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Discrepancy in ABO Blood Grouping: A Report of B Subgroup with Cold Alloantibody

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Abstract

The ABO antigens are the most important in both blood transfusion and transplantation as their antibody can cause severe haemolytic transfusion reaction when ABO-incompatible transfusion occurs. It has been claimed that variants of the ABO blood group are very rare, including B subgroups. We reported a young healthy female with B subgroup and underlying cold-reacting alloantibody causing challenges in determining of her ABO blood group. Initial ABO blood grouping was misinterpreted as group O. Presence of alloantibody in her plasma was suspected after revealing positive antibody screening. Repeated blood grouping at 37°C revealed a discrepancy of the forward and reverse grouping, which suggested B subgroup. Cross-matching showed difficulties in finding compatible blood with both group O and B cells due to the presence of clinically significant cold alloantibody. Determining of correct ABO blood group is mandatory in transfusion practices as haemolytic transfusion reaction can be prevented.

Keywords:
ABO Blood Group, Blood Group Discrepancy, Cold Alloantibody, Subgroup of B

Introduction:
The ABO antigens and antibodies are the most significant blood group system in human blood transfusion and transplantation practices. ABO blood grouping is performed on all donors and transfusion recipients, and it is by far the most common test in transfusion practices. It has been shown that not all A or B antigens are identical by classical serology and these observations led to the division into different ABO subgroups or variant phenotypes[1]. ABO subgroups are rare and usually wrongly typed as O since the RBC lacks A or B antigen and the presence of both anti-A and anti-B in the plasma. It has been claimed that variants of

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blood group B are very rare and proposed that the B subgroups should be used as follows: B_3, B_x and B_{el} or B_3, B_x, B_{el} and B_m[2,3].

In transfusion practice, both forward (red cells typing) and reverse (plasma typing) ABO blood grouping are required and important because each grouping serves as a check on the other. ABO discrepancy is defined when the results or reactions of forward (red cells typing) do not agree or are unexpected with reverse grouping (plasma typing) reaction[4]. This leads to uncertainty in determining of ABO blood group and puts the patient's transfusion safety at risk. ABO discrepancies must be further investigated thoroughly and resolved promptly to ensure safe transfusion practice. ABO blood group discrepancies can be due to 1. technical problems, 2. weak or missing antigen (e.g., ABO subgroups, Bombay, para-Bombay), 2. extra or unexpected antigen (e.g., acquired B phenotype, B (A) phenotype, stem cell transplantation, out-of-group transfusion), 3. weak or missing antibody (e.g. neonate, elderly, hypogammaglobinaemia), and 4. extra antibody (e.g. alloantibody, autoantibody, rouleaux)[5].

Resolving ABO discrepancies will face more challenges if patients have multiple problems simultaneously, which interfere with the ABO grouping, as shown in this reported case. This case report highlight the rarity of B subgroup and the presence of a wide thermal range of cold alloantibody in the plasma, which leads to the wrong initial ABO grouping. Determining of correct ABO blood group is mandatory in transfusion practices as haemolytic transfusion reaction (HTR) can be prevented.

Case Report

An 18-year-old healthy Malay female was admitted to the orthopaedic ward after being involved in a motor vehicle accident. She sustained an open fracture of the right femur and a closed fracture of the proximal phalanx of her fourth right toe. Wound debridement was planned, with fixation of the femur fracture. Intravenous antibiotics (cefuroxime) were administered prophylactically in case of infection. There was no history of transfusion before. Her pre-operative haemoglobin (12.3 g/dL, reference range: 11.6-15.1 g/dL) and platelet ($317x10^9$/L, reference range: $150-400x10^9$/L) level were normal.

Group, screen and hold (GSH) was requested for pre-operative preparation. ABO and Rhesus D (RhD) blood group was identified before surgery using the gel card method (Diamed-ID Gel micro typing system, Switzerland). Initial blood grouping at room temperature (RT) showed no agglutination with anti-A and anti-B (forward grouping), while the serum showed potent anti-A and a mixed-field (MF) reaction with B cells (reverse grouping). The group was therefore initially interpreted as Group O. However, after revealing that antibody screening was positive (Diamed-ID Gel micro typing system, Switzerland), ABO blood grouping was then repeated at 37°C as suspecting that cold-reacting antibody might interfere the initial blood grouping result. Repeated ABO grouping showed a discrepancy between forward and reverse grouping. There was no agglutination with anti-A and anti-B (group O reaction), and the serum only showed potent anti-A (group B reaction). The initial MF reaction with B cell had disappeared. Extended blood grouping was performed, and the result is summarized in Table 1. This reaction is suggestive of a variant of group B, probably the B subgroup with the presence of cold-reacting alloantibody.

