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Review

# Management of the Sensitized Cardiac Transplantation Recipient

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**Abstract**

Preoperative sensitization of the cardiac transplant recipient, defined as the presence of anti-Human Leukocyte Antigen (HLA) antibodies before transplant, represents a significant management challenge for physicians. Sensitization prolongs the pre-transplant wait time and is associated with postoperative transplant complications and death. It is critical that sensitized heart transplant candidates be identified and optimized before surgery. In this review, we describe the risk for sensitization, discuss the means through which sensitization may be diagnosed, and highlight some of the new therapeutic options for managing the sensitized cardiac transplant patients.

**Keywords**
Sensitization; cardiac transplant; postoperative complications; HLA antibodies

## 1. Clinical Vignette

A 27-year-old female heart transplant candidate with non-ischemic adriamycin-induced cardiomyopathy supported by HeartMate II left ventricular assist device (LVAD) before transplant received an organ offer. She was blood type O and her calculated panel reactive antibodies (CPRA) of 87% based on MFI cut-off of 3,000 due to pregnancy. Due to high CPRA, serial serum dilutions are performed, and unacceptable antigens are listed in UNET based on 3,000 MFI cut-off in 1:16 diluted serum. Virtual crossmatch (VXM) is positive with undiluted serum due to the presence of donor-specific antibody (DSA) reactive with HLA-A25 (4,500-5,000 MFI), but negative in 1:16 diluted serum (MFI = 200). The flow cytometric crossmatch (FC-XM) is predicted to be T-cell, B-cell positive, and complement-dependent cytotoxic crossmatch (CDC-XM) is predicted to be negative. There is no time for prospective physical crossmatch. Is it safe to proceed?

## 2. Definition

"Sensitization" is an immunological state among potential heart transplant recipients in which circulating anti-HLA antibodies are either present in the blood or when rapid production of HLA antibodies is possible via an immune memory response. Sensitization may occur as a result of pregnancy, exposure to blood transfusions, a previous organ transplant, or the placement of a ventricular assist device, vascular allografts, valve implants, or previous open-heart surgery [1]. Patients with preformed antibodies against donor HLA antigens may experience hyperacute or accelerated acute antibody-mediated rejection (AMR). The preformed antibody can be detected by testing patient sera against a panel of HLA typed cells using CDC assays or against solubilized HLA antigens attached to solid supports (solid-phase assays). Percent PRA is calculated based on percent of positive reactions from the tested panel. On the contrary, percent CPRA is calculated based on the frequency of unacceptable antigens (HLA antigens against which candidate has antibodies) in various ethnic donor populations [2]. Both percent PRA and CPRA provide an estimate of the percentage of likely incompatible donors. A recent study demonstrated that CPRA values greater than 50% are associated with significantly lower rates of heart transplantation and higher waitlist mortality [3].

## 3. The Role of HLA Antibodies in Transplantation

HLAs are a class of cell surface glycoproteins involved in immune regulation. HLA antibodies fall into two classes: class I, which includes antibodies to antigens HLA-A, -B, and -Cw (constitutively expressed in all nucleated cells and recognized by Cluster of Differentiation (CD) cytotoxic T cells), and class II, including antibodies to HLA-DR, -DQ, and -DP (expressed on B cells, monocytes, dendritic cells, and other antigen-presenting cells, and recognized by CD4 helper T cells). Class I and II HLA proteins are encoded by a set of highly polymorphic genes within the major histocompatibility complex on chromosome 6 [3, 4].

