Case Report

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Quick Response Code:

Website: www.ajts.org

DOI: 10.4103/ajts.AJTS_95_17

A positive complement dependent cytotoxicity immunoglobulin G crossmatch due to auto-antibodies with a negative luminex bead assays in a renal transplant recipient: A Diagnostic dilemma

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Abstract:

Transplant recipients are always at a risk of developing anti-human leukocyte antigen (HLA) antibodies due to prior sensitizing events such as blood transfusions, multiple pregnancies, or transplantation. Unexpected positive outcomes can be seen in complement dependent cytotoxicity (CDC) based assays due to underlying autoimmune disorders or pharmacological treatment (rituximab/intravenous immunoglobulin/anti-thymocyte globulin administration), therefore, limiting its value. CDC based assay results strongly depend on the vitality of the donor lymphocytes, highlighting another major limitation of this assay. Thus, as an alternative approach, solid phase based crossmatch assays were introduced which function independently of the cell quality and have higher sensitivity and specificity in detecting anti-HLA antibodies. We describe a case where the patient awaiting renal transplantation from living related donor was evaluated by pretransplant histocompatibility testing for the detection of anti-HLA antibodies. The histocompatibility testing revealed positive CDC anti-human globulin and flow crossmatch along with negative Luminex based assays (HLA antibody screen, luminex crossmatch, and luminex single bead assay). Detailed histocompatibility workup revealed immunoglobulin G autoantibodies which were complement activating and lympocytoxic in nature.

Keywords:

Complement-dependent cytotoxicity, crossmatch, histocompatibility testing, luminex, immunoglobulin G autoantibodies, lymphocytotoxic, renal transplantation

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Submission: 18-07-2017
Accepted: 10-11-2017

Introduction

In the late sixties of the last century, the complement dependent cytotoxicity (CDC) assay was introduced as a standard technique to exclude anti-human leukocyte antigen (HLA) antibodies.[1] The existence of HLA antibodies is considered a major contraindication to solid organ transplantation due to a high risk of hyperacute or acute rejection. Thus, pretransplant screening is extremely important for such patients to determine the donor-recipient compatibility.[1] With the introduction of Luminex based platforms, the approach for pretransplant screening was revolutionized as it has a higher sensitivity and specificity for detection of HLA antibodies in patients awaiting solid organ transplantation.[1,2] However, false positivity and negativity have been reported in sensitive technologies like Luminex.[3] We report a case where the CDC antihuman

How to cite this article: Chowdhry M, Makroo RN, Kakkar B, Thakur Y, Kumar M, Singh M. A positive complement dependent cytotoxicity immunoglobulin G crossmatch due to auto-antibodies with a negative luminex bead assays in a renal transplant recipient: A Diagnostic dilemma. Asian J Transfus Sci 2018;12:160-4.

globulin (AHG) and flow crossmatch were positive while Luminex based assays were negative.

Methods

CDC crossmatch was performed using neat, and dithiothreitol (DTT) treated patients' sera and lymphocytes (B and T-cells were separated) of the donor or patient (for autocrossmatch).

B and T lymphocyte separation: patients' blood sample was mixed with McCoy's media and T and B rosettes in separate falcon tubes for each T and B cells, which were then incubated at room temperature for 20 min. After incubation, the sample was transferred to another falcon tube containing histopaque solution for each T and B cells. Thereafter, each tube was centrifuged at 2300 rpm for 16 min to separate the lymphocyte layer of T and B cells, respectively. The cells were then suspended in the McCoy media for downstream application. Appropriate controls (positive and negative) were used. For tittered crossmatch dilutions up to 1:128 were taken.

