Annals of Biomedical and Clinical Research

Original article

ABCR Vol 5; No 1; 2026;11-17

Analysis of Molecular Blood Group Determination in Patients at the University Clinical Hospital Mostar over a 2.5-year Period

Mirela Sušac[1], Ivana Primorac[2], Jadranka Knežević[1]

[1]*Department of Transfusion Medicine, University Clinical Hospital Mostar,* [2]*Faculty of Economics and Business, University of Rijeka.*

ABSTRACT

Background: The study aimed to analyze the frequency of ABO and RhD genotypes, including RHD variant alleles, and to evaluate the clinical value of molecular genotyping in comparison with standard serological findings.

Methods: A total of 162 patients were included, all of whom underwent comprehensive serological and molecular typing of the ABO and RhD systems using PCR-based methods to identify weak, partial, and DEL RhD variants as well as other rare alleles, with correlation to transfusion history and clinical indications.

Main findings: The results demonstrated a predominance of blood group A and O, while blood groups B and AB were less frequent, with the majority of patients being RhD positive. A significant number of weak and partial RhD variants were identified, particularly among transfusion recipients and patients with a positive Coombs test. Molecular genotyping enabled clarification of serological discrepancies and precise determination of the Rh phenotype, ensuring a more rational use of RhD-negative blood units.

Principal conclusion: These findings confirm the importance of molecular analysis of the ABO and RhD systems in optimizing transfusion practice, reducing the risk of alloimmunization, and supporting an individualized approach to patient care.

Key words: ABO blood group; RhD variants; RHD genotyping; molecular typing; transfusion medicine; alloimmunization; weak D; partial D; DEL variant

Article processing history:
Received April 12, 2026
Revised May 10, 2026
Accepted June 12, 2026

ORCID IDs of the authors:
M.S. 0009-0000-1505-1554
I.P. 0000-0003-4420-6302
J.K. 0009-0006-3811-9463

Corresponding author:
Mirela Sušac
E-mail: mirela.susac@fzs.sum.ba

Cite this article as: Sušac M, Primorac I, Knežević J. Analysis of Molecular Blood Group Determination in Patients at the University Clinical Hospital Mostar over a 2.5-Year Period. Annals of Biomedical and Clinical Research. 2026;5:11-17.

https://doi.org/

INTRODUCTION

The International Society of Blood Transfusion (ISBT) recognizes over 43 blood group systems with more than 360 distinct antigens, highlighting the complex immunogenetic basis of erythrocyte antigens (1, 2). Traditionally, blood group determination relies on serological methods; however, these techniques face significant limitations in clinical scenarios involving erythrocyte deficiency, recently transfused cells, autoimmune hemolysis, transplantation, or complex prenatal testing (3, 4). Molecular genotyping, based on the DNA analysis of single nucleotide polymorphisms (SNPs), insertions, deletions, and recombination events, has emerged as a transformative approach that enables the accurate prediction of blood group phenotypes even when serology is inconclusive (5–7). By utilizing various DNA sources—such as leukocytes, buccal cells, or cell-free fetal DNA from maternal plasma—genotyping allows for the detection of weakly expressed, atypical, or "silent" antigens (8–12). This is particularly critical in transfusion medicine for multi-transfused patients and in preventing hemolytic disease of the fetus and newborn (HDFN) (13,14). Specifically, within the ABO and Rh systems, molecular analysis using PCR-based techniques or next-generation sequencing (NGS) provides precise recognition of weak, partial, or atypical phenotypes (15–18). Such precision directly impacts clinical outcomes by optimizing blood selection, rationalizing the use of RhD-negative units, and supporting rare donor registries (9, 14, 17). Despite its advantages, the integration of molecular methods into routine practice requires rigorous quality assurance and international standardization. Current efforts focus on internal quality controls (IQC) and external quality assessment (EQA) to ensure the reliability of DNA interpretation and inter-laboratory comparability (19–30). While challenges remain regarding the harmonization of bioinformatics systems and the high cost of specialized equipment, the transition toward personalized transfusion medicine suggests that genotyping will increasingly become a routine standard in clinical laboratories (31–36). The aim of this study is to analyze the frequency of ABO and RhD system genotypes, including RHD variant alleles, in a specific patient population. Furthermore, this research evaluates the clinical value of molecular genotyping in comparison with standard serological findings to determine its role in enhancing transfusion safety and optimizing patient care.