HLA antibodies can cause antibody-mediated allograft injury via the binding C1q, which activates the classical complement cascade. This in turn leads to the production of anaphylatoxins C3a and C5a [5], as well as the formation of the C5b-9 membrane attack complex (MAC) [6]. However, not all episodes of AMR are C1q mediated [7]. Class I HLA antibodies may additionally promote smooth muscle proliferation via fibroblast growth factor binding and receptor expression, leading to the development of cardiac allograft vasculopathy (CAV) [8]. Other proposed mechanisms for anti-HLA antibody-mediated allograft injury include monocytes, neutrophils, natural killer (NK) activation [9, 10], and HLA crosslinking which activates pro-inflammatory transcription factors, mobilizes adhesion molecules and promotes cytoskeletal remodelling [11]. Conversely, low levels of anti-HLA antibodies may exert a protective effect via the promotion of cell survival protein expression [12, 13]. These mechanisms, when taken in concert, may have a synergistic effect on graft injury through AMR and CAV [14] which are considered long-term transplant morbidities rather than a postoperative mortality (which is typically dominated by infection and acute rejection) [7]. Although non-HLA antibodies also could affect the outcomes, their role is reviewed in detail elsewhere [15].

Sensitization can exert problematic effects across all phases of organ transplantation; its presence preoperatively reduces the likelihood of identifying a compatible donor organ, leading to increased wait times and concomitant increase in wait-list morbidity and mortality, and in the postoperative setting, anti–HLA DSA can increase the likelihood of decreased survival as a result of development of AMR and CAV [16]. Numerous analyses of large cardiac transplant registries have demonstrated the negative impact of DSAs on pre-transplant wait times, pre- and post-transplant survival, and the increased risk of rejection and CAV [14, 17-19]. The correlation between anti-HLA antibodies and poor postoperative outcomes has been most recently verified in a prospective multi-institutional clinical trial [20]. The relevance of various subtypes of HLA antibodies is still not well understood, nor is the relevance of the clinical milieu in which HLA antibodies manifest themselves. A recent United Network for Organ Sharing (UNOS) study has demonstrated that in patients with HLA antibodies in the setting of mechanical circulatory support prior to transplantation, allosensitization did not independently predict worse post-transplant outcomes; however, in patients without mechanical support, allosensitization was associated with worse post-transplant survival [21].

Overall, however, HLA antibodies are associated with significantly worse outcomes, and their identification and the amelioration of their harmful effects is of paramount importance in improving post-transplant outcomes.

## 4. Crossmatching

To detect DSA in a recipient's serum against a given donor's HLA antigens, a crossmatch (XM) procedure is performed. Several types of XM tests detect antibodies with different sensitivity and specificity: CDC-XM, FC-XM, and solid-phase XM. A CDC-XM was developed to identify recipients who are likely to develop hyperacute vascular rejection of a graft from a given donor due to the presence of high titer DSA [22]. The donor's cells separated into specific subsets such as T cell and B cell lymphocytes are incubated with the recipient's serum and then complement is added. If a donor-specific antibody is present, it binds to donor cells and activates the complement cascade resulting in cell lysis, which is detected by adding vital dyes. The positivity score is calculated based on the percentage of dead cells, with a score of 0 corresponding to less than 10% of dead cells;

scores of 2 correspond to approximately 20% of dead cells (generally taken as the cut-off for a positive result). Scores 4, 6, and 8 represent increasing levels of lysis, with a score of 8 representing all cells dead which indicates the strongest possible reaction. The scoring system allows a semi-quantitative analysis of the strength of the reaction. In addition, CDC-XM is performed using serial 2-fold dilutions of the recipient serum (titrations) to evaluate how many dilutions are required for CDC-XM to become negative and evaluate the likelihood of achieving negative XM with desensitization procedures. CDC-XM may be positive due to immunoglobulin M (IgM) autoantibodies, which can effectively activate complement and induce cell death, but have no clinical significance in transplantation. Typically, dithiothreitol (DTT)-treated or heat-inactivated serum is used to distinguish positive CDC-XM reaction due to IgM autoantibodies vs. immunoglobulin G (IgG) alloantibodies. In general, a negative CDC-XM suggests that a recipient does not have DSA and can proceed to transplantation, whereas a positive CDC-XM indicates a high risk for graft loss due to hyperacute rejection, and, therefore, transplantation should not be attempted without desensitization [23].