Analysis of the MICA (MHC Class I polypeptide-related sequence A), HLA antibody screening, luminex crossmatch (LumXm), luminex single antigen bead (L-SAB) assay and panel reactive antibody (PRA) was based on the method of Luminex's xMAP® Technology using the Life codes LSATM MIC, Life codes Life Screen Delux and Life codes Class I ID and Life code Class II ID v2 (Immucor) and PRA ELISA (lambda antigen testing – LAT), respectively. Flow Crossmatch (anti-CD3 PerCP, anti-CD19 phycoerythrin and FITC-goat-anti-human immunoglobulin G (IgG) polyclonal antibody; BD FACSCanto II instrument, BD Biosciences) was also performed. Additional tests such as red cell antibody screening were performed using CAPTURE-R Ready screen, Neo, Immucor to rule out any blood incompatibility due to Lewis antigens. All the tests were performed according to manufacturer's instructions using appropriate controls.

Case Report

A 44-year-old male, hypertensive for 11 years complained of weakness and joint pain-on and -off for 2 months and was admitted with above-mentioned complaints in November 2014. On evaluation, patient was found to be anemic (hemoglobin - 7.1 mg/dL) with deranged renal parameters (blood urea - 165 mg/dL; serum creatinine - 13.8 mg/dL; serum sodium - 146 mEq/L; serum potassium - 6.1 mEq/L). On examination, blood pressure – 160/100 mmHg, pulse rate – 96/min, pallor present, itching all over the body, no pedal edema. Cardiovascular and respiratory examination revealed no abnormality. The patient was diagnosed with a case of end-stage renal disease and started on maintenance hemodialysis, and the possibility of renal transplantation was discussed with the patient. The transplant workup was started for ABO compatible living donor related renal transplantation. The patient had a history of blood transfusion 5–6 months back (3–4 units of packed red blood cells). Infectious marker screen (hepatitis b, hepatitis c, and human immunodeficiency virus) was negative. Autoimmune markers (anti-nuclear antibody, ANA; rheumatoid factor, RF; anti-double-stranded DNA, anti-dsDNA; antineutrophil cytoplasmic antibody, ANCA; anti-signal recognition particle, anti-SRP) were also negative. As per the routine departmental histocompatibility testing protocol, initial testing included CDC AHG crossmatch, HLA antibody screening and LumXm. Initial testing revealed positive CDC AHG crossmatch and negative luminex based assays (HLA antibody screening and LumXm). Figure 1 shows the algorithmic approach for histocompatibility workup performed for the patient. Detailed histocompatibility workup was suggestive of IgG auto-antibodies, which were complement activating and lymphocytotoxic in nature. Due to unexplained strong positivity in CDC and flow crossmatch, renal transplantation was not performed.

Discussion

Formation of anti-HLA antibodies is seen due to the history of prior blood transfusion, multiple pregnancies and previous organ transplantation.[4] The aim of reporting this case is to highlight the algorithm to be followed for histocompatibility workup for unexpected reactivity encountered either among CDC-based techniques, flow crossmatch or Luminex based assays.

CDC crossmatch reveals the functional potential of anti-HLA antibodies in activating complement and the results obtained are used as a guide to decide for performing transplantation and predict graft survival.[1,5] Pretransplant screening consisting exclusively of testing based on CDC based technique is associated with several drawbacks, such as detection of antibodies only activating complement, less sensitivity in comparison to solid phase assays and false positivity.[1]

Followed by the introduction of CDC assay, flow cytometric crossmatch was introduced in early eighties of the last century as an alternative approach. However, both the techniques (CDC and flow cytometric crossmatches) had a major disadvantage that valid results are not obtained if the donor lymphocytes are of poor quality/vitality.[6]

In our patient, CDC AHG crossmatch was found to be positive and as per routine departmental protocol, titered CDC crossmatch was performed to know the potency

