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Original Research Article

Resolving ABO discrepancies by serological workup- an analysis of few cases

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ABSTRACT

Background: ABO discrepancies occur whenever the results of red cell grouping and serum grouping are in disagreement. The reasons for discrepancies both clinical and technical have to be sorted out. Further analysis is essential to resolve such discrepancies. If discrepancies are encountered, the interpretation of the ABO grouping has to be delayed until the same has been resolved. The aim of the study was to resolve ABO discrepancies encountered, by serological work up.
Methods: All cases of discrepant samples received between August 2014 and May 2016 at the Department of Transfusion Medicine, The Tamilnadu Dr. MGR Medical University, Chennai, India were analyzed to determine the etiology by serological workup.
Results: A total of twenty-one samples were analyzed and resolved. Fifteen cases of Type IV discrepancy, two cases of Type II discrepancy, one case Type III discrepancy, one case Type I discrepancy and two cases of technical errors were identified.
Conclusions: ABO discrepancies can be resolved serologically if properly worked up. As ABO blood grouping is indispensible in blood transfusion service, it is imperative to resolve such discrepancies before transfusion.

Keywords: ABO blood group, ABO discrepancy, Serology

INTRODUCTION

The ABO system contains four major ABO phenotypes: A, B, O and AB. The four phenotypes are determined by the presence or absence of two antigens (A and B) on red cells. ABO system is also characterized by the presence or absence of naturally occurring antibodies termed iso-hemaagglutinins, directed against missing A and B antigens. It is believed that the immunizing source for such naturally occurring antibodies is gut and environmental bacteria which have been shown to possess ABO like structures on their lipopolysaccharide coats.[1] Donor blood samples are routinely grouped for ABO at the time of donation. Recipient blood samples are grouped for ABO before transfusion. ABO grouping requires both antigen typing of red cells for A and B antigen (red cell Grouping or forward grouping) and screening of serum for the presence of Anti- A or Anti-B isoagglutinins (serum grouping or reverse grouping).[2]

Both forward and reverse grouping are necessary for donors and patients because each grouping serves as a check on the other. A discrepancy exists when the results of red cell grouping do not agree with serum grouping. The discrepancy may arise because of technical errors or clinical conditions of the patients. All technical factors

that may have given rise to the ABO discrepancy should be reviewed and corrected. It is also essential to obtain information regarding the patient's age, diagnosis, transfusion history, medications and history of pregnancy.[2] If the discrepancy appears to be due to an error in specimen collection or identification, a new sample should be drawn from the patient and the forward and reverse grouping repeated.

In solving ABO group discrepancies, the following causes to be considered:[2]

- Improper identification of blood specimen at patient's bedside.
- Common sources of technical errors at blood bank

1. Clerical errors.
2. Mix up in the sample.
3. Missed observation of hemolysis
4. Failure to follow manufacturer's instruction.
5. Cell suspension either too heavy or too light.
6. Uncalibrated centrifuge.
7. Failure to add reagents.
8. Contaminated reagents.
9. Warming during centrifugation.
10. Fibrin clots.

- Due to problems in blood samples s/red cells) (Table 1)

Table 1: Categories of abo discrepancy.[2]

Type	Reasons	Conditions
Group I discrepancy	Weak reacting or missing antibodies.	-Chimerism (due to blood transfusion, transplanted bone marrow, exchange transfusion, feto maternal bleeding) -New born infants. -Elderly patients -Hypogammaglobulinemia (leukemia, immunodeficiency diseases)
Group II discrepancy	Weak reacting or missing antigens.	-Subgroups of A or B -Leukemia – excess amount of B, -Acquired B phenomenon (in gram negative septicemia, intestinal obstruction and cancer of colon or rectum).
Group III discrepancy	Protein/ plasma abnormality leading to rouleaux formation	-Elevated globulin level (in multiple myeloma, Waldenstrom' macroglobulinemia, plasma cell dyscrasias, Hodgkin lymphoma). -Plasma expanders like dextran, polyvinyl pyrrolidone –Wharton's jelly (in cord blood)
Group IV discrepancy	Miscellaneous problems	-Exposure of hidden erythrocyte T antigen (Polyagglutination) -Cold and warm autoantibody (AIHA) -Transfused foreign antigen. -Unexpected ABO iso-agglutinin and alloantibody. -Antibody other than anti-A & anti-B (E.g.: acriflavin antibody) -cis – AB individuals.