It is possible to have a negative CDC-XM in the presence of DSA when the antibody titer is too low to activate complement or when the antigen is expressed at low levels on the donor's lymphocytes (e.g., HLA-Cw, -DQ, -DP) [24] and also the DSA may be of a subtype that does not activate complement (e.g., IgG2 and IgG4). Although the risk of hyperacute rejection is low in these situations, the risk of accelerated acute, acute, and/or chronic rejection is increased compared to recipients who are transplanted without DSA [25].

The sensitivity of CDC-XM can be enhanced by the addition of anti-human globulin (AHG), which binds to the DSA bound to the donor cells, thereby increasing the likelihood of complement activation and cell death. AHG-CDC XM can increase the detection of low-titer DSA and IgG2/4 subtypes.

An FC-XM involves adding a recipient serum to donor lymphocytes followed by an incubation with fluorescein-labelled antibodies against human IgG (anti-human IgG F(ab)2/ fluorescein iso-thiocyanate (FITC). The presence of DSA in the recipient serum is detected by flow cytometry. The FITC-labelled antibody binds to all the IgG antibodies in the recipient serum, irrespective of DSA titer and subtype. The read-out of FC-XM is usually reported as positive or negative based on the intensity of fluorescence above negative control, referred to as median channel shift (MCS). It can be further quantitated using serial dilutions of recipient serum to generate a negative result.

When a recipient has anti-HLA class I DSAs, both T- and B-cell FC-XM should be positive, because class I HLA molecules are expressed on all nucleated cells. When a patient has anti-HLA class II DSA, only B-cell XM is expected to be positive because class II molecule expression is restricted to antigen-presenting cells. A negative B-cell XM in the presence of a positive T-cell XM suggests a technical error or non-HLA reactivity with T-cells.

While positive CDC-XM due to anti-HLA IgG alloantibodies is considered a contraindication to transplantation, proceeding with the transplant when CDC-XM is negative, and FC-XM is positive is not entirely clear-cut. In sensitized patients who ultimately have a positive FC-XM, adjunctive therapies may provide benefit, although there is a dearth of large multi-center studies to support this claim. Some of these reported options include the use of induction therapy with interleukin-2 receptor antagonists or cytolytic agents such as Campath (anti-CD52 antibody) or anti-thymoglobulin (ATG), plasma exchange with intravenous immunoglobulin (IVIG), with or without rituximab (anti-CD20 B-cell targeting monoclonal antibody) [26].

XM can also be performed using Luminex donor-specific antibody crossmatch (DSA-XM) tests. In this test, beads conjugated with monoclonal antibodies specific for class I HLA or class II HLA are first incubated with the lysates of the donor's lymphocytes to capture donor's HLA molecules. After the wash, the patient serum is added to the HLA- conjugated beads for 30 min. Following another wash, the diluted antihuman IgG-phycoerythrin (PE) conjugate is added to the beads to detect anti-HLA antibodies. After a final 30 min incubation followed by a wash, the beads are acquired and analyzed on the Luminex instrument. In contrast to cell-based assays, solid-phase XM is supposed to be HLA specific; however, a recent study identified non-specific reactions even in this type of XM [27]. Overall, this test has not been sufficiently validated for clinical use.

Because cell-based XM tests are not HLA-specific, the presence of DSA should be determined by solid-phase antibody tests to confirm that anti-HLA antibodies are the cause of the positive XM. Although the significance of positive FC-XM and CDC-XM without HLA antibodies is not well understood, they are generally considered as not a contraindication to transplantation. As discussed above, patients with a positive T-cell CDC-XM are at high risk for hyperacute rejection and patients with positive FC-XM (and negative CDC-XM) are at increased risk for acute rejection. If both assays are run simultaneously the results could be interpreted as follows: all candidates with a positive T-cell CDC-XM are ruled out unless they are also liver recipients, and all patients with a negative T-cell flow crossmatch have a very low risk of antibody-mediated graft injury and can be transplanted. The patients with a negative T-cell CDC-XM and a positive T-cell FC-XM fall into a gray zone (Table 1), and the physicians must determine how much risk they feel is warranted to get these patients transplanted. When patients are transplanted across positive FC-XM, they should be followed up closely post-transplant for indicators of immune activation and treated promptly to prevent or reverse the majority of rejection episodes.

