



## RESOLUTION OF SEROLOGICAL ABO DISCREPANCY IN A TERTIARY CARE SET-UP OF WESTERN ODISHA

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### ABSTRACT

**Background:** This study was conducted to identify the patient's precise blood type, prevent the recipient from receiving incompatible blood transfusions, avoid HDFN (Hemolytic Disease of the Fetus and Newborn), and identify rare blood phenotypes such as Bombay and para-Bombay, among others.

**Methods:** This hospital-based prospective study tested blood grouping using 25,559 patient blood samples from the Department of Transfusion Medicine at the Veer Surendra Sai Institute of Medical Science and Research, Burla, Odisha. The study was conducted from October 2016 to May 2018, after obtaining participant written informed consent and institutional ethics committee approval.

**Results:** The common cause of sample-related blood group disagreement was cold autoantibody. The primary causes of variations in red cell testing in our investigation were malignancy and autoagglutinin or excess protein coating red cells. In 1.92% of cases with weak red cell reactivity, malignancy was present. Increased red cell activity in autoagglutinins was primarily responsible for the most prevalent red cell disparities (40.38%). The two primary causes of weak or absent serum reactivity that interfered with serum testing in our investigation were hypogammaglobulinemia and the elderly. In hypogammaglobulinemia and senior patients, the frequency of disagreement was 2.8% and 5.76%, respectively. In our investigation, the infectious patient's serum revealed 3.84% group differences. Five of the 52 cases with inconsistencies were caused by elderly patients. Out of the five old patients, four belonged to group-O and one to group B. The senior patients' ages ranged from 70 to 107 years, with a mean age of 92 years (92.4).

**Conclusion:** It's critical to identify and address inconsistent outcomes. To avoid ABO incompatibility, a person must have their blood type labeled correctly. It is critical to always pay attention to the intensity of the response, because weaker responses typically raise questions.

**Keywords:** Resolution, Serological ABO Discrepancy.

## INTRODUCTION

Pre-transfusion testing, such as blood grouping, compatibility testing, and recipient antibody screening, is required to reduce the likelihood of any transfusion reaction. Blood transfusion services aim to ensure the availability of an adequate supply of safe blood and blood components that are free from transfusion transmitted infections. The red cell membrane's blood group antigens play a crucial role in membrane transport, protein channel ligands, receptor adhesion molecules, enzyme activity, and structural protein synthesis. ABO, Rh, Kell, Kidd, Duffy, MNS, Lewis, and Lutheran blood groups are regarded as therapeutically significant blood group systems. Of the 347 red cell antigens recognized by the International Society of Blood Transfusion, 308 antigens are clustered in 36 blood group systems.<sup>[1,2,3]</sup> Testing for both predicted and unexpected antibodies is necessary before releasing plasma for transfusion-related purposes.<sup>[4]</sup> Karl Landsteiner discovered the ABO blood group system in 1900; it is the most significant blood group system in transfusion therapy.<sup>[5]</sup> Antigens A, B, and Rh make up the ABO system. There are four blood group phenotypes of corresponding antigens based on these antigens.<sup>[6]</sup> There are two methods for grouping blood: forward grouping and reverse grouping. The outcomes of these methods should match.<sup>[7]</sup> A blood group discrepancy occurs when serum and red cell test results differ, typically as a result of unexpectedly positive or negative forward or reverse typing results. An incompatibility between the Rh and ABO blood groups is linked to incompatible transfusion reactions.<sup>[8,9]</sup> A significant portion of blood group differences are caused by human error.<sup>[8,10]</sup> In order to avoid transfusion reactions, blood group discrepancies should be addressed before transfusion and appropriately labeled.<sup>[11]</sup> Technical issues and sample-related issues could be the cause of the blood group difference. Poor or inaccurate blood specimen identification, sample mixing, reagent contamination, and missing reagent additions frequently lead to technical errors. Unexpected reactions in cells or serum grouping are the second category of sample-related problems. Group I, group II, group III, and group IV are the four main categories into which discrepancies can be arbitrarily separated. Group I inconsistencies are linked to an unanticipated response in the reverse grouping as a result of absent or weakly responding antibodies. Differences in Group II are linked to an unanticipated response in the forward grouping as a result of absent or weakly responding antigens. However, there are issues with both forward and reverse grouping groups III and IV because of unanticipated isoagglutinins, plasma proteins, and cold autoantibodies, respectively. When a discrepancy arises during testing, the divergent result needs to be noted, but the blood group interpretation needs to wait until the divergence is cleared up. To rule out the possibility of technological problems during testing, the first step in resolving the difference should be to repeat the test with the same sample. If repeat testing on the same sample does not resolve discrepant results, a new sample should be sought for testing. Blood grouping is hampered by the patient's medical diagnosis, history of transplants, and prior transfusions related to blood type. Many articles have been written about blood group differences among willing blood donors in India and outside, but not much study has been done on patient blood samples. In Western Odisha, research on blood group differences and how they are resolved has not yet been done. This study is the first of its kind in our area.

