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Research Article

Maternal-fetal isoimmunization – a risk for the development of hemolytic disease of the newborn

Maria Hristova Savcheva[1], Angelina Georgieva Kirkova-Bogdanova[2], Maria Stamova Vakrilova Becheva[3], Yozlem Ali Kobakova[4], Maria Georgieva Moneva-Sakelarieva[4], Stefka Achkova Ivanova[5], Maria Sevdelinova Chaneva[4], Ventseslava Petrova Atanasova[6], Yonko Tsvetanov Yonchev[4], Radoslav Fedeev Todorov[4], Nikolay Zarkov Bashev[4], Ismail Elhanov Bashov[4], Diana Nikolaeva Teneva[4], Silviya Milchova Pavlova[4], Konstantin Ivanov Kostov[7], Kiril Todorov Atliev[8], Petar Yordanov Atanasov[4], Adel Habib Ibrahim[9], Pavel Bogdanov Pavlov[10]

1 *Transfusion Hematology Laboratory, Saint Sofia Medical Center, Sofia, Bulgaria*
2 *Department "Medical Informatics, Biostatistics and E-learning", Faculty of Public Health, Medical University – Plovdiv, Plovdiv, Bulgaria*
3 *Medical College, Medical University – Plovdiv, Plovdiv, Bulgaria*
4 *Clinic of Internal Diseases, UMHATEM "N. I. Pirogov", Sofia, Bulgaria*
5 *Bulgarian Pharmaceutical Science Society, Sofia, Bulgaria*
6 *Bulgarian Pharmaceutical Union, Sofia, Bulgaria*
7 *Department of General, Visceral and Emergency Surgery, UMHATEM "N. I. Pirogov", Sofia, Bulgaria*
8 *Department of Epidemiology and Disaster Medicine, Medical University – Plovdiv, "Saint Georgi" University Hospital, Plovdiv, Bulgaria*
9 *Pharmacy of Hospital UMHATEM "N. I. Pirogov", Sofia, Bulgarias*
10 *UMHATEM "N. I. Pirogov", Sofia, Bulgaria*

Corresponding author: Stefka Achkova Ivanova (ivanovastefka_pharm@yahoo.com)

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Abstract

Hemolytic disease of the newborn is a condition caused by maternal alloimmunization against fetal red blood cell antigens. Transplacental hemorrhage, for example, can provoke an immune response in the mother. As a result, IgG-class antibodies directed against fetal erythrocyte antigens are synthesized. The hemolysis that develops in the perinatal period in the fetus is a consequence of the transplacental migration of these anti-erythrocyte antibodies into the fetal circulation. Depending on the perinatal stage in which it occurs, hemolytic disease of the newborn can lead to hemolytic anemia in the infant or hydrops fetalis with subsequent fetal death. Screening for anti-erythrocyte antibodies during pregnancy, as well as the direct Coombs antiglobulin test, are important methods for monitoring alloimmunization and ensuring timely diagnosis of hemolytic disease in the fetus and newborn.

This study reflects the prevalence and trends in alloimmunization among pregnant and postpartum women over a three-year period at MHAT "St. Sofia." The aim was to determine the frequency of alloimmunization and summarize results based on specificity and clinical factors.

A retrospective analysis was performed over three years, tracking blood grouping, antibody screening, and antibody identification in pregnant women, mothers, and newborns. Standard diagnostic methods were used, including the enzyme test at 37 °C, the direct and indirect Coombs antiglobulin test, and agglutination testing. The tests were performed both prenatally and postnatally.

AB0 blood group incompatibility is the most common and usually occurs in pregnant women with blood group 0, whose fetus has blood group A, B, or AB. This is due to the fact that antibodies produced by individuals with blood group 0 are typically IgG, while those produced by individuals with group A or B are more often IgM.

Targeted prenatal care and prophylaxis – pre- and postnatal with anti-D immunoglobulin – have significantly reduced the risk of D-alloimmunization. However, anti-D remains one of the most frequently detected antibodies in pregnant women. To maintain proper use of anti-D prophylaxis, continuous education and awareness are needed among healthcare professionals caring for pregnant women and mothers.

