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Case Report

Transfusion Medicine Section

Resolving an Unexpected Blood Grouping Discrepancy and a False-positive Compatibility Test in Pretransfusion Workup: A Case Report

VIDHI JAIN[1], DEVANSHI GOSAI[2]

ABSTRACT

Column Agglutination Technology (CAT), commonly known as the gel card method, is widely used in transfusion medicine for its sensitivity, standardisation, and reproducibility, but its heightened sensitivity can occasionally generate false-positive cross match results that present a diagnostic challenge in urgent settings. This report describes a 76-year-old man with symptomatic severe anaemia (haemoglobin 5.2 g/dL), cholelithiasis, and Acute Kidney Injury on Chronic Kidney Disease (AKI on CKD), who had been transfused approximately one month earlier and was referred for pretransfusion workup. Forward grouping identified the blood group as O, Rh-D positive, while reverse grouping by CAT initially showed mixed-field agglutination; a strongly positive anti-H lectin reaction excluded the Bombay (Oh) phenotype. CAT cross matching demonstrated persistent 4+ incompatibility against two separate O Rh-D positive units across two independent samples. All recognised causes of grouping discrepancy and cross match incompatibility were systematically evaluated, and the Direct Antiglobulin Test (DAT) was negative. Conventional Tube Technique (CTT) resolved the apparent grouping discrepancy, confirmed blood group O Rh-D positive without ABO discrepancy, and demonstrated full serological compatibility, thereby establishing a gel-specific false-positive result. One unit of O Rh-D positive Packed Red Blood Cells (PRBC) was transfused uneventfully; the patient subsequently died of his underlying illness during the same admission. This case underscores the importance of recognising false-positive CAT cross match results and the continued value of conventional tube testing as a confirmatory method, and highlights the need for clear standard operating procedures to prevent unnecessary withholding of serologically compatible blood from patients who urgently require transfusion.

Keywords: Agglutination tests, Anaemia, Coombs test, Erythrocyte transfusion, Immunoglobulin G, Iso-antibodies

CASE REPORT

A 76-year-old man presented with altered sensorium of 15 days' duration and was referred to the blood centre for blood grouping and cross matching in view of symptomatic severe anaemia, diagnosed on complete blood count (haemoglobin 5.2 g/dL), requiring transfusion. He had known cholelithiasis and AKI on CKD of 15-20 days' duration, and had received three units of PRBC approximately one month earlier. Blood group of previously transfused units was O positive; no adverse transfusion reaction had been documented on that occasion. The altered sensorium was attributed to uraemic encephalopathy in the setting of AKI on CKD, and the anaemia was considered secondary to anaemia of chronic kidney disease with associated iron deficiency, consistent with the microcytic hypochromic picture.

Laboratory evaluation revealed severe anaemia with a haemoglobin of 5.2 g/dL (reference range 13.0-17.0 g/dL) and a haematocrit of 17.6% (reference range 40-50%). The peripheral smear showed a microcytic hypochromic red cell morphology. Markers of haemolysis were as follows: serum lactate dehydrogenase 525 U/L (reference range 140-280 U/L), indirect bilirubin 0.10 mg/dL (reference range 0.1-1.0 mg/dL), haptoglobin 30 mg/dL (reference range 30-200 mg/dL), and reticulocyte count 4% (reference range 0.5-2.5%). When corrected for the degree of anaemia, the reticulocyte count corresponded to a corrected value of approximately 1.6% and a reticulocyte production index below 1, indicating a hypoproliferative marrow response rather than the compensatory erythropoiesis expected in haemolysis. Together with a normal indirect bilirubin and a negative DAT, these findings argued against active haemolysis and favoured a hypoproliferative anaemia of chronic kidney disease with associated iron deficiency; the isolated elevation of serum LDH was regarded as nonspecific.

On initial testing by CAT, forward grouping identified the blood group as O, Rh-D positive, while reverse grouping showed mixed-field agglutination. The mixed-field appearance with pooled O cells raised the possibility of the Bombay (Oh) phenotype. Because group O red cells express the greatest quantity of H antigen, a strong 3–4+ reaction with anti-H lectin (Ulex europaeus) is expected in group O individuals, whereas the Bombay phenotype shows no reactivity with anti-H. In this patient, a strongly positive (3+) anti-H lectin reaction confirmed abundant H antigen and excluded the Oh phenotype. Anti-A1 lectin showed no reaction.

The Indirect Antiglobulin Test (IAT) was 3+ positive and the DAT was negative. Antibody screening was performed using a three-cell reagent panel and showed pan-reactivity by CAT; an antibody identification panel was performed and did not reveal a specific alloantibody; and the auto control was negative.

Blood grouping was then repeated by CTT [Table/Fig-1,2].

Anti-A	Anti-B	Anti-AB	Anti-D1	Anti-D2	Auto control
0	0	0	4+	4+	0

[Table/Fig-1]: Forward grouping - conventional tube method.

A1 Cells	B Cells	O Cells
4+	4+	0

[Table/Fig-2]: Reverse grouping - conventional tube method.

The CTT confirmed blood group O, Rh-D positive with no ABO discrepancy. The mixed-field appearance seen on reverse grouping by CAT was not reproduced by the tube method, indicating that the apparent discrepancy was a gel-technique artifact that resolved on conventional testing.

Cross matching was performed by the antiglobulin (indirect antiglobulin) phase using a Low Ionic Strength Solution (LISS)/ Coombs gel card, i.e., a full serological cross match. CAT cross matching with an O Rh-D positive PRBC unit showed 4+ incompatibility, and a second O Rh-D positive unit was also incompatible by CAT. Fresh patient samples were obtained and retested, with identical results.

