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ABO BLOOD GROUPING DISCREPANCIES AMONG PATIENT SAMPLES AT A TERTIARY CARE CENTRE IN CHENNAI: A DESCRIPTIVE RECORD-BASED STUDY

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ABSTRACT

Background: ABO discrepancies occur when there is a mismatch between forward and reverse grouping or an unexpected reaction prevents definitive group assignment and reporting. In patients, age, disease, treatment, transfusion, and unexpected antibodies may alter antigen or antibody reactions.

Methods: This study is a descriptive, record-based analysis using all 17,940 patient blood-grouping samples received from outpatient and inpatient services at a tertiary care centre in Chennai between 1 November 2023 and 31 December 2024 as the workload denominator. Blood-donor samples and neonatal or infant samples for which reverse grouping was not performed were excluded from discrepancy assessment. Samples with discordant or unexpected reactions on automated testing were re-investigated by tube testing and additional serology as indicated. Grouping discrepancies were classified into Groups I, II, III &IV and summarised using frequencies and percentages.

Results: 34 ABO grouping discrepancies were identified, with a prevalence rate of 0.19% (34/17,940). Group I was the most common type (16/34; 47.06%), followed by Group IV (12/34; 35.29%) and Group II (6/34; 17.65%); no Group III discrepancy was detected. The key underlying causes identified included chemotherapy or immunosuppressive therapy (7), ABO subgroup or altered antigen expression (3), non-ABO alloantibodies (3), cold-reactive antibodies (3), and recent transfusion (2).

Conclusion: Overall ABO discrepancies are uncommon, yet they are clinically important. The predominance of Group I and IV discrepancies in the study reflected patient-related serological interference, emphasising the importance of correlating serological findings with the patient's clinical history, underlying disease, treatment history, transfusion history and relevant laboratory parameters for accurate resolution of ABO grouping discrepancies.

KEYWORDS: Blood grouping; ABO grouping discrepancy; Immunohematology; patient samples; reverse grouping; transfusion safety; pretransfusion testing.

INTRODUCTION

Accurate ABO typing is essential for pretransfusion safety. Routine grouping includes forward typing, which detects A and B antigens on patient red cells, and reverse typing, which detects the expected naturally occurring anti-A and/or anti-B in plasma. There must be a correlation between these complementary tests. A mismatch, unexpected reaction, or inability to assign the same ABO group from both tests constitutes an ABO grouping discrepancy [1,2]. The discrepancy must be resolved before a definitive group is reported, because an inaccurate ABO group assignment can expose a patient to an acute haemolytic transfusion reaction.

ABO discrepancies are commonly classified according to their primary serological pattern. Group I discrepancies involve weak or missing antibodies in reverse grouping; Group II discrepancies involve weak or missing antigens in forward grouping; Group III discrepancies arise from plasma or protein abnormalities that produce rouleaux or pseudo-agglutination; and Group IV comprises miscellaneous mechanisms such as cold-reactive antibodies, unexpected alloantibodies, mixed-field reactions after transfusion & chimerism[1,3].

Sources of clerical and technical errors must be ruled out first. Clerical issues can include mislabelled specimens or test tubes or Improper recording of test results. Technical issues can include failure to follow the manufacturer's instructions; Deleted procedural steps; missed or under-interpreted weak reactions; incorrect interpretation of serological reactions; missing or incorrect reagents in test samples & equipment malfunction, centrifuge time or incorrect speed.

The present study was undertaken to determine the prevalence, pattern, and documented associated factors of ABO grouping discrepancies among all outpatient and inpatient patient samples received by the blood bank of a tertiary care centre in Chennai. By evaluating the prevalence of ABO discrepancies within our inpatient and outpatient population, this study aims to guide a standardised, patient-focused strategy for safe blood transfusion and discrepancy resolution.

MATERIALS AND METHODS

A descriptive, record-based study was conducted in the blood bank of Saveetha Medical College and Hospital, Chennai, from 1 November 2023 to 31 December 2024. The sampling frame comprised all patient blood grouping samples received from outpatient and inpatient clinical services during the study period. Blood donor grouping samples were not part of this analysis.