Keywords

AB0, antibodies, anti-D, alloimmunization, blood group, hemolytic disease of the newborn, incompatibility, pregnancy

Introduction

Hemolytic disease of the newborn represents an immune reaction caused by blood group incompatibility between mother and fetus. When the fetus inherits paternal blood group factors absent in the mother, prenatal or intranatal feto-maternal hemorrhage can provoke an immune response (Myle and Al-Khattabi 2021). This immune reaction results in maternal antibody production – a process known as alloimmunization – whereby antibodies cross the placenta to varying degrees and enter the fetal circulation. Depending on the antigenicity and quantity of antibodies transferred, hemolytic disease of the newborn may develop, manifesting as complications such as anemia, jaundice, or, in severe cases, fetal hydrops, hyperbilirubinemia, and kernicterus (Committee on Practice Bulletins – Obstetric 2017; ACOG Practic 2018). It can also lead to late complications such as protracted aplastic anemia.

When antibody production shifts from an initial IgM to a subsequent IgG response, maternal IgG antibodies cross the placenta. During the second and third trimesters, IgG antibody production increases, accelerating transfer and potentially causing hemolysis of fetal erythrocytes.

The most common type of hemolytic disease of the newborn is AB0 incompatibility, followed by Rh incompatibility. Although AB0 incompatibility is common, it results in neonatal hemolytic disease less frequently, likely due to a moderate immune response to AB0 antigens. Rh incompatibility typically affects subsequent pregnancies, since maternal sensitization occurs during the first pregnancy, leading to more severe complications later, and sometimes intrauterine fetal hydrops. However, AB0 incompatibility may affect the first pregnancy since maternal antibodies often preexist.

Newborns present with lethargy, jaundice, and hepatosplenomegaly. Diagnosis can be made before or after birth through laboratory and imaging studies (Hall et al. 2025). Invasive obstetric procedures such as amniocentesis and chorionic villus sampling increase the risk of maternal sensitization, underscoring the importance of prevention (Kemper et al. 2022; de Winter et al. 2023).

Forty years ago, hemolytic disease of the newborn was commonly associated with RhD incompatibility. The introduction of postnatal and antenatal immunoprophylaxis has reduced RhD alloimmunization to 0.1% (Chavez et al. 1991).

With advanced diagnostic technologies, cases of hemolytic disease caused by anti-erythrocyte alloantibodies are increasingly well recognized in clinical practice. AB0-related hemolytic disease occurs when the mother is blood group 0 and the fetus carries A, B, or AB antigens (Loua et al. 2007). Blood group testing is a standardized mandatory screening during the first trimester. Women with group 0 produce antibodies against A and B antigens, and their newborns should be monitored for hemolytic disease at and after birth. Their antibodies are usually IgG and cross the placenta. By contrast, group A mothers produce anti-B antibodies, typically IgM, which do not cross the placenta (Murray and Roberts 2007).

Affected newborns may develop hyperbilirubinemia from hemolysis. In some cases, exchange transfusions may be needed to control bilirubin and correct anemia. Maternal IgG antibodies (anti-A and anti-B) cross the placenta, bind paternal A or B antigens on neonatal red blood cells, and trigger erythrocyte destruction. (Kumpel et al. 1996; de Haas et al. 2015) Unconjugated bilirubin is neurotoxic and may cause acute bilirubin encephalopathy. Early diagnosis and timely phototherapy effectively control bilirubin levels, often preventing the need for immunotherapy (IVIg) (de Haas et al. 2015).

AB0 incompatibility occurs in about 20% of pregnancies, but AB0-related hemolytic disease is more frequent in newborns of African or Asian descent (3–5%) compared to Europeans (<1%) (de Haas et al. 2015). The reason for this difference is unclear.

Before the introduction of anti-D prophylaxis and intrauterine transfusion, perinatal mortality from Rh hemolytic disease was about 50% (Stockman 2001; Bowman 2003). The underlying mechanism involves fetal blood carrying paternal Rh-positive antigens, which the Rh-negative maternal immune system recognizes as foreign. Initial antibodies are IgM and cannot cross the placenta. In subsequent pregnancies, however, sensitized mothers produce IgG antibodies, which cross and destroy fetal erythrocytes (ACOG Ptactic 2018; Moinuddin et al. 2019). Severe fetal anemia (<7 g/dL hemoglobin) can

cause hydrops, with diffuse edema, pleural and pericardial effusions, and ascites (Nicolaides et al. 1988).

The milder presentation of AB0-related disease is likely due to lower AB0 antigen expression on fetal cells or widespread antigen expression in tissues, reducing specific targeting of fetal erythrocytes (Metcalf et al. 2019).

