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CASE REPORT

Phantom Antibodies: A Clinical Dilemma

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Abstract

Background: Detection of clinically insignificant antibodies unrelated to blood group antigens, such as antibodies against preservatives of reagent red cell constituents during routine red cell antibody screening, can cause serological interference, reagent expenditure, and wasted human resources. Interpreting these antibodies often requires extensive immunohematological workup to exclude clinically significant antibodies. **Objectives:** This study aimed to analyse the interference of clinically insignificant antibodies against preservatives in reagent red cells at our Immunohematological Regional Reference Centre. **Materials and Methods:** A retrospective study was done in which we reviewed our Immunohematology laboratory records for cases involving anti-preservative antibodies against reagent red cells. Data for a period of 12 months (from January 2024 to December 2024) were reviewed. **Results:** During this period, we received a total of 113 cases. Among these, 3 cases with anti-preservative antibodies against reagent red cells were identified. Among the 3 cases, one case was referred to from an outside hospital in view of a grouping discrepancy, and the other two had positive antibody screening. These antibodies are clinically insignificant and cause hindrance in the regular immunohematological workup. **Conclusion:** The detection of clinically insignificant antibodies against preservatives in red cell constituents can not only impede the efficient conduct of routine immunohematological investigations but also lead to unnecessary consumption of resources and, consequently, delay critical patient care, and it can be overcome by using in-house pooled cells.

Keywords: Antibody Identification, Antibody Screening, Anti-Preservative Antibodies, Clinically Insignificant, Reagent Red Cells

1. Introduction

Antibody screening in antenatal mothers and before transfusions is performed to identify alloimmunization against blood group antigens. A reactive antibody screen necessitates antibody identification to determine specificity. Subsequent testing will depend on the specific antibody. However, antibodies unrelated to blood group antigens are sometimes encountered during routine pre-transfusion testing, lacking well-defined serological characteristics[3]. Resolving such discrepancies can lead to unnecessary additional immunohematological investigations, increased reagent costs, and inefficient use of human resources. If a patient has an antibody that reacts with a chemical present in commercial RBC suspensions, antisera, or potentiators (low ionic strength solution), it can lead to serious errors, incompatible crossmatches, delays in blood transfusion, and, in the case of antenatal women, potential Rh- negative mothers may miss crucial RhoGAM prophylaxis. We present three such cases where the patient's plasma/sera reacted solely with components of the reagent red cell suspension medium.

2. Aim and Objectives

To analyse the interference of clinically insignificant antibodies against preservatives in reagent red cells

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at our Immunohematological Regional Reference Centre.

3. Review of Literature

Kandasamy *et al.* stated that the Commercial reagent red cells used in antibody screening and identification are usually stored in buffered preservative suspension medium to maintain their functionality and cellular integrity. Therefore, antibodies to chemicals will react with reagent red cells only in the presence of chemical[2]. Garatty *et al.* have reported antibodies against chemicals in suspension medium, including the sodium azide preservative. These chemicals are not covalently bound to reagent red cells and can be easily removed by washing the cells[3]. Pham *et al.* have reported instances where antibodies against co-trimoxazole in commercial LISS mimic an antibody against a high-prevalence antigen. Antibodies to these antibiotics, if present in a healthy individual, may react with reagent red cells but not with donor red cells[3,6]. Antibiotics are included in the commercial reagents to inhibit bacterial growth. The presence of antibodies to these drugs in a patient's serum, even in healthy individuals, is not an uncommon phenomenon[4].

Umlas *et al.* stated that when a patient's serum contains a temperature-independent antibody reacting with all antibody screening and antibody identification panel cells but with few or no units of blood used for crossmatching, one should suspect an antibody against a constituent of the suspension medium of the reagent blood cells[5]. Sachan *et al.* reported that the drug-induced antibodies may recognise different RBC components and differ in blood group specificity; therefore, it is important to know the formula of the media used in reagents and laboratories to interpret the data correctly. These anti-preservative antibodies should be assessed carefully, and despite the absence of clinical events, the avoidance of the drug should be recommended[6]. Mehra *et al.* determined that the antibody found in their study was not targeting the column matrix or enhancement media of gel cards but was instead directed against chemicals in the suspension medium of the commercial cell panel. These nonspecific "nuisance" antibodies can cause significant confusion and delays in patient care, particularly in resource-limited settings. Recognising the potential for these antibodies is crucial for prompt detection and management, ensuring timely patient care[7].