Inclusion criteria:

- All patient blood-grouping samples received from outpatient and inpatient services during the study period were included.
- Samples were assessed for an ABO grouping discrepancy when both forward and reverse grouping results were available.
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Exclusion criteria:

- Blood-donor samples were excluded.
- Neonatal and infant samples in which reverse grouping was not performed according to departmental practice were excluded.
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Sample identification and serological work-up

Initial ABO and RhD grouping was performed on an automated analyser according to departmental standard operating procedures. A discrepancy was defined as a mismatch between forward/reverse grouping or a weak, missing, extra, or mixed-field reaction that prevented unequivocal ABO assignment.

Discrepant samples were rechecked using the conventional tube testing method. Additional testing was selected according to the observed reaction pattern and included anti-A, anti-B, anti-AB, anti-A1 lectin, and anti-H lectin where appropriate. Antibody screening was performed by column agglutination technology using the Bio-Rad IH 500 platform when unexpected antibodies were suspected. The clinical information reviewed included age, diagnosis, history of chemotherapy or immunosuppressive treatment, and any recent history of transfusion. All the cases were resolved serologically, along with collection of relevant clinical history, after which the final classifications were assigned.

Classification and data analysis

The discrepancies were grouped into four categories: Group I, comprising weak or missing antibodies; Group II, comprising weak or missing antigens; Group III, involving plasma or protein abnormalities; and Group IV, which included miscellaneous causes. Information on the discrepancies was obtained from the blood bank register and immunohematology logbook, while relevant patient details were obtained from the hospital information management system. The data were anonymised and entered into Microsoft Excel for analysis. Descriptive statistics were used, with prevalence calculated using the total number of blood grouping samples as the denominator. Categorical data were presented as numbers and percentages. No inferential statistical tests were performed.

RESULTS

Over the 14-month study period, a total of 17,940 blood grouping samples were received from both outpatient and inpatient services. Of these, 34 showed blood group discrepancies, giving an overall prevalence of 0.19%.

Table 1. Distribution of ABO grouping discrepancy categories (n=34)

Discrepancy category	Number	Percentage
Group I: weak or missing antibodies	16	47.06%
Group II: weak or missing antigens	6	17.65%
Group III: plasma/protein abnormalities	0	0.00%
Group IV: miscellaneous	12	35.29%
Total	34	100.00%

Among the 34 cases, Group I accounted for the largest proportion (16/34;47.06%), followed by Group IV (12/34; 35.29%) and Group II (6/34; 17.65%). No cases were classified under Group III (Table 1 and Figure 1).

Figure 1. Proportional distribution of ABO grouping discrepancy categories

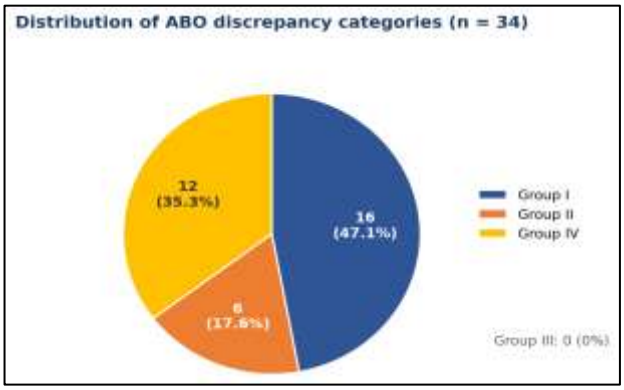

Table 2. Documented factors associated with ABO grouping discrepancies

Documented associated factor	Category	Number	% of all discrepancies
Chemotherapy or immunosuppressive therapy	Group I	7	20.6%
ABO subgroup or altered antigen expression (including acquired B)	Group II	3	8.8%
Cold-reactive antibodies	Group IV	3	8.8%
Recent transfusion	Group IV	2	5.9%
Non-ABO alloantibodies	Group IV	3	8.8%

Note: The table presents the associated findings recorded in the study data. These entries are descriptive and are not intended to form a mutually exclusive or exhaustive causal classification of all 34 discrepancies; therefore, no residual 'no reason' category was calculated.

Among the documented associated findings, chemotherapy or immunosuppressive therapy was the most frequent (7/34; 20.6%). Three cases showed an ABO subgroup or altered antigen expression, comprising A2B with anti-A1, a weak B reaction, and an acquired-B pattern. Non-ABO alloantibodies and cold-reactive antibodies were documented in three cases each, while recent transfusion accounted for two cases (Table 2).