**Table 1** Immunologic Risk Stratification.

| DSA levels in undiluted serum | DSA levels in 1:16 serum | Predicted FC-XM | Predicted CDC-XM | Risk assessment |
|---|---|---|---|---|
| <1,000 MFI (antibodies against shared epitopes are not present) | n/a | NEGATIVE | NEGATIVE | ▪ Low risk of hyperacute or acute rejection |
| 1,000-3,000 MFI | n/a | Most likely NEGATIVE | NEGATIVE | ▪ Low risk of hyperacute rejection<br>▪ Moderate risk of acute rejection<br>▪ If FC-XM is Negative–close DSA monitoring<br>▪ If FC-XM is Positive, consider plasma exchange or IVIG/rituximab |

| | | | | |
|---|---|---|---|---|
| >3,000 MFI | <3,000 MFI | POSITIVE | NEGATIVE | ▪ Low risk of hyperacute rejection<br>▪ Elevated risk of acute rejection<br>▪ Consider peri-operative plasma exchange or IVIG/rituximab |
| >3,000 MFI | 3,000-7,500 MFI | POSITIVE | NEGATIVE | ▪ High risk of accelerated acute rejection and acute rejection<br>▪ Do not proceed without peri-operative TPE |
| >3,000 MFI | >7,500 MFI | POSITIVE | POSITIVE | ▪ High risk of hyperacute rejection<br>▪ Do not proceed |

**5. Antibody Testing**

The goal of HLA antibody testing is the identification of donor specific-antibodies, assessment of their relative strength, and inference of their potential pathogenicity. This allows quick exclusion of incompatible donors and provides clinicians with a tool for assessing the risk of antibody-mediated rejection.

HLA antibody testing has undergone significant evolution since its inception, and its use is currently supported by consensus guidelines [28]. Traditionally, HLA antibody detection used cell-based CDC assays to assess the percentage of PRA [29]. This test provided highly clinically relevant results; however, the results of this test were variable, insensitive, and nonspecific. Currently, anti-HLA antibody detection is performed via solid-phase assay [30].

The choice of solid-phase assay is dictated by the amount of information that is necessary at a given stage of screening. Luminex PRA is often used as an initial screening for potential candidates; it uses beads coated with the equivalent of a cell's class I or class II HLA antigens. This provides either a positive or negative initial screening result. In order to identify antibody specificities in the patient's serum, serum is added to fluorescently-labelled microparticle latex beads coated with individual HLA antigens using a single antigen bead (SAB) assay. Antibodies in the recipient serum bind to the beads if there is a sufficient antibody-antigen affinity. After washing, the beads are incubated with a secondary antibody, usually with a phycoerythrin (PE)-labelled anti-human IgG, which is detected via flow cytometer or fluoroanalyzer (Luminex) instrument [31]. The latter instrument utilizes two lasers, one of which excites the fluorochrome in the bead and the other laser excites the PE bound to the detection antibody. A degree of PE fluorescence is expressed as a mean fluorescence intensity (MFI), which is normalized using negative serum and is proportional to the number of antibody molecules bound to the HLA antigen. Published guidelines recommend using positive cut-off for HLA antibody at 1,500 MFI when technical limitations of SAB are addressed [32]. However, it is important to note that MFI values do not reflect the antibody's titer or functionality (e.g., ability to activate complement).

