronger evidence sources (Wu et al., 2024; Rao et al., 2012). This outcome-oriented synthesis ensures that predictive markers discussed in this review are not only statistically significant but also clinically relevant.

Results 1. Maternal – Fetal Blood Group Incompatibility

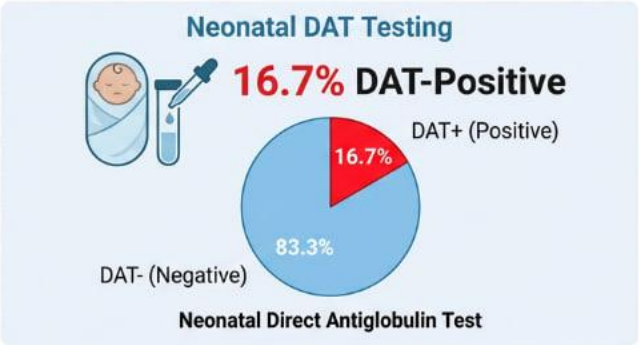
ABO HDN occurs predominantly in infants with blood group A or B born to group O mothers. In a cohort of 10,891 births, 14% were ABO incompatible, and DAT positivity was observed in 16.7% of cases. DAT-positive infants had significantly higher rates of hyperbilirubinemia and hemolytic anemia.

Rh incompatibility remains clinically severe when present, especially in anti-D mediated cases. Across the reviewed studies, neonates diagnosed with HDN demonstrated significantly altered hematological profiles compared to non-hemolytic controls. Decreased hemoglobin levels and elevated reticulocyte percentages were consistently observed, reflecting active hemolysis and compensatory erythropoiesis. In ABO-incompatible neonates, reticulocytosis greater than 7% was frequently associated with immune-mediated hemolysis (Mutiawati, 2018). Additionally, Liu et al. (2023) identified RET% as an independent risk factor for ABO-HDN, confirming its role as an early laboratory marker of increased red blood cell turnover.

Serum lactate dehydrogenase (LDH), a marker of cellular breakdown, was significantly elevated in hemolytic cases and positively correlated with RET% (Liu et al., 2023). This correlation supports the biological link between erythrocyte destruction and bone marrow compensation. Furthermore, γ-glutamyltransferase (γ-GT) levels were higher in neonates with hemolytic disease, suggesting hepatic stress secondary to bilirubin overload. The combined elevation of RET%, LDH, and γ-GT demonstrated superior diagnostic performance compared to any single parameter. ROC analysis showed that the triple-marker combination yielded the highest predictive value for early ABO-HDN (Liu et al., 2023).

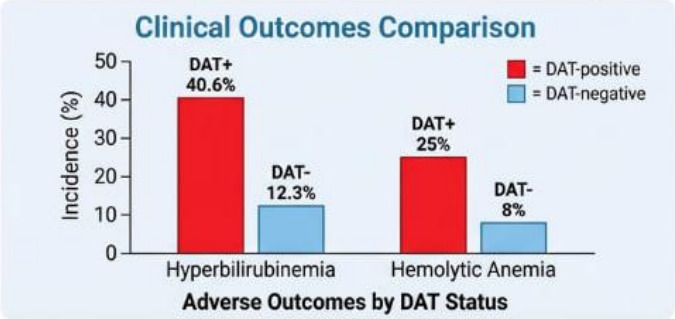
Direct Antiglobulin Test (DAT) and Clinical Correlation

DAT positivity was significantly associated with adverse clinical outcomes. In ABO-incompatible pregnancies, 16.7% of neonates were DAT-positive, and these infants showed higher rates of hyperbilirubinemia and hemolytic anemia (see Picture 1).



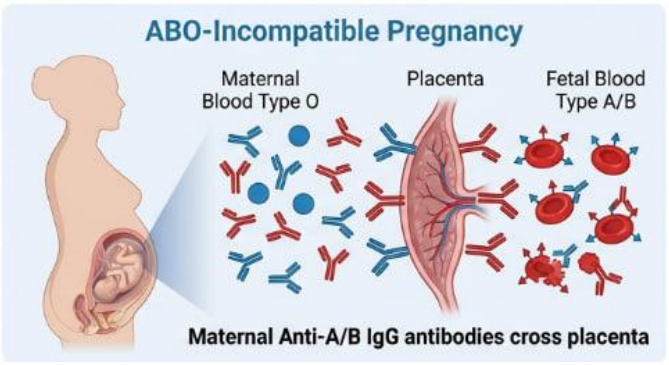
Picture 1. Neonatal DAT Testing

Specifically, hyperbilirubinemia occurred in 40.6% of DAT-positive infants compared to 12.3% of DAT-negative infants (see Picture 2).



Picture 2. Clinical outcomes comparison

However, despite its strong association, DAT demonstrated limited sensitivity (41.3% for hyperbilirubinemia and 70.5% for hemolytic anemia), indicating that DAT-negative neonates may still develop clinically significant HDN. (see Picture 3).



Picture 3. ABO – incompatiable pregnancy

In cases of irregular antibody-mediated HDN, DAT positivity was more frequent and correlated with earlier onset jaundice, more severe anemia, hepatosplenomegaly, and greater treatment requirements, including enhanced phototherapy and exchange transfusion (Wu et al., 2024). These findings suggest that DAT may better reflect disease severity rather than serve as a standalone screening tool (see Table 1).

Table 1

Evidence Synthesis

No.	Source	Key Insight
1	Clinical Value of Combined Predictors of RET%, γ-GT, LDH (Liu et al., 2023)	Combined RET% + LDH + γ-GT provides superior early diagnostic accuracy for ABO-HDN compared to single markers.