## AIMS AND OBJECTIVES

- To figure out the exact blood group of the patient.
- To prevent incompatible blood transfusions in the recipient.
- To prevent HDFN.
- To detect rare blood phenotypes like Bombay, para-Bombay etc.

## MATERIALS & METHODS

This hospital-based prospective study tested blood grouping using 25,559 patient blood samples from the Department of Transfusion Medicine at the Veer Surendra Sai Institute of Medical Science and Research, Burla, Odisha. The study was conducted from October 2016 to May 2018, after obtaining participant written informed consent and institutional ethics committee approval.

### Exclusion Criteria

The hemolyzed sample was the sole criterion for exclusion. For patients whose samples had hemolyzed, a new sample was required.

### Statistical Methods

The data was entered into MS Excel and examined using SPSS software. The results were presented in tables.

## RESULTS

Causes Due to Sample Related Problems		Number	Percentage (%) (N = 32)
Weak/missing antibody		5	15.62
Weak antigen expression		1	3.12
Rouleaux		1	3.12
Cold autoantibodies		20	62.5
Cold alloantibodies		3	9.37
Bombay phenotypes		2	6.25
<i>Distribution of Discrepancy in Relation to Sample Related Problem</i>			
Group	Cause	Number	Frequency (%)
I	Weak/missing antibody	5	9.61
II	Weak/missing antigen	1	1.92
III	Rouleaux	1	1.92
IV	Miscellaneous problems	25	48.07
<i>Different Categories of Sample Related Problems (N = 52)</i>			
<i>Table 1</i>			

Thirty-two of the 52 disparities in the current study were the result of sample-related issues. Among these sample-related issues, we discovered weak or missing antibodies, weak antigen expression, rouleaux, cold autoantibodies, cold alloantibodies, and Bombay phenotype, with frequencies of 15.62%, 3.12%, 3.12%, 62.5%, 9.37%, and 6.25%, respectively. The most frequent reason for sample-related blood group differences was cold autoantibody.

Four main categories were used to categorize ABO discrepancies: group I, group II, group III, and group IV. In our study, 9.61% of the participants in group I had weakly responding or missing antibodies, which was linked to an unexpected reaction in the reverse grouping. Group II discrepancies were found to be 1.92%, indicating that weakly responding or absent antigens caused an unanticipated reaction in the forward grouping. Group III inconsistencies were observed to occur often (1.92%), primarily as a result of rouleaux. Group IV, which included 48.07% of the issues, included various issues like cold autoantibodies, cold alloantibodies, and Bombay phenotypes.

First, additional samples were requested from patients who had auto-antibodies that were reactive to the cold. Because prewarming might have inhibited autoantibody adhesion to patient red cells in vitro, the serum and red cells were kept apart and at 370 °C. Before red cell testing, the patient's red blood cells were washed three or more times with warm (370 °C) saline to get rid of any autoantibodies that had previously attached themselves to the red blood cells. Anti-A, anti-B, and anti-D were used in a 1:1 ratio to forward group the washed red blood cells, while a parallel experiment with 6% bovine albumin was conducted. If the control group responded favorably, then the average cleaning of red blood cells was insufficient. In this instance, the test was conducted once more after the washing stage was completed several times using warm saline.