Antibody identification showed no reaction at anti-human globulin (AHG) phase but pan agglutination with papain-treated cell (1+ to 2+) and the reactions were enhanced at 4°C (3+ to 4+) with negative auto control (AC) (Diamed-ID Gel micro typing system, Switzerland). Cross-matching revealed difficulties in finding compatible blood with both group O and B packed red cell (PC). However, we managed to prepare compatible group O PC (only 4 out of 12 cross matching) for her but she did not require any blood transfusion since the surgery was uneventful with minimal blood loss.

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Table 1: ABO blood grouping workup in this patient

Technique	Forward grouping					Reverse grouping			AC	Blood group
	Anti-A	Anti-B	Anti-D	Anti-AB	Anti-H lectin	A cell	B cell	O cell		
Initial grouping (RT)	0	0	4+	ND	ND	4+	MF	ND	0	type as O, RhD positive
Repeat grouping (37 °C)	0	0	ND	ND	ND	4+	0	ND	0	suspected B subgroup
Extended grouping (RT)	0	0	ND	0	4+	4+	MF	2+	0	suspected B subgroup with additional cold type alloantibody

RT=room temperature; 0=no agglutination; +=positive agglutination; MF=mix-field; ND=not done; RhD=Rhesus D; AC=auto control

B subgroup was then confirmed after the adsorption-elution test and molecular testing. Adsorption of patient's red cell with anti-B and anti-A, and an elute was prepared from the adsorbed patient's cells which agglutinated with B cells but did not agglutinate with A or O cells (Table 2). Molecular testing (BAGene ABO-type, PCR-SSP technology, Germany) confirmed B blood group (Figure 1), however the B subtype was unable to specified due to limited resources.

Table 2: Other immuno-haematological workup in this patient

Test	Reaction			Interpretation
Antibody screening	1+ in all 3 cell panels			
Antibody identification	0 in all 11 cell panels (LISS)			presence of cold-reacting alloantibody with no specificity
	1+ to 2+ in all 11 cell panels (papain)			
	3+ to 4+ in all 11 cell panels (4 °C)			
Auto control	0			
RBC phenotype	R1R1 (CDe/CDe), Lu^{a-b+}			
Adsorption-elution test result	O cells	A cells	B cells	
RT (immediate spin)	0	0	0	
RT (30 minutes incubation)	0	0	0	
4 °C	0	0	1+	Presence of B antigen

LISS=low ionic strength saline; RBC=red blood cell; RT=room tempearture

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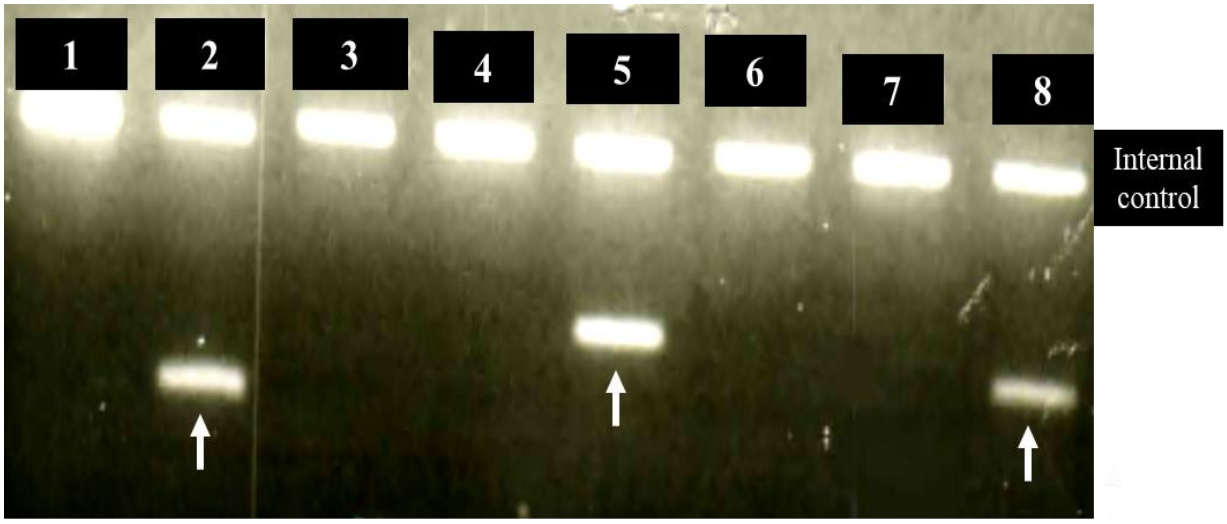