Subramaniyan *et al.* strongly emphasised the inclusion of CTT in suspected cases of Antibiotic-Dependent Antibody, enabling timely blood provision to these patients[8]. Anuragaa *et al.* reported that reagent-dependent reactivity can be recognised and resolved early during the investigation of ABO discrepancies, positive Red Blood Cell (RBC) antibody screens, antibody identification panels, and crossmatch reactivity. In their case, both a three-cell panel and an 11-cell panel were pan-reactive. A probable cause for this reactivity was an antibody against cell preservatives used to store RBCs[9].

4. Materials and Methods

A retrospective study was conducted to review Immunohematology laboratory records for cases involving anti-preservative antibodies against reagent red blood cells. Data from 12 months (January 2024 to December 2024) were analysed. ABO cell grouping was performed using anti-A, anti-B, and anti-D reagents (Tulip Diagnostics, India). ABO serum grouping was performed using In-house-pooled (3 donors) red cells of the A, B, and O group were used by the Conventional Tube Technique (CTT) and Column Agglutination Technology (CAT) (DIAHEM, Diagnostic products, Switzerland).

A Direct Antiglobulin Test (DAT) was performed on the patient's red blood cells using polyspecific antiglobulin reagents (anti-IgG and C3d). Autocontrol (AC), Indirect Antiglobulin Test (IAT), and crossmatch were performed by both CTT and CAT methods. Antibody detection and identification were carried out using the Column Agglutination Technique (CAT) with polyspecific Coombs gel cards (Bio-Rad, Cressier, Switzerland), commercially available 3-cell panels (Biorad Diacell), 11-cell panels (Biorad Diapanel) and Surgiscreen cells using the glass bead method (Ortho Clinical Diagnostics).

5. Results and Observations

Over 12 months (January 2024 to December 2024), we encountered three cases of anti- preservative antibodies against reagent red blood cells. One case was a referral from an outside hospital due to a blood grouping discrepancy, and the other two involved a positive antibody screen.

- Case 1: 29-year-old female with primary infertility and hypothyroidism. This case was referred to by an outside hospital due to a blood grouping discrepancy that arose during reverse grouping using commercial reagent red blood cells (Biotestcell from Bio-Rad) with a positive antibody screen. The patient had no history of blood transfusion or pregnancy.
- Case 2: 42-year-old female G2A1, a known case of hypothyroid with no history of previous blood transfusion, referred from outside hospital for antibody screening.
- Case 3: 27-year-old female Primigravida referred from outside hospital in view of positive antibody screening. The patient had no history of blood transfusion.

Clinical profile of the cases (Table 1). In all cases, there were no blood grouping discrepancies as shown in Table 2, and the Direct Antiglobulin Test (DAT), autocontrol (AC) and Indirect Antiglobulin Test (IAT) were negative. However, antibody screening and identification tests were panreactive, suggesting the possibility of antibodies against high- frequency antigens or reagent red cell constituents. However, a negative IAT with O-pooled cells ruled out the possibility of high-frequency antigens as in Table 3.

Crossmatching was performed for all the cases using four group-specific packed red blood cells with a Low-Ionic-Strength Solution (LISS)/Coombs gel card and Conventional Tube Technique (CTT). All units were found compatible with the patient's serum as in Table 4. As a pan reaction was observed only

Table 1. Clinical profile of the cases

Case No	Age(Years)/ Gender	Blood group/Rh type	Diagnosis	History of previous blood transfusion	Referral reason
1	29/F	O Rh D Positive	Primary Infertility and Hypothyroidism	No	Blood grouping discrepancy
2	42/F	O Rh D Positive	G_2A_1 and hypothyroidism	No	Positive antibody screening
3	27/F	A_1B Rh D Positive	Primigravida	No	Positive antibody screening

Table 2. Serological profile of the cases

Case No	Blood grouping (CTT and CAT)					
	Forward grouping			Reverse grouping (In-house pooled cells)		
	Anti-A	Anti-B	Anti-D	A1 cells	B cells	O cells
1	0	0	4+	4+	4+	0
2	0	4+	0	4+	0	0
3	4+	0	0	0	4+	0