<b>Discrepancy in Red Cell Testing</b>	<b>Cause</b>	<b>Present Study</b>	<b>Heo Min-Seok et al.</b>
Weak red cell reactivity	Malignancy	1 (1.92%)	3 (5.5%)
Extra red cell activity	Autoagglutinins or excess protein coating red cells	21 (40.38%)	2 (3.5%)
<b>Type of Discrepancy Due to Read Cell Testing among Total Discrepancies (N = 52)</b>			
<b>Discrepancy Due to Serum Testing</b>		<b>Present Study</b>	<b>Heo Min-Seok et al</b>
Cold alloantibody		3 (5.76%)	3 (5.1%)
Cold autoantibody		20 (38.46%)	2 (3.6%)
Excess serum protein		1 (1.92%)	4 (7.3%)
Infection		2 (3.84%)	1 (1.8%)
Weak or missing serum reactivity		2 (3.84%)	
*Hypogammaglobinemia		3 (5.76%)	3 (5.5%)
*Elderly			1 (1.8%)
<b>Type of Discrepancy in Serum Testing among Total Discrepancies (N = 52)</b>			
<b>Table 2</b>			

The primary causes of the variations in red cell tests in our investigation were autoagglutinins, excess protein coating red cells and malignancy. Malignancy was seen in 1.92% of cases when there was low red cell reactivity. The most frequent red cell disparity, auto-agglutinin (40.38%), was mostly caused by increased red cell activity.

Maximum differences were attributed to cold autoantibodies (44.23%) as an additional reaction in serum grouping. Due to an excess of serum protein, there was a 1.92% difference in blood typing. Two major factors that affected our study's serum reactivity and interfered with serum testing were hypogammaglobinemia and aging. The differences were 3.84% and 5.76%, respectively, in the aged patient and the hypogammaglobinemia patient. Elderly patients with lymphoma, gallbladder cancer, and other diseases were linked to a lack of antibodies. In our investigation, there were 3.84% group disparities in the serum of infected individuals.

	<b>Present Study</b>	<b>Esmaili et al. <sup>67</sup></b>
<b>Age</b>	<b>60-80 Years (Mean: 68.3)</b>	<b>88-113 Years (Mean: 93.5)</b>
A	0	22
B	1	20
O	4	28
<b>Age Related Weak or Missing Antibodies</b>		
<b>Blood Group</b>	<b>Number</b>	<b>Percentage (%)</b>
O +ve	29	55.76
B +ve	14	26.92
A+ve	6	11.53
AB-ve	1	1.92
Oh+ve	2	3.84
<b>Actual Blood Group of 52 Cases</b>		
<b>Table 3</b>		

Five of the 52 cases with inconsistencies were caused by elderly patients. Of the five senior patients, four belonged to group-O and one to group B. The patients' ages ranged from 70 to 107 years, with a mean age of 92 years (92.4).

In the event that there were weak or absent antibodies, serum testing was conducted and the incubation period was extended from five minutes to thirty minutes at room temperature. If the issue persisted,

the serum test was run again at a lower temperature of 40 °C. A negative auto-control result could rule out autoantibodies, while a negative group O cell screening result could rule out allo-antibodies. In Rouleaux, serum testing was carried out using the saline replacement approach, and red cell testing was resolved by repeating the process of washing red cells four to six times with normal saline (0.9%). Due to insufficient antigen expression, we discovered one instance of group IV discrepancy in a group-A patient who was diagnosed with acute leukemia. In order to determine whether or not a subgroup was present, we retested the red cell tests using monoclonal anti-AB, anti-A1 lectin, and anti-H lectin. Red blood cells were tested with anti-H lectin to determine the Bombay phenotypes.

## DISCUSSION

A number of interrelated procedures are necessary for safe blood transfusion, beginning with the right medical judgment on blood therapy and accurate patient blood typing in order to provide the appropriate blood components to the right patients. The most significant blood group system is the ABO system. Both forward and backward grouping should be a part of ABO grouping. The outcomes of the forward and backward techniques need to line up. An ABO discrepancy suggests that the reverse technique and forward typing are at odds.