The aim of this study was to analyze serologically the discrepancies between forward and reverse ABO grouping, to determine the etiology of discrepancies and resolving it for accurate and reliable ABO grouping.

METHODS

Retrospective analysis of ABO discrepancies observed in the samples received between august 2014 and May 2016 at the Department of Transfusion Medicine, the Tamilnadu Dr MGR medical university, Chennai, India which is a specialist centre for transfusion medicine, where other centers experiencing difficulty in resolving ABO discrepancies, refer samples for work up and resolution. All discrepant samples received at our blood bank between August 2014 And May 2016 was included in study. EDTA and clotted Blood samples were received from every patient for forward and reverse grouping respectively. In the event of samples being hemolysed, fresh samples were obtained. All samples were analyzed as soon as possible, or stored at 1°C to 6°C to reduce deterioration of weak antibodies or false reaction due to contamination of the specimen.[1] Blood grouping was performed by conventional tube technique. Forward grouping was performed by using standard anti-A and anti-B reagents to demonstrate the presence or absence of the A and B antigens on the patients' red cells.

Reverse grouping was done by using A_1, B and O group pooled red blood cells to demonstrate the presence or absence of anti-A and anti-B antibodies in the patients' serum or plasma. Antibodies were screened by column agglutination technology (CAT) using commercially available three cell antigen panel (Asia -ID-DiaCell I, II, III Bio-Rad, California, United States) by coombs' gel card. Whenever antibody screening was positive, extended eleven cell panel was used for antibody identification using low ionic strength saline (LISS). In the case of discrepancy between forward and reverse blood grouping, the clinical details were analyzed to

ascertain the etiology, to classify the discrepancy and to resolve the same.

RESULTS

Discrepant blood samples from all twenty-one (n=21) patients received during the study period were analyzed and resolved (Table 2). Of these 21 samples, the discrepancy for one (n=1) sample was due to improper identification of the patient at bed side, 'wrong blood in tube' (WBIT) error. Another discrepancy in one (1) sample was due to inadvertent cell suspension. These were technical errors which were resolved by obtaining properly labeled fresh samples.

Table 2: Summary of serological workup of ABO discrepancies (n=21).

Case details	Forward grouping			Reverse Grouping			Remarks	Interpretation	Type of discrepancy
	Anti-A	Anti-B	Anti-AB	Ac	Bc	Oc			
20/Male regular blood donor. Sample received for confirmation of blood grouping	0	4+	4+	2+	0	0	• Forward Group: B • Reverse Group: weak agglutination with 'A' cells • After rechecking every step, the fault was found to be in 'A' cell suspension. As 'A1' packed red cell units were reserved by a catering hospital, the Lab technician who prepared 'A' cell suspension that morning had pooled samples from 'A' RBC units without checking their subtypes, which later turned out to be 'A2' cells. • When the reverse grouping was repeated with A1 cells, it was found to be compatible with forward grouping.	B Group	Technical Error
24/Male posted for exploratory laparotomy with request for packed red blood cells	0* 0#	4+* 0#	4+ 0	4+ 4+	0 4+	0 0	• *Given sample showed B Group. • However, while verifying the request, group of the patient mentioned was O Group. • Hence, ordered for another fresh sample to resolve the discordant grouping. • # Grouping on the fresh sample showed O Group • Concluded as wrong blood in tube	O Group	Clerical Error
66/Female. Sample received for confirmation of blood grouping	0	0	0	0*	0*	0	• *In reverse grouping agglutination occurred, with A1 and B cells, only when the amount of serum was doubled. This could be due to depressed antibody production, since the patient's age was 66 years.	O Group	Group I
37/Female. sample received for confirmation of blood grouping	4+	0	4+	1+	4+	0	• With anti – A1 lectin, no agglutination. • With anti-H lectin, 2+ agglutination. • Resolved as A2 subgroup with anti-A1 antibodies	A2 Group	Group II
32/Male. prospective	4+	0	4+	2+	4+	0	• With anti – A1 lectin, 2+ agglutination	Probable Aint Group	Group II