PARTICIPANTS AND METHODS

Participants

This retrospective observational study was conducted using data from the Immunohematology Laboratory at the University Clinical Hospital Mostar, covering the period from January 31, 2023, to July 1, 2025. The study population was derived from a total pool of 24,207 clinical samples processed in 2024, which served as the baseline for evaluating screening volume. The primary inclusion criteria focused on patients of both sexes and all age groups for whom definitive blood group determination was not achievable through standard automated serological protocols. Specifically, the study included 1,001 samples (857 outpatients and 144 inpatients) that exhibited ambiguous, weak, or discordant serological reactions. Exclusion criteria were applied to samples with clearly defined serological results that did not require molecular clarification. The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the University Clinical Hospital Mostar (Ref. No: (12/2026)).

Methods

The primary objective was to evaluate the clinical utility of molecular genotyping in resolving serological discrepancies. Secondary

objectives included determining the frequency of ABO and RhD genotypes and identifying specific variant alleles. Initial serological screening for ABO and RhD antigens was performed using the ERYTRA automated analyzer (Grifols, Barcelona, Spain) employing BIO-RAD (Cressier, Switzerland) and IMMUCOR (Norcross, GA, USA) reagents via standard column agglutination technology. For all samples meeting the inclusion criteria, molecular genotyping was conducted using the RBC FluoGene® system (Inno-Train Diagnostics, Kronberg im Taunus, Germany). This method utilizes sequence-specific priming polymerase chain reaction (PCR-SSP) coupled with fluorescence detection. Detailed analysis focused on RHD and RHCE gene variants, specifically identifying weak D, partial D, and DEL variants, as well as C/c and E/e polymorphisms. All genetic nomenclature followed the standards established by the ISBT Working Party on Red Cell Immunogenetics and Blood Group Terminology.

Statistical analysis

Statistical processing was performed using IBM SPSS Statistics v.24.0. Descriptive statistics were used to summarize the frequency and distribution of blood group genotypes and serological discrepancies.

RESULTS

Socio-Demographic Characteristics of Participants

A total of 162 participants were included in the study. The analysis of residence showed that more than half of the participants originated from the Herzegovina-Neretva County (n = 89; 54.9%), followed by West Herzegovina County (n = 52; 32.1%) and Herzeg-Bosnia County (n = 16; 9.9%). The remaining participants (3.1%) were from other counties or countries. Regarding the city of residence, Mostar was the most frequent (n = 54; 33.3%), followed by Široki Brijeg (n = 27; 16.7%), and Livno (n = 12; 7.4%). Detailed demographic data and residence distributions are summarized in Table 1.

Table 1. Residence and Demographic Characteristics of Participants

Category	n	%
County of residence		
Herzegovina-Neretva County	89	54.9
West Herzegovina County	52	32.1
Herzeg-Bosnia County	16	9.9
Tuzla County	1	0.6
Central Bosnia County	1	0.6
Other country	3	1.9
City of residence		
Mostar	54	33.3
Široki Brijeg	27	16.7
Livno	12	7.4
Posušje	11	6.8
Konjic	9	5.6
Čitluk	8	4.9
Grude	8	4.9
Ljubuški	7	4.3
Rama	5	3.1
Jablanica	4	2.5
Čapljina	4	2.5
Other	3	1.9
Stolac	2	1.2
Glamoč	1	0.6
Srebrenik	1	0.6
Kupres	1	0.6
Drvar	1	0.6
Žepče	1	0.6
Bugojno	1	0.6
Sarajevo	1	0.6
Neum	1	0.6

Clinical Department and Indications for Testing

The samples originated from a wide range of clinical departments, reflecting the heterogeneity of indications for molecular testing. The highest frequency of participants was referred from the Transfusion Outpatient Clinic (n = 40; 24.7%), followed by the Maternity Ward (n = 17; 10.5%), Hematology (n = 16; 9.9%), and Neonatology (n = 14; 8.6%). Other contributing departments included intensive care and emergency units (6.2%), cardiology (3.7%), and various surgical subspecialties (1.2%–3.7%).

Transfusion History

Within the study population, 68 participants (42.0%) had a recorded history of blood transfusion. This subgroup included several multi-transfused patients with significant transfusion exposure, reaching up to 47 recorded episodes in individual cases. The most common blood groups among transfusion recipients were O RhD-positive and A RhD-positive.