Anti-D immunoglobulin prevents sensitization in Rh-negative women. Prophylaxis reduces Rh hemolytic disease by 80–90%, along with a two-thirds drop in mortality. Administering anti-D during antigen exposure (e.g., after delivering an Rh-positive baby) blocks the maternal primary immune response, preventing antibody formation in later pregnancies (Wysowski et al. 1979). Rh alloimmunization remains a problem in regions with limited access to prophylaxis (Pegoraro et al. 2020).

The prognosis for hemolytic disease is good with early detection and treatment. Untreated cases may lead to permanent neurological dysfunction, though rare today thanks to improved monitoring and prevention (Zwiers et al. 2017). Prognosis in subsequent pregnancies depends on successful prevention of fetal hydrops (Dumitru et al. 2021).

Aim

Hemolytic disease of the fetus and newborn can have severe prenatal and postnatal consequences. The main aim of this study is to analyze the clinical significance of hemolytic disease of the newborn in order to create algorithms for management and prevention, as well as to determine the prognostic value of the direct antiglobulin (Coombs) test in diagnosing hemolytic disease of the newborn.

Materials and methods

The main goal of immunohematological testing in prenatal patients is to detect antibodies directed against their own erythrocytes. If antibodies are present that could hemolyze fetal red blood cells, their reactivity must be assessed. Determining and analyzing the fetal antigen status has direct clinical importance for pregnancy outcomes. The key method for detecting bound antibodies is the direct Coombs antiglobulin test.

Over a three-year period (2022, 2023, 2024), blood group typing, screening, and identification of allo- and auto-antierythrocyte antibodies were performed in pregnant women, mothers, and newborns.

A total of 1,796 pregnant and postpartum women were included in the study. Of these, 1,546 (86%) were RhD-positive and 250 (24%) were RhD-negative. The number of newborns tested for blood group and with a direct Coombs test was 352. Of these, 15 newborns (4.3%) had a positive direct Coombs test.

All RhD-negative women underwent prenatal screening for anti-erythrocyte alloantibodies. Prenatal screening for anti-A and anti-B antibodies was not performed; these were assessed postnatally.

Routine and specific immunohematology methods were applied, including agglutination testing, direct and indirect Coombs tests, and enzyme tests at 4 °C, 18 °C, and 37 °C.

Results

Between 01.01.2022 and 31.12.2024, blood group testing was performed on 1,796 samples from pregnant and postpartum women, using an agglutination method at 37 °C.

Table 1. Distribution of blood groups by RhD antigen.

RhD	Number of patients
RhD(+)	1546
RhD(-)	250

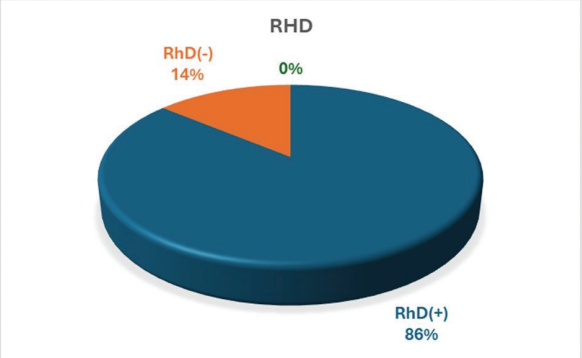

Figure 1. RhD status in pregnant and postpartum women.

The total number of patients screened for alloantibodies was 250 (24%). Tests included the enzyme test at 37 °C, indirect and direct Coombs antiglobulin tests. Alloantibodies were found in 24 women (9.6%).

Table 2. Distribution of antibodies by antigen specificity.

Antibody	N
Anti-A	7
Anti-B	3
Anti-D	6
Anti-M	3
Anti-N	2
Nonspecific	3

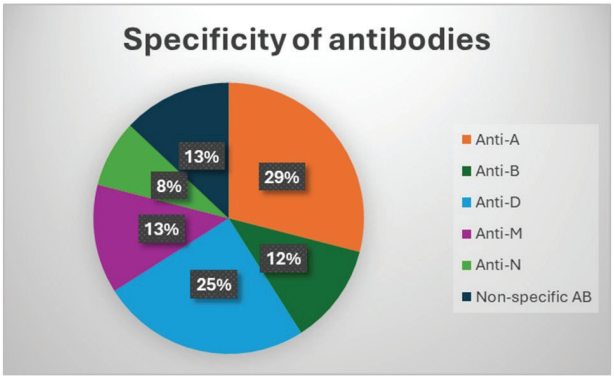

Figure 2. Specificity of antibodies according to blood group systems.