Figure 1: The polymerase chain reaction sequence-specific primer (PCR-SSP) was performed using SSP at base pair (bp) of 1. 134bp (O^1), 2. 133bp (non O^1), 3. 194bp (O^2), 4. 193bp (non O^2), 5. 195bp (B^1), 6. 194bp (non B^1), 7. 172bp (A^2), and 8. 173bp (non A^2). Electrophoresis through gel-based methods showed positive band (white arrow) at 133bp (non O^1), 195bp (B^1), and 173bp (non A^2), which confirmed the presence of homozygous for *B* allele, specifically *ABO*B101/B101*, indicated of B group. However, using this method with the available primers, the other variant *B* alleles can be hidden behind the PCR-result of *B101,* hindering the B subgroup from being confirmed.

The saliva secretor study for the patient and ABO grouping for the family members were planned. The mother's blood group was O RhD positive. However, saliva secretor study and the father's and sibling's blood grouping could not be explored since they did not turn up for the investigations.

Discussion:

This reported case shows the ABO discrepancy is due to combination of weak antigen (B subgroups) and the presence of unexpected extra antibody (cold-reacting antibody) in the patient's plasma which is highly uncommon and causing patient mistyped as group O. Presence of clinically significant cold alloantibody make more difficulties in searching compatible blood to patient.

Subgroups of B are very rare and much less frequent than A subgroups[2,6]. It has been suggested that ABO subgroup typing is usually carried out based on various IH tests include: 1. degree of red cell agglutination with antiseras, 2. presence or absence of agglutinins in serum, 3. presence of A or B and H subtances in saliva of secretors, 4. adsorption-elution studies and 5. family studies[7]. Subgroups of B (B_3, B_m, B_x and B_{el}) are usually recognized by variations in reaction strength using anti-B and anti-AB monoclonal antisera, the presence anti-A1 and-A2 isoagglutinins in the plasma, the presence of B and/or H antigen in saliva secretor, and the adsorption-elution studies with polyclonal anti-B antisera or group O and A sera[2,7-9].

It is concluded from the present investigations that the patient was a B subgroup, which might have B_{el} phenotype since forward grouping showed no reaction with anti-B and anti-AB. Usually subgroup B_3, B_x, and B_m have weak or MF reaction respectively with anti-B and anti-AB[2,9]. B subgroup secretors expected to have B and H substance in their saliva except for B_x and B_{el}, where they have only H substances in their saliva[9]. However, patient did not turn up for saliva-secretor test for further evaluation. O Bombay and B para-Bombay are excluded since patient's red cell showed reaction to anti-H indicating presence of H

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antigen as shown in her extended blood grouping. Figure 2 shows the suggested recommended algorithm of procedure for resolving ABO discrepancies with an emphasis on the ABO subgroup which required step-by-step procedure to resolve the discrepancies results.