A major limitation of the SAB assay is the so-called prozone-like effect, whereby the detection of high titer complement-fixing HLA antibodies is compromised due to deposition of complement split products (from C3 and C4 components), which interfere with the reporter anti-IgG-PE antibody binding. Strategies to minimize prozone include serum titration or treatment with heat, DTT, or ethylene diamine tetra acetic acid (EDTA) [33]. In addition, low-level antibodies against shared epitopes may not be effectively detected by SAB due to dilutional effects when a low-level antibody is distributed over many different beads, each bearing the same epitope. As a result, no single bead achieves a significant quantity of antibody to be assigned as positive; nevertheless, such antibody may often result in a positive FC-XM and increase the risk of AMR post-transplant [30, 34]. Alternative antibody-testing strategies can help to identify such reactivity leading to a more accurate histocompatibility consultation. In addition to false-negative reactions, SAB is prone to false-positive reactions due to the presence of naturally occurring antibodies against denatured HLA antigens, known as "cryptic epitopes" [35]. As a result, clinically irrelevant antibodies may be detected that bind to the denatured but not intact antigen. Oaks et al [35] identified 21% of patients awaiting a heart transplant as having at least one antibody to cryptic epitopes, which are thought to falsely increase CPRA by 5% in the 21% of patients. The prozone-like effect, shared-epitope effect, and the effect of cryptic epitopes must be controlled as much as possible because false negative or false positive testing can significantly affect the ultimate allotment of organs [36].

When EDTA-treated serum is used, MFI values obtained using SAB testing provide information about relative antibody levels. Serial serum dilution tested by SAB assay provide information about titers that better reflect antibody strength. Finally, C1q binding assay can identify high titer complement-fixing antibodies [37]. Every transplant center has to define mean fluorescent intensity (MFI) cut-off values or antibody titers that represent low, moderate, and high immunologic risk. In other words, transplant programs have to define which antigens must be listed as unacceptable to avoid hyperacute and accelerated acute rejection, and which antigens have to be avoided to lower the risk of acute, and/or chronic rejection [38].

## 6. Virtual XM

One of the biggest challenges in identifying an organ suitable for transplantation in a sensitized patient has been the need for a prospective XM to confirm compatibility. This historically required the harvest and transport of donor cells to the site performing the XM, markedly increasing the time for crossmatching. Because of the time-sensitive nature of transplantation, this imposed major limitations on geographic catchment areas from which transplant organs could be procured. However, the use of SAB assays has greatly alleviated that requirement by using VXM.

VXM is a process by which recipient HLA antibodies detected by Luminex SABs are compared with donor HLA antigens. The VXM has excellent negative and positive predictive value (92% and 79%, respectively) and is suitable as a "first-pass" XM to more rapidly facilitate organ acquisition transport. VXM appears to be an accurate predictor which also improves the accessibility of donors.

When VXM is reported as "negative", it means that a recipient does not have detectable DSA against the donor, suggesting a low immunologic risk for such transplant. When sensitized patients are transplanted without detectable DSA, their risk of developing early AMR is similar to non-sensitized patients [38]. When VXM is reported as positive, it usually means there is a detectable or clinically significant DSA present. However, there are no clear guidelines on how to recognize a

clinically significant DSA at the time of donor's offer based on SAB results. The strength of MFI in undiluted serum does not necessarily correlated with the ability of that antibody to activate the complement cascade and predict the development of AMR. One of the tools to identify the clinical significance of certain antibodies is the C1q assay which detects antibodies capable of binding C1q, the first step in activating the membrane attack complex [39]. Similar SAB assays have been used for detecting C3d and C4d binding anti-HLA antibodies [40]. Recent studies have demonstrated that DSA delineated according to titration studies and C1q assays better define low-level DSA that are well tolerated to cross. This strategy to assign unacceptable antigens (UAs) improves the transplantation rates for highly sensitized recipients [38, 41-43].