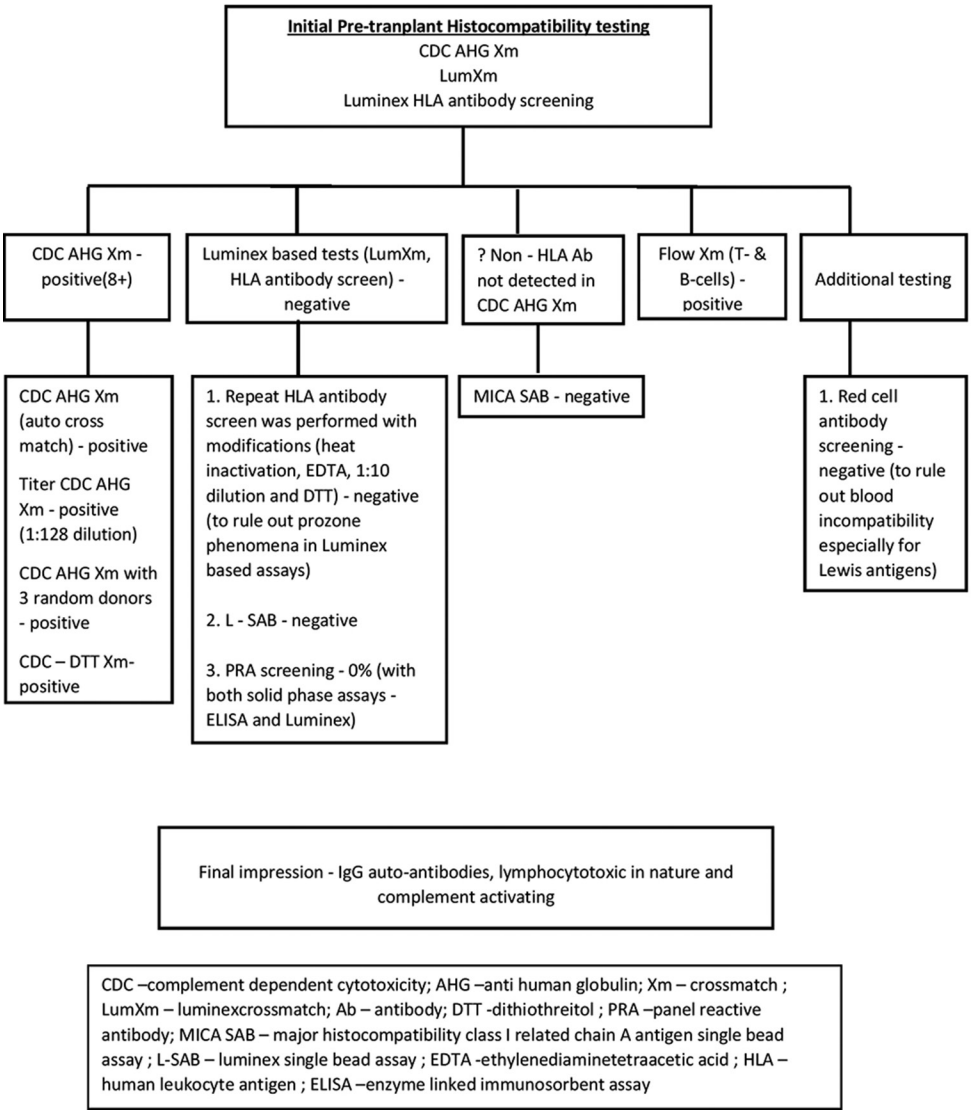

Figure 1: Details of histocompatibility workup performed for the patient

of the antibodies and to determine whether there is any role of desensitization. CDC AHG crossmatch was also performed with 3 random donors and was found to be positive. The patients flow crossmatch was also positive for T and B cells. False positivity in CDC crossmatches has been noted in patients presenting with autoimmune disorders such as systemic lupus erythematosus or administration of drugs such as rituximab, antithymocyte globulin (ATG), and intravenous immunoglobulin (IVIg).[1,5] However, in our patient, autoimmune markers were negative and there was no history of administration of rituximab/ATG/IVIg before histocompatibility testing, thus ruling out them as the cause of false positivity.