donor for liver transplant. Sample received for confirmation of blood group.							• With anti-H lectin, 3+ agglutination • Resolved as subgroup of A with anti-A1 antibodies		
65/Female diagnosed as a case of Multiple Myeloma. Sample received for confirmation of blood group	4+	0	4+	1+	4+	1+	• In view of the case being Multiple Myeloma 1+ agglutination in reverse grouping, when observed under microscope showed Rouleaux formation. • Rouleaux formation disappeared after replacing serum with saline (saline replacement technique)	A Group	Group III
35/Female for evaluation of anaemia	2+* 0#	4+* 4+#	4+* 4+#	4+* 4+#	4+* 0#	4+* 0#	• At Room Temperature, Auto Control: 4+. • # The discrepancy was resolved by repeating the grouping and typing at 37ºC. • Resolved that the discrepancy was due to Cold Agglutinins. • Cells in forward and reverse grouping were washed with warmed normal saline to 37ºC. In forward and reverse grouping respective sera were also brought to 37ºC.	B Group	Group IV
55/Male for evaluation of anemia and jaundice	2+* 0#	4+* 4+#	4+* 4+#	4+* 4+#	4+* 0#	2+* 0#	• At Room Temperature. Auto Control: 4+. • # The discrepancy was resolved by repeating the grouping and typing at 37ºC. • Resolved that the discrepancy was due to Cold Agglutinins. • Cells in forward and reverse grouping were washed with warmed normal saline to 37ºC. In forward and reverse grouping, respective sera were also brought to 37ºC.	B Group	Group IV
23/Female suffering from fever with anemia for evaluation	1+* 0#	1+* 0#	1+* 0#	4+* 4+#	4+* 4#	3+* 0+#	• At Room Temperature – Auto Control: 2+. • # The discrepancy was resolved by repeating the grouping and typing at 37ºC. • Resolved that the discrepancy was due to Cold Agglutinins. • Cells in forward and reverse grouping were washed with warmed normal saline to 37ºC • In forward and reverse grouping, respective sera were also brought to 37ºC.	O Group	Group IV

27/Female G3P1L1A1 35 weeks of gestation. Sample received for confirmation of blood group	4+	0	4+	1+	4+	2+	• Autocontrol: 0 • With anti-A1 lectin: 4+ • Antibody screening and identification panel cells showed pan reactivity suggestive of multiple warm reactive alloantibodies or antibody to high prevalent antigen. Red cell phenotyping with Rh, Kell, Kidd, Duffy, Lewis, Lutheran and MNS antisera suggested "D deletion (D-)" phenotype. • Resolved that the discordant reverse grouping result was suggestive of the presence of antibody to high prevalent Rh antigens.	A1 Group	Group IV
48/Female for evaluation of anemia	4+	0+	4+	0	4+	2+	• DAT Negative. IAT: Positive, Autocontrol: Negative. With A1 lectin: 4+ agglutination. • Antibody screening and identification showed Anti-C and Anti- D. • Resolved that the discordant reverse grouping result was due to the presence of anti – C and anti-D.	A1 Group	Group IV
26/Female G2P1A0L1 8 months of gestation. Sample received for confirmation of blood group	4+	0	4+	0	4+	1+	• DAT Negative. IAT: Positive, Autocontrol: Negative. With A1 lectin: 4+ agglutination. • Antibody screening and identification showed Anti-Leb. • Resolved that the discordant reverse grouping result was due to the presence of Anti-Le b.	A1 Group	Group IV
15/Female with Thalassemia major. Sample received for confirmation of blood group	0	4+	4+	4+	0	1+	• DAT Negative. IAT: Positive, Autocontrol: Negative. • Antibody screening and identification showed Anti-c and Anti-Jka. • Resolved that the discordant reverse grouping result was due to the presence of Anti-c and Anti-Jka.	B Group	Group IV
62/Male. Sample received for confirmation of blood group	0	0	0	0	3+	0	• No agglutination with anti-H lectin. • Serum blood grouping with O cells incubated at 4^0C showed 1+ agglutination suggestive of anti HI antibody • Lewis antigens status: Le (a-b-) • Secretor Status of saliva showed the presence of H and A antigens. • Resolved that the discordant reverse grouping result was due to Para-Bombay OhA	Para-Bombay OhA Phenotype	Group IV