2	The Clinical Spectrum of ABO Incompatibility (Rao et al., 2012)	DAT positivity significantly associated with hyperbilirubinemia and anemia; limited sensitivity as standalone predictor.
3	Hemolytic Disease of the Newborn (Ajmani, 2020)	Rh-mediated HDN remains severe; laboratory confirmation requires blood grouping and antiglobulin testing.
4	Hemolytic Disease of the Newborn (Mutiawati, 2018)	ABO incompatibility common but usually mild; early jaundice and anemia are key clinical clues.
5	Clinical Characteristics of HDN Caused by Irregular Antibodies (Wu et al., 2024)	Irregular antibodies cause earlier and more severe manifestations with higher DAT positivity and treatment intensity.

Discussion

Single indicators such as DAT are insufficient alone due to limited sensitivity. However, combining markers reflecting immune activation and hemolysis significantly enhances diagnostic precision.

The findings of this review emphasize that early prediction of hemolytic disease of the newborn (HDN) requires a multidimensional diagnostic strategy rather than reliance on a single laboratory parameter. Although maternal–fetal blood group incompatibility remains the primary etiological factor, the clinical expression of HDN varies significantly. ABO incompatibility is common but often mild, whereas Rh and irregular antibody – mediated cases tend to present with earlier and more severe anemia and hyperbilirubinemia (Ajmani, 2020; Wu et al., 2024). Therefore, risk stratification must integrate immunohematological, biochemical, and clinical indicators to identify neonates at highest risk. (see Table 2).

Table 2

Integrated early prediction framework for hemolytic disease of the newborn

No.	Domain	Key Components	Clinical Significance	Purpose in Early Prediction
1	**Maternal Risk Assessment**	O blood group; Rh-negative status; Presence of irregular antibodies	Identifies pregnancies at risk of alloimmunization and fetal RBC incompatibility	Enables prenatal risk stratification and closer monitoring during pregnancy
2	**Cord Blood Testing**	ABO/Rh typing; Direct antiglobulin test (DAT)	Detects maternal antibodies bound to neonatal erythrocytes	Confirms immune-mediated hemolysis immediately after birth
3	**Early Biochemical Markers**	Reticulocyte percentage (RET%); Lactate dehydrogenase (LDH); γ-glutamyltransferase (γ-GT)	Reflects hemolysis (LDH), compensatory erythropoiesis (RET%), and	Improves diagnostic accuracy when combined with immunohematological markers

			hepatic stress (γ-GT)	
4	**Clinical Monitoring**	Jaundice within 24 hours; Anemia; Hepatosplenomegaly	Indicates early and clinically significant hemolysis	Guides timely therapeutic intervention (phototherapy, transfusion, exchange transfusion)

One important observation is the limited sensitivity of the direct antiglobulin test (DAT) when used as a standalone screening tool. While DAT positivity strongly correlates with hyperbilirubinemia and hemolytic anemia, a substantial proportion of affected neonates may be DAT-negative. This limitation underscores the need for adjunct biomarkers. The addition of reticulocyte percentage (RET%), lactate dehydrogenase (LDH), and γ-glutamyltransferase (γ-GT) provides a more comprehensive reflection of the hemolytic process. Elevated RET% indicates active bone marrow compensation, increased LDH reflects erythrocyte destruction, and γ-GT elevation suggests hepatic stress secondary to bilirubin metabolism. Together, these markers capture different stages of the pathophysiological cascade of HDN.

Another key discussion point concerns the timing of clinical manifestations. Early-onset jaundice within the first 24 hours of life is a critical warning sign of immune hemolysis. The rapid rise in indirect bilirubin levels is not merely a laboratory abnormality but a predictor of neurological risk, particularly kernicterus. Studies demonstrate that irregular antibody – mediated HDN presents with earlier jaundice and more aggressive disease progression compared to ABO incompatibility. Therefore, close monitoring during the first 48 – 72 hours of life is essential, especially in neonates with known maternal alloimmunization.

From a clinical management perspective, early prediction directly influences therapeutic decision-making. Prompt identification of high-risk neonates allows earlier initiation of phototherapy, prevention of bilirubin neurotoxicity, and timely preparation for blood transfusion or exchange transfusion if needed. The combined biomarker model (RET% + LDH + γ-GT) proposed by Liu et al. (2023) may serve as a foundation for developing standardized risk-scoring systems. However, further prospective validation studies are required to determine optimal cutoff values and establish universal screening algorithms.

Conclusion

Early prediction of hemolytic disease of the newborn (HDN) is fundamental to reducing neonatal morbidity and preventing life-threatening complications such as severe anemia, hydrops fetalis, and kernicterus. The evidence reviewed in this article demonstrates that while traditional tools such as maternal blood group screening and the direct antiglobulin test (DAT) remain essential, they are insufficient when used in isolation. A comprehensive predictive strategy that integrates maternal immunohematological risk factors with early neonatal laboratory biomarkers significantly enhances diagnostic accuracy and clinical preparedness.

The incorporation of hemolysis-related biochemical markers – particularly reticulocyte percentage (RET%), lactate dehydrogenase (LDH), and γ-glutamyltransferase (γ-GT) – represents a promising advancement in early detection models. These markers reflect different physiological dimensions of HDN, including erythrocyte destruction, compensatory bone marrow activity, and hepatic involvement in bilirubin metabolism. Studies indicate that

combined biomarker models outperform single-parameter testing, supporting the development of structured risk-scoring systems for clinical practice.

Moving forward, implementation of standardized screening protocols that combine prenatal antibody assessment, cord blood testing, and early postnatal biochemical evaluation could significantly improve neonatal outcomes. Future prospective and multicenter research is needed to validate predictive thresholds and optimize clinical algorithms. Ultimately, an integrated, evidence-based approach to early HDN prediction aligns with precision neonatal care and offers the potential to minimize preventable neurological and hematological complications in affected newborns.

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