The largest proportion of antibodies came from the AB0 system (anti-A and anti-B) – 10 (41%), which is characteristic of the Western European population (Roberts 2008). Next were Rh system antibodies (anti-D, 25%), followed by the MNS system (21%) and non-specific antibodies (13%).

The total number of newborns during the study period (2022–2024) was 2,470. Of these, 352 (14.3%) were tested for blood group and direct Coombs test. Fifteen newborns (4.3%) had a positive Coombs test. All 15 infants showed mild to moderate jaundice.

Among the mothers, alloantibodies were detected in 5 (33% of positive cases; 1.4% of all studied) with anti-D specificity. In 10 mothers (67% of positive cases; 2.8% of all studied), immune anti-A and anti-B antibodies were found. These 10 mothers had group 0, while their newborns had group A or B. Overall, the frequency of AB0-related hemolytic disease of the newborn during the study period was <1%, consistent with previously reported studies (de Haas et al. 2015).

The highest frequency of positive Coombs tests occurred in 0/A and 0/B incompatibilities, supporting previous findings that infants of group 0 mothers are more likely to test Coombs-positive (Jackson et al. 2020; Talwar et al. 2022).

Table 3. Antibodies identified in mothers of newborns with positive Coombs test.

Antibody	N
Anti-A	7
Anti-B	3
Anti-D	5

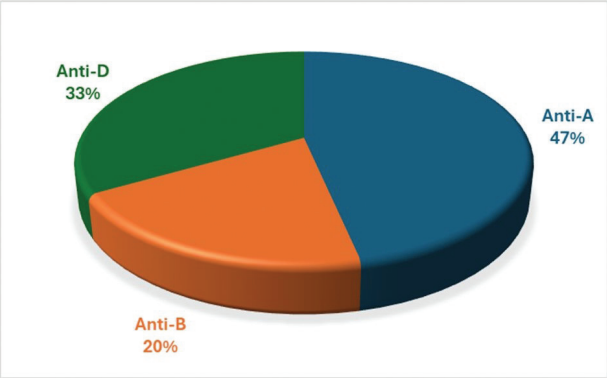

Figure 3. Antibodies identified in mothers of newborns with a positive direct Coombs antiglobulin test.

Clinical course:

- 12 newborns (80%) had mild jaundice and required no specific therapy.
- 3 newborns (20%) had moderate disease and received fractionated phototherapy with good effect. In all 3, immunization was due to AB0 incompatibility with anti-A or anti-B antibodies.

Discussion

Screening tests for pregnant women and newborns in the context of hemolytic disease of the newborn aim to focus the attention of obstetricians and gynecologists on the most serious problems of alloimmunization. This represents the optimal prevention of potential consequences resulting from fetal red blood cell hemolysis.

Conclusions

Screening in the first trimester of pregnancy for AB0 and RhD blood group is a key factor in assessing the potential risk of hemolytic disease of the newborn. Laboratory monitoring enables timely diagnostic and therapeutic interventions, ensuring a good prognosis for the fetus during pregnancy, a favorable pregnancy outcome, and a normal postpartum course for the newborn.

A positive direct antiglobulin (Coombs) test in a newborn of a mother with group 0 and an infant with group A or B represents a risk factor for mother–fetus AB0 incompatibility and the development of hemolytic disease of the newborn. The direct Coombs test in newborns shows high sensitivity in diagnosing hemolytic disease. Therefore, performing the test in cases of 0/A and 0/B incompatibility can be applied as a routine method in transfusion hematology laboratories.

Timely identification and follow-up of the type and titer of antibodies can reduce harmful effects on the newborn.

Immunohematology testing plays a vital role in prenatal detection, monitoring, and in providing compatible blood and blood components for transfusion in such cases.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statements

The authors declared that no clinical trials were used in the present study.

The authors declared that no experiments on humans or human tissues were performed for the present study.

Informed consent from the humans, donors or donors' representatives: UMHATEM "N. I. Pirogov" – Sofia, Bulgaria

The authors declared that no experiments on animals were performed for the present study.

The authors declared that no commercially available immortalised human and animal cell lines were used in the present study.

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Author contributions

All authors have contributed equally.

Author ORCIDs

Nikolay Zarkov Bashev https://orcid.org/0009-0007-8029-9856
Konstantin Ivanov Kostov https://orcid.org/0000-0001-8752-8323
Petar Yordanov Atanasov https://orcid.org/0009-0006-8337-2089

Data availability

All of the data that support the findings of this study are available in the main text.

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