Distribution of ABO Blood Groups

All major ABO blood groups were represented in the study population (N = 162). Blood group A was the most prevalent, followed by group O, while groups B and AB were identified at lower frequencies. A notable occurrence of A2 and A2B subgroups was observed. These cases were characterized by discrepancies between cell and serum typing, weak reactions with anti-A reagents, or the presence of anti-A1 antibodies.

RhD System and Serological Discrepancies

Initial serological testing identified a high frequency of weak or ambiguous RhD expressions. These were categorized as "weak D," "RhD W," "D 2+," or "unclear RhD" reactions. In a significant proportion of these cases, standard hemagglutination was unable to differentiate between weak D, partial D, or DEL variants, necessitating molecular intervention.

Molecular Interpretation of RHD and RHCE Genotypes

Molecular typing resolved all cases of serological ambiguity. The allelic distribution of the RHD gene showed substantial heterogeneity:

- Weak D Variants: The most frequently identified alleles were RHD 1W.1, 1, and 1.2, corresponding to Weak D type 1. This was followed by the RHD 01W.3/3.1 allele, which corresponds to Weak D type 3.
- Partial D Variants: Several highly immunogenic partial D variants were identified, including D VII (RhD 07.01), DNB, DAR2, and 4.1.
- DEL Variants: The RHD 11 allele (DEL variant) was identified in a small number of cases where the D antigen was serologically undetectable.

RHCE Gene Analysis

Analysis of the RHCE gene confirmed the expected distribution of C, c, E, and e antigens. The E allele was present at a lower frequency relative to other major Rh antigens. Additionally, the allele encoding the Cw antigen was identified in several participants, correlating with weak reactions observed during the antiglobulin phase of serological testing.

DISCUSSION

This study evaluated the clinical utility of molecular genotyping in resolving serological ambiguities within the ABO and RhD blood group systems. Our primary findings demonstrate that molecular analysis successfully resolved 100% of serological discrepancies in the cohort (N = 162), identifying a high degree of RHD allele heterogeneity that would otherwise remain misclassified by standard hemagglutination techniques.

The observed distribution of ABO blood groups, characterized by the predominance of groups A and O, aligns with established epidemiological data for the Southeast European region (37,38). While the majority of participants were RhD-positive, the clinical significance of our results lies in the detection of specific variant alleles, such as Weak D types 1, 2, and 3, as well as highly immunogenic partial D and DEL phenotypes (39,40).

A critical aspect of this study is the differentiation between variant groups. While Weak D types 1, 2, and 3 are typically associated with a low risk of anti-D alloimmunization, our identification of partial D variants (e.g., D VII, DNB, and DAR2) highlights a significant

clinical risk. These patients, if incorrectly classified as RhD-positive by serology, are at risk of developing anti-D antibodies upon exposure to the complete D antigen (41–44). Furthermore, the detection of DEL variants (RHD 11) in serologically D-negative individuals underscores the risk these donors pose to RhD-negative recipients, as even minimal antigen expression can trigger a primary immune response (41,42,45).

Our findings confirm that molecular genotyping is an essential adjunct to serology in complex clinical scenarios, such as in multi-transfused patients where donor cell interference masks the recipient's true phenotype, or in neonates with a positive Coombs test (43,46). By providing a definitive genotype, we can ensure the rational use of RhD-negative blood units—a limited and costly resource—by safely managing Weak D type 1 and 2 recipients as RhD-positive where appropriate, while strictly reserving RhD-negative units for partial D and DEL variant patients.

Study Limitations and Future Perspectives

Despite the high accuracy of the PCR-based methods used, this study has limitations. The molecular screening focused on known common and regional variants; therefore, extremely rare or novel RHD mutations may not have been captured. Additionally, the retrospective nature of the study and the specific focus on "problematic" samples means the frequencies reported here do not reflect the general population but rather a high-risk clinical subgroup. Future research should incorporate larger, prospective cohorts and potentially employ Next-Generation Sequencing (NGS) to detect novel alleles that escape current PCR-SSP designs.

Conclusion and Clinical Implications

In conclusion, this study supports the integration of molecular genotyping into routine transfusion protocols for specific indications. Based on our data, we propose a standardized molecular reflex testing protocol for:

1. All patients exhibiting weak, discordant, or ambiguous RhD serological reactions.
2. Multi-transfused hematological and oncological patients to prevent alloimmunization.
3. Pregnant women with weak D expressions to determine the necessity of Rh immunoglobulin prophylaxis.