A physical XM may be performed after a VXM, especially in the presence of DSA. This is typically performed either before or immediately after transplantation. A positive FC-XM is associated with a significantly higher risk of early rejection and it often requires complex immunosuppression, including peri-operative plasma exchange, high dose of IVIG, and/or rituximab in addition to induction therapy and maintenance immuno-suppressive drugs. A negative FC-XM in the presence of DSA requires close DSA monitoring post-transplant.

## 7. Managing the Sensitized Patient

In suitable patient's desensitization therapy may be considered to increase the chances of negative crossmatch, expand the number of suitable donors and improve post transplantation outcomes [1]. B cells, Plasma cells, antibodies and complement activation are important components of the humoral immune response and potential targets for desensitization therapy. Current strategies involve removal of alloantibodies and downregulation of antibody producing cells [8] Highly sensitized patients with CPRA>50-80% who have had multiple positive crossmatches are reasonable candidates for desensitization therapy.

### *7.1 Plasmapheresis*

The technique of mechanically removing antibodies from the patient's circulation. There are variant modalities including therapeutic plasma exchange (TPE), double filtration plasmapheresis and immunoadsorption plasmapheresis. TPE is typically performed to exchange 1-2 times the plasma volume (PV) with albumin or fresh frozen plasma given as replacement fluid. TPE removes circulating alloantibodies but does not remove antibodies in the extravascular compartment nor halt the ongoing production of antibody which can result in a rebound after cessation of therapy in 11 days [44]. While there is little consensus on the number, frequency or duration of TPE treatments it appears the benefit plateaus after 3-4 sessions and high titer antibodies do not reduce to a clinically useful level [45]. The American Society of Apheresis guidelines recommend a TPE protocol for desensitization of 1.0 to 1.5 PV performed daily or alternate days with duration of therapy guided by patient response [46]. Side effects such as bleeding, hypotension, blood borne pathogen transmission and allergic responses have been reported [47]. There is however little to support TPE as monotherapy in the sensitized heart transplant candidate [48].

### *7.2 Intravenous Immunoglobulin (IVIG)*

When used in desensitization protocols IVIG is used in combination with other immune therapies. Data for IVIG use among patients awaiting heart transplantation are limited. A study by John et al [49] showed that there was a 33% reduction in circulating IgG antibody within 1 week; however, the efficacy decreased progressively by the end of the 4th week. When used in combination with TPE, IVIG has been reported to reduce the incidence of rejection after transplantation across a positive crossmatch [50].

### *7.3 Rituximab*

Rituximab is a monoclonal antibody against the B-cell marker CD20 and acts by depleting B-cell lymphocytes through a combination of antibody dependent cell cytotoxicity, complement mediated lysis and induction of apoptosis [51]. Urban et al [52] showed that Rituximab and IVIG were effective in lowering pre-transplantation PRA from a mean of 70.5% to 30.2% while allowing for negative cross matches and successful transplantation. Combination protocols with TPE, IVIG and Rituximab have been reported [53] along with successful use of rituximab as salvage therapy in AMR [54].

### *7.4 Bortezomib*

Bortezomib is a reversible 26S proteasome inhibitor which acts by disrupting protein processing and degradation. By acting on the mature plasma cell which is the major alloantibody producing cell Bortezomib is able to overcome the limitation of desensitization therapies [44]. In a limited experience reported by Patel et al [55] a bortezomib desensitization protocol was used in 7 patients with a PRA>50% (MFI >5000). This protocol resulted in rapid antibody reduction compared to other regimes such as IVIG and rituximab. There was a mean reduction in PRA from 62% to 35% in six of the 7 patients.

### *7.5 Novel Therapies*

Other novel therapies have been used; Carfilzomib, another 26S proteasome inhibitor with lesser neuropathy compared to Bortezomib has been used in a single case study in a patient who had 100% CPRA leading to successful transplantation [56]. Eculizumab, a monoclonal antibody that binds complement C5 has been used in sensitized pediatric heart transplant recipients [57] and is being studied (NCT02013037) to evaluate the efficacy in preventing AMR and cell mediated rejection in highly sensitized adult heart transplant patients.