From January 2015 to December 2016, 25,559 patients had their blood groups tested. The etiology and primary cause of the fifty-seven cases of differences between the forward and reverse methods were assessed. The incidence of blood type differences was found to be 0.20% in the current investigation, which was similar to the findings of a Korean study by Heo et al.<sup>[12]</sup> that found it to be 0.14%. A comparable study with an incidence of 0.08% and 0.05%, respectively, was carried out in Korea by Kim et al.<sup>[13]</sup> in Saudi Arabia by Bashawari et al.<sup>[14]</sup>

The most frequent kind of disagreement found in our investigation was sample-related inaccuracy (61.53%). Technical mistakes accounted for the other form of difference, with a frequency of 38.46%. Technical errors included mislabeling, patients with the same medical number, patients with two or more different medical numbers, contaminated reagents, inaccurate results recording, and changing medical record numbers. Phlebotomy and administrative errors during patient registration involving the use of different insurance cards are the causes of ABO discrepancies.<sup>[15]</sup> According to data submitted to the FDA (Food and Drug Administration), from 1990 to 1991, one avoidable transfusion-related death occurred for every 600,000 transfusions due to sample misidentification.<sup>[16]</sup> Of the clerical errors in sample misidentification in our analysis, mislabeling had the highest rate (5.76%). ABO discrepancies resulting from incorrectly diagnosed specimens can affect anywhere between 1 in 517 and 1 in 3,400 blood tests.<sup>[17,18]</sup> The frequencies of additional reasons for incorrectly identifying sample errors were determined to be 1.92%, 3.84%, and 3.84%, respectively. Our research was similar to that of Bashawari et al., who discovered that the primary source of error in sample misidentification (14.6%) was mislabelling. Errors in sample collection account for 0.09% of the disparities in another global investigation with 62 hospitals.<sup>[19]</sup> Labeling the tubes incorrectly or combining together sample collection led to mislabelling. This mistake can be prevented by having two different phlebotomists separately collect and label the sample, or by employing a handheld electronic device to create pre-transfusion sample labels based on information from the patient's wrist band at the bedside.<sup>[20,21]</sup> Some hospitals have a protocol requiring a second specimen to recheck the ABO/Rh on all patients receiving type-specific red cells who have never had their blood bank history in order to prevent mistakes.<sup>[22]</sup> Sample-related issues led to differences in both forward type (cell grouping) and reverse type (serum/plasma grouping). According to the current investigation, the Bombay phenotype, weak or missing antibodies, poor antigen expression, rouleaux, cold autoantibodies, and cold alloantibodies were sample-related issues. Of the 32 instances of sample-related issues, 1.92% had poor antigen expression. Only in forward grouping was there a discrepancy due to the weak antigen expression. In our study, group disparity in reverse typing was found for weak or missing antibody, cold alloantibody, and Bombay phenotype, with respective frequencies of 15.62%, 9.37%, and 6.25%. In our investigation, we found that the most common source of sample-related issues, including discrepancies in both forward and reverse types, was cold autoantibodies (62.5%). Rouleaux, caused by anomalies in plasma or proteins, is the stacking of erythrocytes that adhere in a coin-like way,

giving the impression of agglutination.<sup>[23]</sup> The Rouleaux formation was discovered to have both forward and reverse grouping anomalies, with a reported 3.12% in this study. Using saline replacement techniques to generate a legitimate reverse grouping, the discrepancy in the cases of rouleaux formation was rectified by washing the red blood cells utilized in the forward grouping.

Another name for the discrepancy in red cell testing is forward grouping discrepancy. Either an excess of red cell antigen during agglutination or a weak or absent red cell antigen can cause this disparity. Malignancy accounted for 1.92% of the causes of weak or absent antigens in our analysis. After analyzing ABO disparities in 35 French hospitals, including 407,769 patients, Chiraoni et al. concluded that there was one instance of ABO discrepancies for every 3,400 individuals.<sup>[24]</sup>

The following factors can lead to disparities in serum tests (reverse type): infection, poor or absent serum reactivity, cold alloantibody, cold autoantibody, and high serum protein. Reverse grouping was inconsistent due to unexpected alloantibodies, primarily cold alloantibodies, in the patient's serum. A higher protein level also caused false agglutination, which hampered serum tests. The study observed that 1.7% of the discrepancies were caused by elevated serum protein.