DISCUSSION

This study evaluated ABO grouping among 17,940 patient samples received from both outpatient and inpatient services and identified 34 discrepancies, yielding a prevalence of 0.19%. Group I discrepancies were most common, followed by Group IV & Group II, while no Group III discrepancy was not observed.

The percentage of ABO discrepancies found in our study matches what other researchers have found in similar hospital-based studies. Heo et al. analysed more than half a million patient blood-grouping tests in a Korean tertiary hospital and reported discrepant events in approximately 0.24% of tested samples, with weak or missing serum reactions as the largest category [4]. In a study done by Makroo et al., 138 discrepancies were reported among 135,853 patient samples (approximately 0.10%) in an Indian tertiary hospital [5]. At a tertiary oncology centre, Desai et al. detected 104 discrepancies among 134,964 patient samples (approximately 0.08%) [6]. These comparable findings suggest that the low prevalence of ABO discrepancies observed in our study is consistent with reports from tertiary-care centres across different geographical regions and populations, including studies from India and other countries.

Mishra et al. also demonstrated that patient grouping discrepancies require correlation with diagnosis, transfusion history, and targeted serological testing rather than interpretation from reaction strength alone [8]. Kaur et al. evaluated a donor cohort and is therefore not directly comparable with the present patient-based prevalence; that study is relevant primarily for its serological resolution methods [7]. The difference in prevalence of discrepant cases should be analysed in relation to case mix, age distribution, malignancy burden, transfusion exposure, testing platform, discrepancy definition, and the intensity of the resolution protocol rather than as direct measures of laboratory performance.

The predominance of Group I discrepancies is biologically plausible in a hospital patient population. Group I reflects weak or absent expected anti-A and/or anti-B in reverse grouping. In the present series, seven of the 16 Group I discrepancies (43.8%) were associated with chemotherapy or immunosuppressive therapy, supporting impaired isoagglutinin production as an important mechanism. Neonatal and infant samples were not classified as discrepant solely because of absent reverse-grouping reactions, as reverse grouping was not performed in this population according to departmental practice. This approach avoids misclassifying the expected developmental absence of ABO isoagglutinins

as a pathological discrepancy. Similar patient-based studies have identified weak or missing reverse-grouping reactions as a major source of discrepancy [4-6].

The association with chemotherapy and immunosuppressive treatment is worth noting, particularly because this study was conducted in a tertiary care setting. Many patients in such settings may have haematological or solid-organ malignancies, receive corticosteroids or cytotoxic drugs, or have disease- related hypogammaglobulinaemia. These factors can reduce antibody production and may result in weaker or lower-titer ABO antibodies. In some patients with malignancy, changes in red cell antigen expression may also occur, making the serological findings more difficult to explain by a single Group I mechanism alone [9]. The AABB Technical Manual, Harmening's Modern Blood Banking and Transfusion Practises, and Mollison's Blood Transfusion in Clinical Medicine also stress the importance of considering the patient's age, immune status, underlying disease, treatment, and previous transfusion history when interpreting serological findings [10-12]. Clinical history should be considered alongside the serological findings, as it can help explain and interpret the observed serological phenotype.

Group IV was the second most common category, accounting for more than one-third of the discrepancies. It included a varied set of findings, with three cases showing non-ABO alloantibodies, three showing cold-reactive antibodies, and two occurring in patients with a recent history of transfusion. Cold-reactive antibodies may agglutinate reagent cells or patient cells at room temperature and thereby create unexpected reactions in forward typing, reverse typing, or both. Non-ABO alloantibodies can react with reagent reverse-grouping cells if the corresponding antigen is present. Recent transfusion may produce mixed-field agglutination when circulating donor red cells differ from the patient's intrinsic ABO phenotype. The high contribution of Group IV therefore reflects the complexity of hospital patients and reinforces the need for an antibody screen and transfusion history when routine repeat testing does not resolve the pattern [1,5,6,10-12].

6 discrepancies were classified as Group II. Three documented findings included an A2B with anti-A1, a weak B reaction, and an acquired-B pattern. Group II abnormalities result from weak, missing, or altered A/B antigen reactivity and may be inherited, as in A or B subgroups, or acquired in association with disease. Anti-A1 and anti-H lectins, anti-AB reagent, extended incubation, adsorption-elution, and molecular methods in selected cases can help identify weak phenotypes [1,7,10-13,16].