False positivity in CDC crossmatch due to IgM type of autoantibodies can be avoided by treating the serum with DTT, thus increasing the sensitivity of the test.[1] In our case, we observed that the CDC AHG autocrossmatch was positive even after DTT treatment, thus implying that the patient had IgG autoantibodies which were complement activating and lymphocytoxic in nature. Nagele *et al*.,[7] have reported that IgG autoantibodies might have the infectious origin as well, however, at the time of evaluation for transplant, the patient was negative for hepatitis B, hepatitis C, and human immunodeficiency virus.

To avoid nonspecific reactivity seen with CDC based techniques, solid phase assays such as ELISA and Luminex were introduced. Higher sensitivity and specificity has been noted with Luminex based assays toward detection of anti-HLA antibodies, and therefore, at present, they are considered to be an important part of pretransplant histocompatibility testing. False positivity has been reported with Luminex single bead antigen assay due to the presence of denatured antigenic epitopes produced during processing. However, such positivity

has been reported less frequently with LumXm assay due to utilisation of native antigens extracted from donor lymphocytes after membrane lysis using detergent.[3] In our case, the Luminex based tests, i.e., LumXm and L-SAB were negative. Furthermore, the patients' serum for PRAs was 0%.

Some patients might present with high titre antibodies which can result in lower than expected or even negative results with Luminex based assays which are known as the "prozone phenomena." The several methods available for overcoming prozone are heat inactivation, serum dilution in a ratio of 1:10, treating the sera with ethylenediaminetetraaectic acid and DTT.[6] No reactivity was observed in patients sera on testing with all these methods, thus ruling out prozone phenomena.

MICA (major histocompatibility complex Class I related chain A) antigens are highly polymorphic in nature and are expressed on the surface of vascular endothelial cells. Since they are not expressed on the surface of lymphocytes, they can be missed by testing only by CDC crossmatch and/or flow crossmatches, as these techniques utilize donor lymphocytes for detection of anti-HLA antibodies.[8] Therefore, to rule out the non-HLA nature of antibody seen in our patient, MICA SAB (single antigen bead) assay was performed utilising Luminex platform, which was also found to be negative.

Lewis antigens are expressed on the cell surfaces of the renal parenchyma in Lewis positive individuals and are capable of inflicting both cell-mediated and humoral immune responses of cytotoxic nature.[9] Thus, renal transplant recipients mismatched for Lewis antigens with their donors might be at risk of diminished allograft function, despite compatibility with other histocompatibility antigen systems.[10,11] Red cell antibody screening was also performed to rule out any blood incompatibility due to Lewis antigens and was found to be negative. However, we were not able to determine the Lewis status to decide for the incompatibility between our patient and donors.

As per the guidelines of British Transplant society, the presence of IgG autoantibodies with positive CDC or flow crossmatch in the absence of HLA alloantibodies by Luminex does not confer any additional risk to renal transplantation.[12] In a study conducted by Süsal *et al*.,[13] role of different isotypes and specificities of anti-IgG autoantibodies were investigated. The pretransplant sera of renal transplant recipients (with well-functioning grafts, with successful treatment of recipients with reversible rejection episodes and irreversible graft rejection) were tested for different anti-IgG autoantibody activity. They observed that IgG-anti-F(ab)$_{2y}$ antibodies have a protective effect on graft survival while high pretransplant IgG-Fc$_{y}$ was associated with low graft survival rate. However, in our case, we were not able to determine the isotype of the antibody present in our patient and determine whether it will have any implications on transplanted organ.

Another major limitation encountered was that the positivity in CDC (not abolished by DTT treatment) and flow crossmatch made the results noninterpretable, highlighting the inherent problem of using these assays and we were also not able to explain the reason for positive outcome in these assays (negative infectious marker screen; negative autoimmune makers, no prior history of administration of rituximab/ATG/IVIg), which deterred the clinician from performing renal transplantation in our patient.

This case highlights the fact that unexpected results can be encountered in different techniques utilised for pretransplantation screening, thus, reminding us that a correlation between the clinical status of recipient along with detailed histocompatibility testing involving different permutation and combination of tests such as CDC based assays, flow crossmatch and/or Luminex based assays may be helpful in resolving such cases.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the jvournal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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