							Phenotype.		
58/Male. Sample received for confirmation of blood group	0	0	0	4+	4+	4+	• No agglutination with anti-H lectin. • Lewis antigens status Le (a-b+ • Secretor status of saliva showed the presence of H antigen + • Resolved that the discordant reverse grouping result was due to Para-Bombay Oh 0 Phenotype.	Para-Bombay Oh0 Phenotype	Group IV
24/Female G3P1L1A1 for confirmation of blood group	0	0	0	4+	4+	1+	• DAT Negative. IAT: Positive, Autocontrol: Negative. • Antibody screening and identification showed Anti-D. • Resolved that the discordant reverse grouping result was due to the presence of Anti-D	O Group	Group IV
46/Male. Sample received for confirmation of blood group	0	0	0	4+	4+	4+	• No agglutination with anti-H lectin. • Lewis antigens status Le (a-b-) • Secretor status of saliva showed the absence of H antigen • Resolved that the discordant reverse grouping result was due to Bombay Oh Phenotype.	Bombay Oh phenotype	Group IV
31/Female. Sample received for confirmation of blood group	0	0	0	4+	4+	4+	• No agglutination with anti-H lectin. • Lewis antigens status Le (a+b-) • Secretor Status of saliva showed the absence of H antigen • Resolved that the discordant reverse grouping result was due to Bombay Oh Phenotype.	Bombay Oh phenotype	Group IV
48/Female posted for hysterectomy. Sample received for confirmation of blood group	0	0	0	4+	4+	2+	• DAT Negative. IAT: Positive, Autocontrol: Negative. • Antibody screening and identification showed Anti-M. • Resolved that the discordant reverse grouping result was due to the presence of Anti-M	O Group	Group IV
19/Female. Primigravida with placenta previa. H/O Acute hemolytic transfusion reaction	4+	4+	4+	1+	3+	3+	• DAT Positive. IAT: Positive. Autocontrol: Negative. • Antibody screening and identification showed Anti-Lea. • Resolved that the discordant reverse grouping result was due to the presence of Anti-Lea	A1B Group	Group IV
50/Female, a case of Non-functional left kidney posted for	0	0	0	4+	4+	1+	• DAT Positive. IAT: Positive. Autocontrol: Positive. • Antibody screening and identification with adsorbed	O Group	Group IV

nephrectomy. Sample received for confirmation of blood group	serum showed Anti-N. • Resolved that the discordant reverse grouping result was due to the presence of autoantibody with Anti-N alloantibody.

Of the remaining 19 discrepant samples, one (n=1) discrepancy was due to old age related weak or missing antibody (4%). This was an example of Group I discrepancy due to very low level of antibody production. Group II discrepancy was observed in two (n=2) of the samples and was due to subgroups of A (A2 and probable Aint) (9%). A Sample (n=1) from a multiple myeloma patient was found to be discrepant because of rouleaux formation and constituted Group III discrepancy (4%). Group IV discrepancy was observed in fifteen (15) of the samples (71%). Discrepancies in three (n=3) (14%) samples were due to cold agglutinins. Two (n=2) (9.5 %) samples were identified to be Bombay phenotype and another two (n=2) (9.5%) samples were of para-Bombay phenotype. The rest of seven (n=7) (33%)discrepant samples were due to the presence of alloantibodies viz., anti-Leb, anti-Lea, anti-D, anti-c and anti-Jka, anti-G (C+D), anti-N and anti-M. Case details are given in the Table 2.

DISCUSSION

Being a referral teaching centre in transfusion medicine, the centre had received the above 21 samples for ABO grouping discrepancies from the month of August 2014 to May 2016. There were two samples which showed discrepancies due to clerical errors such as 'wrong blood in tube' (WBIT) at the time of bed side sample collection and inadvertent cell suspension preparation at the blood bank. In a study by Chiaroni et al, wrong blood in tube was the most common technical reason for ABO discrepancies.[3] Moghaddam et al has also reiterated that clerical errors, technical errors are common even in regional transfusion centres and proper pre-transfusion testing is very important to limit errors.[4]

There was a sample which showed Group I ABO grouping discrepancy due to the deficient amount of antibody production in an elderly patient, which was resolved when the amount of serum was doubled. In the studies by HA Esmaili et al and Rahgozar et al, the most common cause for ABO discrepancies was due to low titer of antibodies in a reverse grouping.[5,6] Khan et al has also implied that increasing the incubation period and lowering the reaction temperature are known techniques to resolve ABO discrepancies.[7] Such discrepancies can be resolved by proper history taking and enhancement of the weak/missing reaction in the serum by extending incubation time at room temperature for approximately 15 to 30 minutes or by adding one or two drops of more plasma or serum. While, resolving such discrepancies, always include autocontrol and "O" cell control concurrently.