The recognised causes of grouping discrepancy and cross match incompatibility were systematically considered as differential diagnoses: weak or absent antigens (ABO subgroups, haematological malignancy, or marrow transplantation); weak or absent antibodies (advanced age, hypogammaglobulinaemia, or recent plasma-containing transfusion); rouleaux or pseudo-agglutination from abnormal plasma proteins; unexpected alloantibodies, cold-reactive autoantibodies, or High-Titre Low-Avidity (HTLA) antibodies such as anti-Chido, anti-Rodgers, anti-York, and anti-JMH; and technical or clerical error. Technical and clerical error was excluded by repeat testing on fresh samples, and the negative DAT argued against an autoimmune haemolytic process. The HTLA antibodies were retained as differential possibilities but were not supported by the subsequent findings.

Cross matching and IAT by CTT (immediate-spin, 37°C, and antiglobulin phases) demonstrated full compatibility, establishing the CAT result as a gel-specific false-positive. One unit of O Rh-D positive PRBC was transfused under clinical observation without any immediate adverse event. During the same hospital admission, the patient succumbed to his underlying illness; the death was not attributed to the transfusion, and the immediate cause of death was not ascertained.

DISCUSSION

Pretransfusion compatibility testing is the cornerstone of transfusion safety, preventing immune-mediated haemolytic reactions caused by incompatible donor red cells. The antiglobulin test remains central to red cell serology [1], and CAT, or the gel card method, has largely replaced the CTT in many laboratories [2,3]. However, the enhanced sensitivity of CAT may occasionally amplify clinically insignificant reactions and produce false-positive incompatibility [3]. Reported here is such a case, in which a persistent CAT cross match incompatibility proved to be a gel-specific false-positive on conventional tube testing.

The gel card method offers several advantages over CTT, including greater sensitivity for clinically significant IgG antibodies, standardised serum-to-cell ratios, objective reading (agglutinates trapped at the top of the gel column indicate a positive result, whereas a pellet at the base indicates a negative result), reduced inter-observer variability, compatibility with automation, and superior documentation. These advantages have driven its widespread adoption in teaching hospitals and larger transfusion centres [2,3].

However, this heightened sensitivity is a recognised limitation when clinically insignificant reactions — cold agglutinins, HTLA antibodies, or nonspecific reactions in patients with elevated serum proteins or recent transfusion — are amplified into apparent incompatibility. [3,4]. Gel-specific false-positive reactions may arise from fibrin strands or particulate material trapping red cells at the top of the column, non-specific protein interactions within the gel matrix, antibodies directed against components of the enhancement medium or the gel matrix itself, and reagent- or technique-related factors [5,6]. In the present patient, advanced age, recent transfusion, and AKI on CKD together plausibly created conditions favouring such non-specific gel-amplified reactivity. The negative DAT and a normal CTT cross match together indicated the absence of a clinically significant allo- or autoantibody.

The transient mixed-field appearance on reverse grouping merits comment. Because the mixed-field pattern was observed on reverse (serum) grouping against pooled reagent O cells, it is best explained by gel-technique amplification of weak, nonspecific serum reactivity - in keeping with the pan-reactive antibody screen - rather than by the two red-cell populations expected after recent transfusion. It was not reproduced by conventional tube testing. The lower sensitivity of the tube method, together with its different reaction milieu, is the likely reason both the mixed-field pattern and the cross match incompatibility resolved on conventional testing.

Comparable observations have been reported from other centres. Anuragaa S et al., described a patient with a negative DAT but positive IAT and auto control, pan-reactive antibody screening, and group-specific incompatibility by CAT that resolved on tube testing, ultimately attributed to an antibody against the low-ionic-strength enhancement medium [5]. Deerej P et al., similarly reported gel-card discrepancies and false-positive cross matches attributed to antibodies targeting the gel-card matrix, which were compatible by CTT and on an alternative gel platform [6]. The present case shares the central features of these reports, a negative DAT, gel-specific incompatibility, and full compatibility by CTT but differs in that a specific anti-medium or anti-matrix antibody could not be characterised and the tube method, although labour-intensive and operator-dependent, remains robust and serves as an essential confirmatory tool when CAT yields unexpected positive results [3,7]. Withholding compatible blood from a patient with severe anaemia solely on the basis of a CAT false-positive carries serious clinical risk; standard operating procedures should therefore mandate CTT confirmation before a CAT-incompatible unit is rejected. A limitation of this report is that the patient died from his underlying illness during the same admission, so long-term transfusion safety and any subsequent alloantibody development could not be assessed; in addition, the specific mechanism underlying the gel-specific reactivity was not definitively characterised.

CONCLUSION(S)

False-positive cross match results by CAT are uncommon but clinically important, particularly in elderly patients with recent transfusion and multiple co-morbidities. When a gel-card cross match is unexpectedly incompatible but the DAT is negative and no clinically significant antibody is demonstrable, conventional tube testing should be used to confirm or refute the result before compatible blood is withheld. The practical message for the blood bank is to treat an isolated CAT incompatibility as a signal for confirmatory tube testing rather than an automatic reason to reject a unit, and to embed this step in standard operating procedures so that patients who urgently need transfusion are not denied serologically compatible blood.

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PARTICULARS OF CONTRIBUTORS:
1. Assistant Professor, Department of Transfusion Medicine, Sumandeep Vidyapeeth Deemed to be University, Vadodara, Gujarat, India.
2. Associate Professor, Department of Pathology, Sumandeep Vidyapeeth Deemed to be University, Vadodara, Gujarat, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Vidhi Jain,
C/15, Pancham Duplex, Parivar Char Rasta, Waghodia Road,
Vadodara, Gujarat, India.
E-mail: vidhidjain269@gmail.com

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