C1 inhibitor has shown promise in animal studies in preventing AMR after transplantation [58]. Imlifidase (IdeS) [59], Belimumab [60], Tocilizumab [61] and Belatacept [62] have been used sporadically and might hold promise in the future.

## 8. Post-Transplant DSA Monitoring

Development of *early* or *de novo* DSA post-transplant increases the risk for rejection (AMR and cellular-mediated rejection (CMR)) depending on the pre-transplant status of DSA and crossmatch positivity. However, it has been shown that there is no difference in overall survival rates for patients whose rejection was successfully treated. When patients are transplanted in the presence

of DSA, frequent post-transplant monitoring is warranted. It has been shown that patients who have DSA-MFI decreased during the first two weeks after surgery, do not develop AMR, while patients with persistent or increased DSA may develop AMR if not treated in timely manner [63]. In addition, sensitized patients transplanted without preformed DSA may demonstrate rapid increase in early DSA due to B-cell memory response activation. Early DSA after transplant is often a result of memory response rather than a true de novo DSA. However, even previously non-sensitized patients may develop de novo DSA at some point post-transplant [64]. Effective detection of DSA using SAB assay, in turn, allows timely diagnosis of AMR and the implementation of therapies to decrease the deleterious impact of DSA on graft outcome. The therapeutic approaches may include therapeutic plasma exchange with IVIG with or without Rituximab or Bortezomib [these strategies were reviewed in [7]. SAB assay can also be used to monitor the response to treatment. Using serial dilutions may provide a more accurate interpretation of DSA response compared to undiluted serum [41].

### *8.1 Interpretation of the Clinical Vignette*

It is not safe to proceed considering VXM results, but clarification is needed to better understand the immunologic risk and the patient's options. Since the patient has a CPRA of 87%, it implies that only 13% of donors will be compatible. The DSA avoidance strategy is likely to result in increased wait time. To increase the candidate's chances to receive an organ offer, unacceptable antigens were listed in UNet$^{SM}$ (online database system developed by UNOS) based on MFI >3,000 at 1:16 serum dilutions. Usually, antibodies >7,500 MFI in 1:16 diluted serum can bind C1q and result in positive CDC-XM. Since DSA against this donor had MFI value of 200 at 1:16 dilution, we predicted that CDC-XM would be negative. At Temple University Hospital, DSA with an MFI >3,000 in undiluted serum usually results in positive FC-XM, so we predicted it as positive. Using immunologic risk stratification (Table 1), we concluded that the transplant could proceed using this donor, but peri-operative desensitization is needed due to a positive FC-XM. In this case, there was a sufficient time to perform a single 1.5 PV TPE prior to surgery. Serum specimens were collected before and after TPE to evaluate the efficacy of treatment. T-cell FC-XM was 189 MCS prior to and 120 MCS after treatment (positive cut-off is 75 MCS). Since T-cell FC-XM remained borderline-positive after plasma exchange, an additional 5 sessions were scheduled every other day post-transplant. The pre-formed DSA against HLA-A25 rebounded on day 7 post-transplant (Figure 1). In addition, 3 early DSAs not detected before transplant rapidly increased along with DSA against A25, suggesting activation of the memory B-cell response (Figure 1). However, patient did not have any signs of AMR on the biopsy. Therefore, after completion of five scheduled sessions of TPE, patient was given a high dose of IVIG to further reduce antibody levels. DSA levels were measured on a weekly basis after IVIG. Since there was no further rebound, rituximab was not given. All DSA decreased to weak levels (<3,000 MFI) within 3 weeks and became negative 2 months post-transplant. The patient never demonstrated AMR on biopsy and remains DSA-negative 2 years post-transplant.

