Red cell antibodies resulting in false-positive complement-dependent cytotoxicity cross-match: A unique case

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Abstract:
A false-positive complement-dependent cytotoxicity cross-match (CDC XM) has a negative impact in donor selection process obliterating healthy, donor compatible population. A 47-year-old male with chronic kidney disease was planned for ABO-compatible renal transplantation from his sister. CDC and donor-specific antibody (DSA) lysate XM were negative 10 days before transplant. The pretransplant CDC XM showed 40% positivity. DSA lysate XM and HLA antibody screen were negative. Patient’s Indirect antiglobulin test (IAT) was positive and anti-M antibody (IgG + IgM) was identified. Therapeutic plasma exchange, intravenous immunoglobulin, and rituximab were used for desensitization. Decrease in positivity of CDC XM and anti-M titer was seen. The transplant was performed successfully. Red cell alloantibody should be considered in differential diagnosis of a positive CDC XM. The utility of DSA lysate XM as a pretransplant monitoring tool is immense in such situations. Institutional policies regarding plan of action in the event of positive CDC XM and negative DSA lysate XM and vice versa should be formed.

Keywords:
Complement-dependent cytotoxicity, cross-match, HLA, positive, renal transplant

Introduction

Antibody-mediated rejection in cases of kidney transplantation is associated with the presence of preformed donor-specific antibodies (DSAs). Hence, recipients are screened before transplantation. The conventional complement-dependent cytotoxicity cross-match (CDC XM) is performed to confirm compatibility between the donor and recipient.

The presence of antilymphocyte antibodies in the recipients’ serum (HLA and non-HLA antibodies) targeting donor antigens results in positive CDC XM. Previous sensitization events in recipients such as pregnancy, blood transfusion, and previous transplant can result in a positive CDC XM. To avoid rejection, an unexpected positive CDC XM must be immediately reported in a prospective setting.

We report here an unexpected positive CDC XM due to the presence of a RBC alloantibody with anti-M specificity.

Case Report

A 47-year-old male patient with chronic kidney disease due to acute pyelonephritis was considered for kidney transplantation with his sister as a prospective donor. The patient had a history of Packed red blood cells (PRBC) transfusion (day: −38). The blood group of the patient and donor was O Rh (D) positive. Low-resolution HLA
typing was performed by polymerase chain reaction SSO method revealing a 5/6 mismatch [Table 1].

The initial CDC XM and the DSA lysate XM performed during the pretransplant work-up were negative; hence, the patient was posted for transplant. Since the initial CDC XM was negative, auto-CDC XM and dithiothreitol (DTT)-modified CDC XM were not performed. As per our institutional protocol, CDC XM is done 48 h before transplant. The pretransplant routine CDC XM (day: −14) showed 40% positivity. To confirm the result, DTT-modified CDC XM assay was performed which also showed 40% positivity. This indicated the presence of IgG antibodies in the recipient’s serum; hence, the transplant was put on hold. The recipient’s sample was sent for blood grouping, antibody screening, and blood reservation. The alloantibody screening revealed the presence of anti-M alloantibody with a titer of 256 and having both IgM (RT) and IgG (37°C) components [Table 2].

The patient was managed with therapeutic plasma exchange (TPE) followed by intravenous immunoglobulin treatment as a desensitization protocol. TPE (1.2× plasma volume) was performed using a continuous flow cell separator (day: −10). Following the treatment on day −8, DSA lysate XM and DTT CDC XM were performed. We found that DSA lysate XM was negative for both HLA Class I (MFI-544) and II (MFI-227) and DTT CDC XM showed 15% positivity, indicating the persistence of IgG antibodies. He was further managed with rituximab and second sitting of TPE (day: −8). This treatment resulted in the reduction of anti-M titer to 8 on day −7 and negative DTT CDC XM on day −3.

The transplant was performed; posttransplant day +3, DSA monitoring was done which was found to be negative. The patient accepted the graft well. Further follow-up was possible till 10 months posttransplant and the graft was functioning well at that time.