Implementing these recommendations will significantly enhance patient safety by reducing the risk of hemolytic transfusion reactions and supporting an individualized approach to immunohematological care (45,46).

ACKNOWLEDGMENTS

None.

FUNDING

The authors received no financial support for the research, authorship, and/or publication of this study. No external funding was requested or received.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTIONS

MS: contribution to study conception and design, data collection and analysis, literature review, interpretation of data, and writing of the manuscript. **JK:** supervision of the research, contribution to study conception and design, critical revision of the manuscript, and approval of the final version. **IP:** statistical analysis of the data, interpretation of statistical results, and critical revision of the manuscript. **A**ll authors have read and approved the final version of the manuscript.

ETHICAL BACKGROUND

Institutional Review Board Statement: The study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the Ethics Committee of the University Clinical Hospital Mostar (Reg. No. 01-1-259/25).

Informed Consent Statement: Due to the retrospective nature of the study and the use of existing data from the hospital information system, individual informed consent was not required. The study was conducted with the approval of the Ethics Committee of the University Clinical Hospital Mostar (Reg. No. 01-1-259/25).

Data Availability Statement: The data supporting the findings of this study are not publicly available due to privacy and confidentiality considerations but may be available from the corresponding author upon reasonable request and subject to applicable ethical and institutional restrictions.