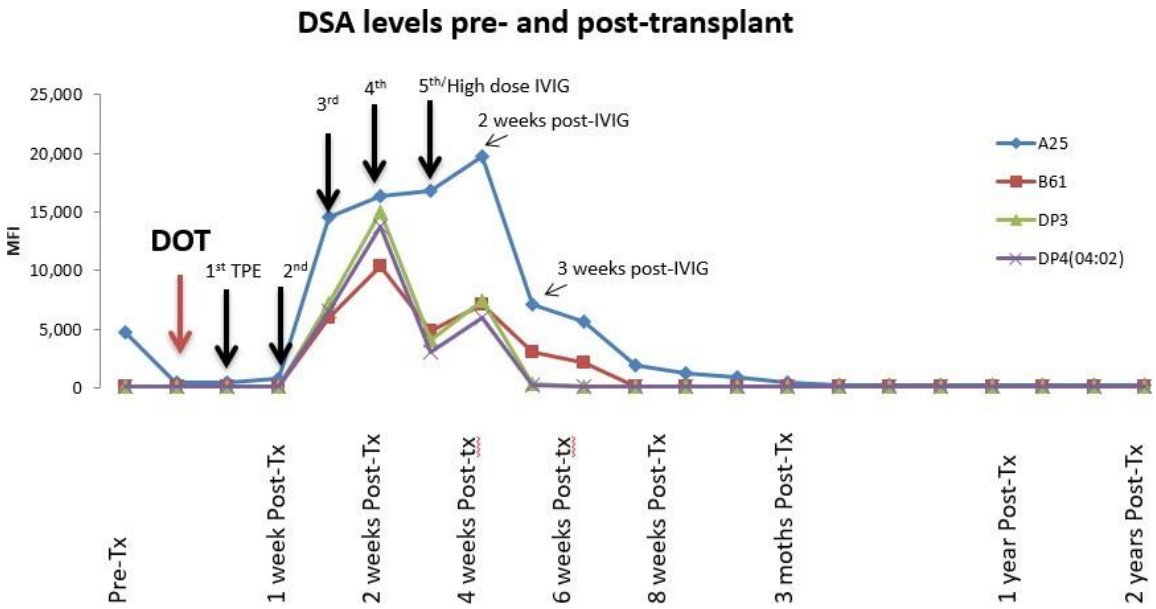


**Figure 1** DSA levels before and after transplant in response to peri-operative treatment. DSA levels were measured in EDTA-treated serum using SAB assay. Peri-operative TPE is shown with red arrow, 5 post-transplant TPE sessions are indicated with bold black arrows. High dose of IVIG was infused after the 5$^{th}$ TPE. DSA levels were measured after each TPE, weekly post-IVIG until 1-month post-transplant and then monthly up to 1 year.

## 9. Conclusion

Virtual crossmatching became a vital tool in assessing the immune compatibility of a particular donor/recipient pairing. It is based on a sensitive and specific detection of anti-HLA antibodies in patient serum; however, clinical relevance of such antibodies is not well understood. The HLA laboratory supporting a heart transplant program has to establish the correlations between DSA MFI/ titer levels and CDC-XM as well as FC-XM results. This can help to identify antibody specificities that have to be avoided to eliminate the risk of hyperacute rejection and identify antibodies that can be crossed with peri-operative desensitization. It has been recently shown that serum dilutions using SAB assay allow to determine which antibodies are likely to respond to desensitization treatment with plasma exchange. Ideally, virtual crossmatch should take into consideration not only MFI values, but also DSA titers, which may help identifying DSA that could respond to peri-operative desensitization and provide accurate prediction of the functional significance of the antibody. This approach will guide transplant physicians to select a peri-operative desensitization strategy and whether to proceed with a transplant in the face of a positive DSA for a specific recipient.

## Author Contributions

Conceptualization: M.M., S.K., O.T., and Y.T. Literature curation and writing of original draft: M.M., S.K., and O.T. Writing–review and editing: S.K., O.T., and Y.T.

## Competing Interests

The authors have declared that no competing interests exist.

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