**Discussion**

The conventional CDC XM technique was established as the prototype of cross-matching in the late sixties and has unquestionably improved the outcome of transplantations since hyperacute and acute rejections have been reduced.[1,2] Positive CDC XM can be observed in recipient with an autoimmune disease or preexisting antibodies not detected by single-antigen bead assay (SAB) due to complement interference or due to previous desensitization protocols.[3] Although false-positive CDC XM due to autoimmune diseases has been mentioned in the literature, it lacks concrete examples which demonstrate different possibilities or reasons for CDC XM positivity (non-HLA antibodies).[4]

**Table 1: HLA typing results of donor and recipient on Luminex 100/200 platform**

| HLA alleles | Donor | Recipient |
|-------------|-------|-----------|
| HLA A       | A*03  | A*02 A*26 |
| HLA B       | B*44  | B*08 B*15 |
| HLA DRB1    | DRB1*03 | DRB1*15 |

**Table 2: Patient management plan summary**

| Investigations | Days | Results |
|----------------|------|---------|
| CDC XM         | −27  | Negative |
| CDC XM         | −14  | Routine CDC-40% positive |
| Alloantibody screening, identification, and titer | −13 | Anti-M antibody detected, total (IgG+IgM) titer=256 |
| Therapeutic plasma exchange performed and IVIG administered | | |
| CDC XM         | −8   | Routine CDC-15% positive |
| DSA XM         | −8   | Class I Class II Result 544 227 Negative |
| Therapeutic plasma exchange performed and rituximab administered | | |
| Antiglobulin titer | −7  | Total (IgG+IgM) titer=8 |
| CDC XM         | −3   | Negative |
| Day: 0 – transplant done | | |
| DSA XM         | +3   | Class I Class II Result 248 403 Negative |

DTT=Dithiothreitol, IVIG=Intravenous immunoglobulin, CDC=Complement-dependent cytotoxicity, CDC XM=CDC cross-match

Whenever a CDC XM is positive, we follow an algorithm; where CDC XM is repeated with freshly drawn samples, CDC XM with and without DTT is done to confirm the positivity. The results are correlated clinically and with the results of DSA lysate XM. If the discrepancy is not resolved, it is further referred for SAB testing and flow cross-match. Since the discrepancy was resolved on red cell alloantibody screening, it was not tested further by SAB and flow XM.

In the present study, CDC XM showed 40% positivity before transplant; even with DTT treatment, the cross-match was positive, due to which transplant was postponed. Initially, the antibody levels were reduced by desensitization protocol, but positivity still persisted. To confirm the positivity, DSA lysate XM was performed which was negative for both Class I (MFI-544) and II (MFI-227), indicating false or doubtful positivity in CDC results. Simultaneously, the patient’s antiglobulin test revealed the presence of anti-M alloantibodies which was reactive for both IgG (37°C) and IgM (RT) type.

In this case, alloantibody may have formed after transfusion of the PRBC unit on day −38. The antibody screening was performed before transfusion which was negative then.
Anti-M antibody is commonly IgM type, but there are case reports available where it has been reported to occur with both IgM and IgG components, as was seen in this case.[3] When such alloantibody exists in recipient’s serum and is of IgG subtype, it is likely to be detected in CDC XM as well (like any other IgG type antibody, e.g., rituximab). In India, the prevalence of alloantibodies has been reported in range of 3.4%–9.8%.[6,7] This case also emphasizes that although the addition of DTT and Anti human globulin (AHG) reagent has improved the efficiency of CDC XM with time, still the probability of counterfactual results persists with this technique. Tests like DSA lysate XM, Red Cell antibody screening and titre, HLA antibody screening are suggested as a prognostic tool in kidney transplantation. This will reduce our dependence on the result of single gold standard test that is CDC XM. Thus, this case highlights that multidisciplinary approach and healthy communication between the transplant and laboratory services is essential for the proper management of transplant cases.

**Conclusion**

Red cell alloantibody should be considered in differential diagnosis of a positive CDC XM. The utility of DSA lysate XM as a pretransplant monitoring tool is immense in such situations.

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**Conflicts of interest**

There are no conflicts of interest.

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