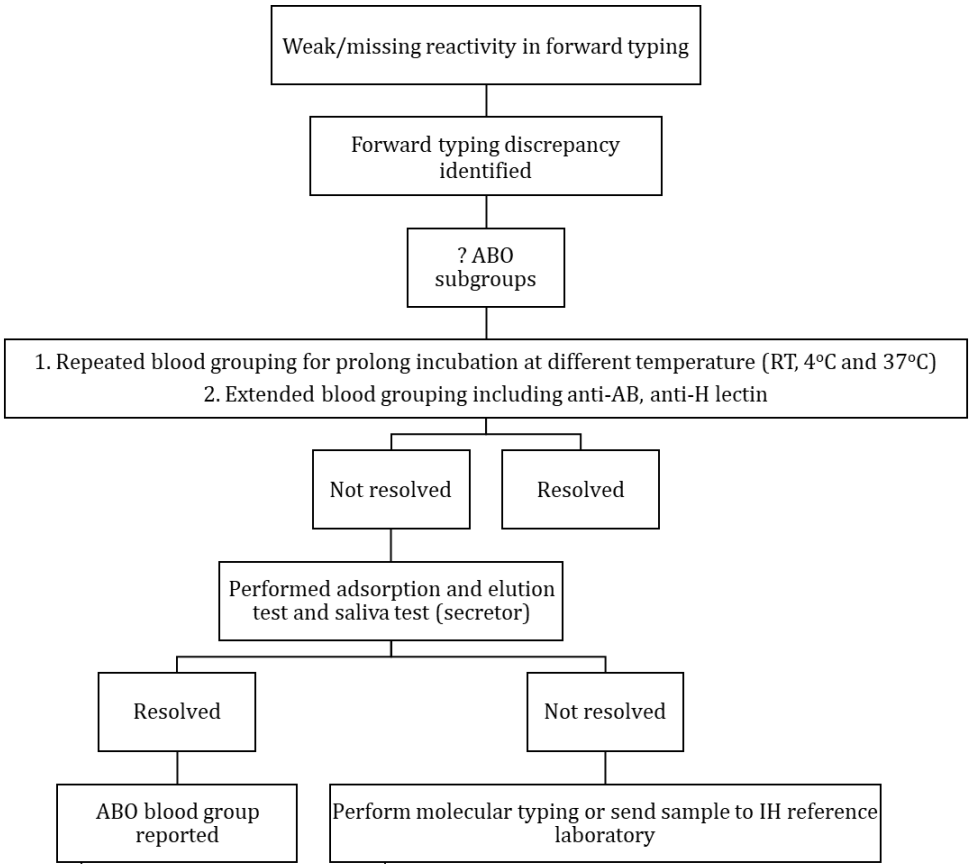

Figure 2: Algorithm for resolving forward ABO typing discrepancies due to weak/missing reactivity suspecting ABO subgroups

Molecular analysis including polymerase chain reaction (PCR) using sequence-specific primer (PCR-SSP) or restriction fragment length polymorphism (PCR-RFLP) and DNA sequencing or PCR- are a few available methods used for confirmation of ABO subgroups genotype[8]. The patient was confirmed to have B blood group by using PCR-SSP. Unfortunately, the other variant *B* alleles cannot be detected using the available specific primers, as they are hidden behind the PCR result of *B101*, necessitating additional molecular testing such as full ABO sequencing to identify any changes in the *B* allele for confirmation of B subgroup. Family member's blood group is also important for exact typing of the subgroups. However, we could not perform subsequent molecular analysis due to limited resources and patient's family members except her mother, did not turn for follow up due to logistic problem.

B subgroup individual can be mistyped as group O individuals because the B antigen on the red cell surface is very weakly present and probably can occur in these three conditions where; 1. only forward grouping is done since the results is not counter checked by doing reverse grouping[6], 2. presence of anti-B in their serum (especially B_x and B_{el}) [8], and 3. presence of cold-reacting antibody in their plasma as shown in this reported case, which is actually cold-reacting alloantibody. The initial MF reaction with B cell (reverse

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grouping) at RT and subsequent disappearance when repeated at 37°C (**Table 1**), is explained the interference by this cold-reacting alloantibody.

This alloantibody reacted best at cold temperature (4°C), but also at AHG phase (incompatible cross matching) indicating the possibility of wide thermal range activity. It is considered as clinically significant and have potential to cause HTR. Makroo et.al had reported cold-reacting alloantibody causing ABO discrepancies in both of patients (4.3%) and blood donors (7.0%), with specificity toward anti-M, anti-N and anti-Le[10]. Antibodies against MN and Lewis antigens reported to react at higher thermal range and is considered as clinically significant which it can induce in vivo haemolysis if transfused PC express the corresponding antigen. Thus corresponding antigen negative PC must be provided[11]. However, this cold–reacting alloantibody in our patient did not shown specificity toward any red cell antigen neither M, N, P nor Lewis and is considered naturally occurring since patient does not have any history of immunization event before. Thus, neither patient's cells nor those of a compatible donor's cells were tested for phenotyping for those antigens. Anti-IH alloantibody is unlikely because the patient's cells express a high level of H antigen. The presence of concurrent anti-B in her plasma could not be excluded with the current testing facilities. Anti-B if present usually clinically is not significant. Autoantibody was excluded since AC was negative.

For the purposes of transfusion, this type of patient with B subgroup should be transfused with blood group O PC rather than group B, as well as group compatible plasma and platelet components. In addition to underlying clinically significant cold-reacting-alloantibody with no specificity is concerned, fully compatible O PC at AHG phase should be provided to prevent the occurrence of HTR. On the contrary, if a blood donor belongs to the B subgroup which usually mistype as O, the unit should not be issued because it may cause hemolytic transfusion reaction if transfused to group O recipients.

Conclusions

As conclusion, in case of any discrepancy between forward and reverse ABO typing, no ABO type should be concluded until it is resolved by extended immunohematology investigations. Identification of ABO subgroup is important because they may be mistyped as group O individual. Use of compatible group O PC is recommended and safe in case of emergency transfusion as long as the discrepancy is not resolved yet.

Conflict of interest

The authors disclose no potential conflicts of interest

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