REFERENCES

1. Quraishy N, Sapatnekar S. Advances in blood typing. Adv Clin Chem. 2016;77:221–69.
2. Yamamoto F. Molecular genetics of ABO. Vox Sang. 2000;78:91–103.
3. Storry JR, Olsson ML. The ABO blood group system revisited. Immunohematology. 2009;25:48–59.
4. Springer GF, Tegtmeyer H. Apparent violation of "Landsteiner's law". Klin Wochenschr. 1974;52:295–7.
5. Wuttke NJ, MacArdle PJ, Zola H. Blood group antibodies are made by CD5+ and CD5- B cells. Immunol Cell Biol. 1997;75:478–83.
6. Branch DR. Anti-A and anti-B: what are they and where do they come from? Transfusion. 2015;55:S74–9.
7. Arend P. Ancestral gene and "complementary" antibody dominate early ontogeny. Immunobiology. 2013;218:755–61.
8. Flegel WA. How I manage donors and patients with a weak D phenotype. Curr Opin Hematol. 2006;13:476–83.
9. Westhoff CM, et al. DIIIa and DIII Type 5 are encoded by the same allele. Transfusion. Xu W, et al. Evaluation of an automated microplate technique in the Galileo system. Clin Lab. 2014;60:241–4.
10. Xu W, et al. Evaluation of an automated microplate technique in the Galileo system. Clin Lab. 2014;60:241–4.
11. Spindler JH, et al. A novel microplate agglutination method for blood grouping. Transfusion. 2001;41:627–32.
12. Mannessier L, Guignier F. Standardization trial of ABO-Rh (D) blood typing using a U-microplate. Rev Fr Transfus Hemobiol. 1992;35:33–8.
13. Garratty G. Do we need to be more concerned about weak D antigens? Transfusion. 2005;45:1547–51.
14. Denomme GA, et al. Partial D, weak D types, and novel RHD alleles among 33,864 multiethnic patients. Transfusion. 2005;45:1554–60.
15. Flegel WA, Roseff SD, Tholpady A. Phasing-in RHD genotyping. Arch Pathol Lab Med. 2014;138:585–8.
16. Reid ME. Milestones in laboratory immunohematology: techniques and procedures. Immunohematology. 2009;25:39–43.
17. Harmening DM. Modern blood banking & transfusion practices. 7th ed. Philadelphia: FA Davis; 2018.
18. Bianco-Miotto T, et al. DNA methylation of the ABO promoter underlies loss of ABO allelic expression in leukemia. PLoS One. 2009;4:e4788.
19. Noiphung J, et al. Novel paper-based assay for simultaneous determination of Rh typing and ABO. Biosens Bioelectron. 2015;67:485–9.
20. Songjaroen T, Laiwattanapaisal W. Simultaneous forward and reverse ABO typing using paper-based device. Anal Chim Acta. 2016;921:67–76.
21. Afshari P, Abolfathi N. A novel method for blood-typing using nitrocellulose. Biomed Chromatogr. 2017;31:e3952.
22. Hertaeg MJ, et al. A rapid paper-based blood typing method from droplet wicking. Analyst. 2021;146:1048–56.
23. Pelton R. Bioactive paper provides a low-cost platform for diagnostics. Trends Analyt Chem. 2009;28:925–42.
24. Khan MS, et al. Paper diagnostic for instantaneous blood typing. Anal Chem. 2010;82:4158–64.
25. Flegel WA. The genetics of the Rhesus blood group system. Transfus Med Hemother. 2011;38:1–10.
26. Poole J, Daniels G. Blood group antibodies and their significance in transfusion medicine. Br J Haematol. 2007;137:3–16.
27. Reid ME, Lomas-Francis C. Molecular insights into the Rh complex. Vox Sang. 2002;82:95–107.
28. Westhoff CM, et al. Weak D types: clinical significance and genotyping strategies. Transfusion. 2013;53:297–306.
29. Flegel WA, et al. Molecular basis of weak D and partial D phenotypes. Immunohematology. 2011;27:6–14.
30. Daniels G. Red cell immunogenetics. Br J Haematol. 2013;161:1–14.
31. Al-Tamimi M, et al. Validation of paper-based assay for rapid blood typing. Anal Chem. 2012;84:1661–8.
32. Li M, et al. Paper-based blood typing device that reports patient's blood type "in writing". Angew Chem Int Ed Engl. 2012;51:5497–501.
33. Daniels G. Human Blood Groups. 3rd ed. Oxford: Blackwell Science; 2013.
34. Reid ME, Lomas-Francis C, Olsson ML. The Blood Group Antigen FactsBook. 3rd ed. London: Academic Press; 2012.
35. Yamamoto F, McNeill PD, Hakomori S. Molecular genetic basis of the histo-blood group ABO system. Nature. 1990;345:229–33.
36. Avent ND, Reid ME. The Rh blood group system: a review. Blood. 2000;95:375–87.
37. Sainath PB, Ramaiyan V. Weak D phenotype in transfusion medicine and obstetrics: Challenges and opportunities. World J Exp Med. 2025;15(2):102345.
38. Stanic HS, Galic ZK, Dogic V, Bingulac-Popovic J, Jukic I. Serological and molecular characterisation of the most prevalent weak D variants in Croatian population. Transfus Med. 2023;33(2):132-6.
39. Kruhonja Galić Z, et al. (Guidelines for the determination of Rh(D) blood group and the application of RhD genotyping). Lijec Vjesn. 2022;144:125-33. (in Croatian)
40. Gonsorcik V, Zhou L. Rh Typing: Overview: Clinical Indications/Applications, Test Performance and Limitations. Medscape. 2025. Available from: https://emedicine.medscape.com/article/1731214-overview. Accessed November 25, 2025.
41. Rodrigues ES, et al. Frequency and characterization of RHD variant alleles in a population of blood donors from southeastern Brazil: comparison with other populations. Transfus Apher Sci. 2021;60(4):103135.
42. Vege S, Sprogøe U, Lomas-Francis C, Jakobsen MA, Antonsen B, Aeschlimann J, et al. Impact of RHD genotyping on transfusion practice in Denmark and the United States and identification of novel RHD alleles. Transfusion. 2021;61:256–65.
43. Lasić L, Lojo-Kadrić N, Silajdžić E, Pojskić L, Hadžiselimović R, Pojskić N. Molecular-genetic variance of RH blood group system within human

population of Bosnia and Herzegovina. Bosn J Basic Med Sci. 2013;13(1):10-3.
44. Guzijan G, Jovanovic Srzentic S, Pavlovic Jankovic N, Djilas I, Lilić M. Implementation of Molecular RHD Typing at Two Blood Transfusion Institutes from Southeastern Europe. Transfus Med Hemother. 2019;46(2):114-20.
45. Tomar A, Devendra R, Parchure D, Kulkarni S. Weak or partial D: Importance of molecular characterization of D variants. Transfus Apher Sci. 2025;64(2):104069.
46. Collodel L, Gessoni G. RHD Genotyping to Resolve Weak and Discrepant RHD Phenotypes: The 'Serenissima' Experience. Int Blood Res Rev. 2024;15(2):30–8.