The acquired-B phenomenon requires separate detailed testing because acquired B is not an inherited ABO subgroup. Bacterial deacetylase can modify the terminal N-acetylgalactosamine of the A antigen, producing a B antigen-like structure that reacts with some anti-B reagents. The resulting pattern may appear as an AB blood group, often with weak or mixed-field anti-B reactivity, while the patient's plasma may retain anti-B. This usually resolves in 8 to 12 weeks after the infection has subsided. So we followed up and rechecked blood grouping after 12 weeks, when the discrepancy resolved. Published reports have described acquired-B discrepancies in septicaemia and necrotising enterocolitis, demonstrating the importance of previous blood-group records, autocontrol, reagent selection, and clinical microbiology findings [14,15]. Several literature reviews on the ABO system also emphasise that apparent phenotype changes must be distinguished from inherited subgroup variation [13,16].

No Group III discrepancy was observed. Group III discrepancies typically result from rouleaux or pseudoagglutination associated with elevated plasma proteins, plasma expanders, or other abnormalities of the suspending medium. Their absence in this series may reflect the relatively small number of discrepant samples, the local case mix, or effective exclusion of rouleaux during tube-method resolution. A zero count should not be interpreted as evidence that Group III mechanisms cannot occur in this patient population; saline replacement and microscopic assessment remain relevant when the reaction pattern suggests rouleaux [1,3,10-12].

The associated findings table should not be treated as a complete causal partition of the 34 discrepancies. The Table should be interpreted as a summary of the commonly recognised causes rather than as a definitive explanation for every case. Some patients may have had a possible underlying cause, such as medication use or a previous transfusion at another hospital, that was not documented in the available medical records. In cases where clinical and serological findings were available, more than one possible explanation may also have been present. Therefore, we assigned all unexplained cases to a separate "no reason" group by simple subtraction from the available records. For future audits and research, it would be useful to record the serological finding, suspected association, confirmed cause, and method by which the discrepancy was resolved as separate variables. This would make the findings easier to interpret and would allow clearer comparisons between groups.

From a practical point of view, a discrepant ABO grouping result should not be reported as final based only on the initial automated reaction pattern. The discrepancy should be investigated systematically, beginning with verification of the patient identification and specimen details, followed by repeat forward and reverse grouping. The sample should also be examined for haemolysis or mixed-field reactions, and the patient's age, diagnosis, medication history, and previous transfusions should be reviewed before the result is finalised. Targeted testing—such as washed-cell typing, altered incubation temperature, anti-A1/anti-H lectins, saline replacement, autocontrol, direct antiglobulin testing, and antibody screening—should then be selected according to the pattern [1-3,10-12]. If transfusion is urgently required before resolution, component selection should follow the institution's emergency policy and be guided by a transfusion medicine specialist.

The principal strengths of this study are the use of all OP/IP patient grouping samples as the workload denominator and the categorisation of discrepancies within routine tertiary-care practice. Neonatal and infant samples without reverse grouping were excluded from forward-reverse discrepancy assessment; consequently, the denominator represents the total grouping workload rather than only samples with paired forward and reverse results. Other limitations include the single-centre design, the small number of discrepant cases, reliance on recorded clinical information, and the non-mutually-exclusive nature of the associated findings. Advanced confirmation such as adsorption-elution, secretor studies, or

molecular genotyping was not documented and may have clarified selected cases. This study was descriptive and cannot establish causality or determine whether particular diagnoses or treatments independently increased discrepancy risk

CONCLUSION

In our study among the 17,940 samples, there were 34 ABO grouping discrepancies identified. Each discrepancy identified represented a clinical barrier. Among the discrepancies, Group I was found to be most frequent, and chemotherapy or immunosuppressive therapy was the most prominent association. Group IV was the second most common, attributed to cold reactive antibodies, non-ABO alloantibodies, and recent history of transfusion. Three of the six Group II discrepancies were associated with findings of subgroups or altered antigen expression, including a case of acquired-B. These results show that standardised serological testing protocols, proper and accurate documentation, and integration of proper patient history while resolving ABO discrepancies result in safe transfusion practices.

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