Group II ABO grouping discrepancy was found in two of the samples in our study. Both the samples were weak subgroups of A namely A_2 and probable Aint. In the studies by Rahgozar et al, Kim MH et al and Sharma et al, weaker expression of ABO antigens was the second most common cause for ABO discrepancies.[6,8,9] Finding out weaker sub groups can be tricky and time consuming but worth the effort as one of our present cases was a prospective donor for liver transplant. With the diminutive organ donation pool, surgeons are now looking out for ABO incompatible solid organ transplants and weaker subgroups have a reduced chance of acute rejection. Presence or absence of corresponding ABO isoagglutinins in reverse grouping, adsorption-elution studies with anti-A/anti-B, saliva studies to detect presence of A (or B) & H substances helps to differentiate weak A/ Weak B phenotypes serologically.

Group III ABO grouping discrepancy was found in one of the samples from a patient with multiple myeloma due to rouleaux formation. Esmaili et al had found 16% of discrepancies to be due to rouleaux formation.[5] Rouleaux formation can be easily resolved with tube technique as they can be examined microscopically and saline replacement technique is possible which will dissociate rouleaux. Thus tube technique can be even more advantageous compared to column agglutination technology in resolving rouleaux.

The remaining 15 samples showed Group IV ABO grouping discrepancies. Most common reason for Type IV grouping discrepancy in our study was due to presence of alloantibodies (n=9). There were three cases of cold agglutinins, which were resolved by repeating the blood grouping with pre-warmed samples. Two samples each of Bombay and para-bombay phenotypes were observed. Sharma et al observed that 10% of ABO discrepancies were due to the presence of alloantibodies while Heo et al in his study, found that cold antibodies were the common cause for ABO discrepancies.[9,10] Cold agglutinins are notorious as they give agglutination with all antisera at room temperatures and new technicians tend to label the sample as AB positive when they do not perform reverse grouping. Unless tests are repeated at 37ºC, resolution is difficult and hemolytic transfusion reactions are a definite risk when cold agglutinins are overlooked. India has its fair distribution of Bombay phenotype individuals who can be easily overlooked as O positive by the uninitiated technicians. Reverse grouping

with O pooled cells and counterchecking with Anti-H lectin can help to pick up these individuals who can then be brought into donor pool.

CONCLUSION

In blood transfusion service ABO blood grouping is the first and foremost investigation to be done. While doing so, if ABO discrepancies are found, they have to be resolved before proceeding further. If such discrepancies are diligently approached with the available resources, most of them can be resolved serologically even before resorting for high end investigations. However, it is essential to strictly adhere to the standard operating procedures, from sample collection to laboratory practices, to avoid delayed turnaround time for issuing blood components.

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REFERENCES

1. AABB Technical Manual. 17th edition. AABB publishers. Available at: http:// aabbimmigrationdna.com/ ?gclid=CjwKEAiAoOvEBRDD25uyu9Lg9ycSJAD0cnBySklGLDkMUt2KucrcpLoVtYcKfJ2spT5P_oVpq2QrbRoC_evw_wcB.
2. Harmening D. Modern Blood Banking and Transfusion Practice. Jaypee publishers. 2013;136-43.
3. Chiaroni J, Legrand D, Dettori I, Ferrera V. Analysis of ABO discrepancies in 35 French hospitals. Transfusion. 2004;44(6):860-4.
4. Moghaddam ES, Khosravi S, Dorgalaleh A. Discrepancies in ABO and Rh Grouping in Southeast Iran, an Analysis of 3 Years' Experience. J Blood Disord Transfus. 2016;7:364.
5. Esmaili HA, Taghipour H, Talghini S, Mohammadhoseyni B. An Analysis of Discrepancies between forward and reverse ABO blood grouping. Res J Pharma, Bio Chem Sci. 2014;5(3):1360-6.
6. Rahgozar S, Yavari FM, Hariri MM, Moafi AR. ABO discrepancy prevalence and qualitative study of relevant factors I blood donors of Isfahan Regional Blood Transfusion center. Scien J Iran Blood Trans Org. 2006;3(1):53-61.
7. Khan MN, Khan TA, Ahmed Z. Discrepancy in ABO Blood Grouping. J Coll Phys Surg Pak. 2013,23(8):590-2.
8. Kim MH, Choi MJ, Kim HO. Analysis of ABO discrepancy (82 cases). Korean J Clin Pathol. 1991;11(2):493-9.
9. Sharma T, Garg N, Singh B. ABO blood group discrepancies among blood donors in Regional Blood Transfusion Centre GTB hospital, Delhi, India. Transfusion and Apheresis Science. 2014;50(1):75-80.
10. Heo MS, Cho D, Park HR. Analysis of ABO discrepancies and Transfusion experiences in Chonnam National University Hospital. Korean J Blood Transfus. 2013;24(3):222-32.

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