Table 3. Antibody screening of the cases

Case No	DAT		AC		IAT (3 O-Pooled cells)		Antibody screening 3-cell panel (Biorad Diacell)	Antibody Identification 11-cell panel (Biorad Diapanel)
	CTT	CAT	CTT	CAT	CTT	CAT		
1	0	0	0	0	0	0	PANREACTIVE(2+)	PANREACTIVE(2+)
2	0	0	0	0	0	0	PANREACTIVE(2+)	PANREACTIVE(2+)
3	0	0	0	0	0	0	PANREACTIVE(2+)	PANREACTIVE(2+)

Table 4. Antibody screening with various reagent red cells, IAT and Crossmatch

Manufacturer		In-house O-pooled cells	Cross-match with group-specific units	Bio-Rad Laboratories ID-Diacell-I-II-III	Ortho Clinical Diagnostics Surgiscreen cells	Washed ID-Diacell-I-II-III
Preservatives		-	-	Trimethoprim, Sulfamethoxazole	Neomycin, Gentamycin, Chloramphenicol	-
Antibody Screen result	Case 1	Negative	Negative	Panreactive in AHG phase	Negative	Negative
	Case 2	Negative	Negative	Panreactive in AHG phase	Negative	Negative
	Case 3	Negative	Negative	Panreactive in AHG phase	Negative	Negative

with Bio-Rad (Diacell) reagent red cells, we repeated the antibody screening test with panel cells from a different manufacturer (Surgiscreen cells, Ortho Clinical Diagnostics), followed by testing with washed Diacell panel cells. Both tests showed a negative reaction, confirming the antibody specificity against the suspension medium of diacell panel cells as in the Table 4.

6. Discussion

Detection of nonspecific antibodies in routine antibody screening is uncommon. Their frequency and immunological characterisation are not well-defined. Literature reports that nonspecific antibodies unrelated to blood group antigens can interfere with pretransfusion testing, from blood grouping to antibody screening and crossmatching[3]. In our study, the patient had no blood grouping discrepancies. The DAT, AC, and IAT were negative, ruling out hemolysis and antibodies against enhancement media. However, panreactive antibody screening with Bio-Rad reagent cells suggested high-frequency antigens or antibodies against reagent red cell constituents.

The IAT with O-Pooled cells and cross-matching with group-specific PRBCs ruled out high-frequency antigens. Antibodies against preservatives in reagent red cell constituents were suspected. Using cells from different manufacturers and washing the Bio-Rad panel cells three times with phosphate-buffered saline both yielded negative antibody screening results, confirming this suspicion. Similar to our study, Kandasamy *et al.* also reported panreactive antibody screening with a negative IAT and positive DAT. Their patient, like ours, had no hemolysis but a drop in haemoglobin due

to surgery. Both studies found that washing the panel cells and using different manufacturers' cells eliminated panreactivity, indicating antibody specificity against the Diacell panel cell suspension medium[2]. Sachan *et al.* suggested that antibodies against preservative drugs can only be detected when patient serum is added to RBCs in the presence of antibiotics, as found in commercial RBC suspension media. These antibodies should be considered when there are conflicting antibody screening results, panreactive patterns, and a lack of matching clinical and biochemical data. These in vitro reactions are similar to those seen in drug-induced immune haemolytic anaemia, which follows an immune complex mechanism[6]. Mehra *et al.* described a similar case where compatible crossmatches and negative results with in-house donor pooled cells and commercial panels from different manufacturers suggested nonspecific, clinically insignificant antibodies targeting the DiaCell reagent red cell suspension medium. However, the specific component of the suspension medium responsible for this reaction remained unidentified[7].

7. Summary and Conclusion

Type and screen tests are crucial for safe blood transfusions and preventing Hemolytic Disease of the Fetus and Newborn (HDFN). However, nonspecific antibodies in a patient's blood can react with the red cell suspension medium, causing false positive results, depleting both the material and human resources and delaying critical care. Using in-house prepared red blood cells for testing can help identify these non-clinically significant antibodies and ensure timely transfusion or Rh prophylaxis. In view of the potential for preservative-related antibodies to interfere with serological testing, it is imperative that red cell manufacturing companies carefully evaluate and standardise the preservatives used in their products to minimise immunogenic response and ensure accuracy in antibody detection.

8. References

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