Patients with reduced or absent ABO antibody production are classified as having weak or absent isoagglutinin. Serum tests can be inconsistent due to decreased antibody production in individuals with hypogammaglobulinemia and older people. Hypogammaglobulinemia was the cause of differences in 2.8% of instances, whereas the prevalence in elderly individuals was 5.76%.

Our study included elderly patients whose ages ranged from 60 to 80 years old, with a mean age of 68 years. This is similar to the findings of a study by Esmali et al.<sup>[25]</sup> that reported the mean age of elderly patients to be 93 years old, with patient ages ranging from 88 to 113 years old. The majority of weak or absent antibodies were found in 4 out of the elderly patients in group O, which is similar to the 28 cases reported by Esmali et al. Anti-A or anti-B titers are low in elderly individuals, particularly those over 65. They may also exhibit weak reactions or lack predicted antibodies. As a result, the forward grouping approach is advised for establishing the ABO blood group in older patients, as the results of back type or reverse grouping may not be trustworthy in these patients. The most effective strategy for resolving the age-related weak or missing antibody group discrepancy is to enhance the response using the reverse procedure, which involves letting the patient's serum sit at room temperature with the reagent cells for 15 to 30 minutes.

If the reagent cells exhibit pan-agglutination and have the matching antigens on their surface, high titer cold auto-agglutinins may also obstruct reverse grouping. Once more, the most frequent source of inconsistencies in serum testing was cold agglutinins, which occurred 38.46% of the time in our sample, higher than the frequency (3.6%) reported by Heo et al. The frequency of cold agglutinin was higher in our population because autoimmune illnesses are more common there.

ABO grouping is influenced by the patient's history and diagnosis since diseases can lead to differences in groupings. Rouleaux development is brought on by AIHA, SLE, anemia, tuberculosis, and pneumonia, and it obstructs both forward and reverse grouping. In the physiological state of pregnancy, we discovered differences in forward grouping. Two cases of hepatocellular and gallbladder carcinoma were reported by Bum et al.<sup>[26]</sup> In both cases, the red cell type was identified as the O group, and the reverse typing revealed anti-A exclusively. A patient with gallbladder carcinoma whose forward type was O group but whose reverse typing revealed B alone was a similar instance that we saw in our investigation. We discovered a case of acute leukemia in our investigation that displayed a discrepancy in red cell tests because of weak A antigen expression. A similar case, titled "49 Women with Blood Group A and Acute Myeloid Leukemia," was reported by Picker et al.<sup>[27]</sup> The women's A antigen was not detected, not even using the tube-spin method.

Blood grouping differences are the main reason for transfusion reactions. ABO incompatibility is the cause of 37% of all transfusion-associated deaths that have been recorded in the USA.<sup>[28]</sup> The required precautions should be taken to prevent the sources of group differences in order to reduce the transfusion reaction caused by ABO incompatibility. To boost the safety and effectiveness of blood transfusions, steps to enhance patient identification and modern technologies like barcodes and radiofrequency technology should be implemented.<sup>[29]</sup> A quality assessment or quality improvement team comprising the transfusion service, transfusion committee, and medical director should set up a

process to monitor, assess, and audit the transfusionist's adherence to institutional blood administration policies. If necessary, the team should also start corrective action, such as arranging for staff education for the medical and nursing departments.<sup>[30,31]</sup>

## CONCLUSION

The technical staff should receive the appropriate training and use cutting-edge technologies, including automation in grouping, to prevent mistakes. We occasionally come across weak or absent antigens. Although not observed in our investigation, the ABO subtype was one of the causes of weak or absent antigens. Advanced studies, like the analysis of saliva and the use of molecular technologies to determine an ABO subgroup, are crucial. It's critical to identify and address inconsistent outcomes. To avoid ABO incompatibility, a person must have their blood type labeled correctly. It is crucial to always pay attention to the intensity of the response because weaker responses typically raise questions. Each donor should receive personalized information on their blood group and a unique blood group card that explained both their donor and recipient status, helping to avoid transfusion reactions that can result from weak subgroups.

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