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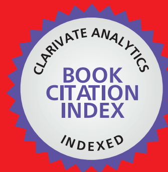

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Chapter

Novel Diagnostic and Therapeutic Approach to Antibody-Mediated Rejections in Heart Transplantation

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Abstract

Despite the improvement of immunosuppressive therapy in heart transplantation (HTx), antibody-mediated rejection (AMR) is still a great obstacle to prolong cardiac graft survival. Anti-donor-specific antibodies (DSAs), especially anti-donor human leukocyte antigen (HLA) antibody, lead to heart graft failure resulting in hemodynamic consequence and often in the recipient death. To prevent hyperacute rejection, prospective complement-dependent cytotoxicity test has been performed in every cardiac donor in Japan. But in other solid organ transplantations, flow cytometry crossmatch has been recently recommended to crossmatch to select the recipient in Japan as well as the world. However, flow cytometry is too sensitive to select the recipient, because not all DSAs determined by flow cytometry are cytotoxic to the cardiac graft. On the first complement classical pathway, alloantibodies bind to HLA antigens on cells of the graft and then recruit C1q, which is essential to make membrane attack complex and kill the cell. We review a role of the novel monitoring method of complement pathway regarding C1q in occurrence of AMR and its diagnostic and therapeutic significance in managing AMR in HTx.

Keywords: heart transplantation, antibody-mediated rejection, sensitization, complement binding donor-specific antibodies, C1q assay

1. Introduction

Although immunosuppressive therapy in heart transplantation (HTx) has been remarkably improved, antibody-mediated rejection (AMR) is still a great obstacle to prolong cardiac graft survival [1, 2]. AMR may develop when recipient antibodies against donor human leukocyte antigen (HLA) on the endothelial cells exist in the recipient serum [3]. The presence of circulating anti-donor-specific antibodies (DSAs) has several impacts on clinical outcomes both before and after HTx. The timing of sensitization against DSAs can be divided into the pre- and posttransplant periods [4]. The standard method to detect preformed antibodies at the time of HTx has been the complement-dependent cytotoxicity (CDC) test using recipient serum and donor leukocytes [5]. In Japan, only the donor heart with negative prospective CDC crossmatching with T lymphocytes has been transplanted, and none of 512 HTx consecutive recipients transplanted between 1999 and 2019 in Japan experienced hyperacute rejection (HAR) [6]. Although flow cytometry has been recently recommended to crossmatch to select the recipient in the world in solid organ

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transplantation, flow cytometry is too sensitive to select the recipient, because not all DSAs determined by flow cytometry are always cytotoxic to the cardiac graft cells [7–9]. Posttransplant antibodies produced before and after HTx are currently screened for evaluating AMR development using single antigen Luminex bead (SAB) assay or panel reactive antibodies (PRA) test to detect DSAs in the recipient serum. However, their clinical impact is not clear and may be less elucidated in HTx. Further optimal protocol for management strategies for AMR to reflect clinical prognosis is needed [4, 9]. A detection of circulating complement binding DSAs may be promising. We review the role of the novel management method using complement binding ability assay in prevention, diagnosis, therapy, and monitoring of AMR in HTx.

2. Overview of AMR in HTx

HAR, the immediate form of AMR may occur within 0 to a few days after HTx if a certain level of preformed DSAs exists in the recipient serum. Early AMR may occur during the first month, usually within 1 or 2 weeks after HTx because of newly production of de novo DSAs or enhanced production of preformed DSAs. These early type AMRs are usually associated with allograft dysfunction and hemodynamic compromise [2–4, 9–11]. Late AMR may occur months to years after HTx, most likely due to enhanced recognition [10, 12–15]. Approximately 50% of HTx recipients who develop rejection later than 7 years after HTx are associated with evidence of AMR [16]. Up to 24% of cases with late AMR has been reported concurrent with cellular rejection [17]. As more sensitive diagnostic tools have become available for detecting AMR in HTx, the evidence that AMR is a wide spectrum of immunologic injury that ranges from subclinical, histological, immunologic, serological findings and/or graft dysfunction was increased [4, 9, 18].

2.1 Clinical features and diagnosis of AMR in HTx

2.1.1 Hyperacute rejection in HTx

HAR is a rare cause of primary cardiac graft failure occurring within minutes to hours of aortic unclamping with a high mortality rate of around 70% in HTx [19, 20]. Preformed antibodies directed against donor HLA class I antigens or ABO antigen expressed on the donor vascular endothelium mediate complement deposition with widespread hemorrhage and thrombosis within the cardiac allograft [20]. HLA class II molecules are not usually expressed on the donor vasculature, but they can be induced by inflammation and injury associated with procurement and preservation of the heart graft. At last, antibodies against non-HLA endothelial antigens may also lead to HAR. Previous blood product transfusions (particularly platelets), mechanical circulatory support (MCS), pregnancy, and previous transplantation may increase the likelihood of the presence of preformed DSAs. The use of leukocyte-depleted transfusions may decrease the risk for DSA production. To prevent HAR, CDC PRA screening is used to determine the presence of circulating DSAs [5]. In Japan, by routinely performing prospective CDC crossmatching with T lymphocytes since the first HTx in 1999, no HTx recipients experienced HAR [6]. A higher PRA is associated with worse AMR rates and poorer overall survival [21]. In patients with a high percentage of PRAs (>10%), perioperative plasmapheresis combined with immunoglobulin therapy may be used to reduce the incidence and severity of HAR. These interventions have allowed for transplantation between donors and recipients with positive crossmatches. HAR is manifested as severe biventricular failure that, if immediate re-HTx cannot be carried out, is usually fatal [4, 9, 20].

Grade	Definition	Substrates
pAMR 0	Negative for pathological AMR	Histological and immunopathologic studies are both negative
pAMR 1 (H+)	Histologic AMR alone	Histological findings present, and immunopathologic findings are negative
pAMR (I+)	Immunopathologic AMR alone	Histologic findings are negative, and immunopathologic findings are positive (CD68+ and/or C4d+)
pAMR2	Pathologic AMR	Histologic and immunopathologic findings are both present
pAMR3	Severe pathologic AMR	Interstitial hemorrhage, capillary fragmentation, mixed inflammatory infiltrates, endothelial cell pyknosis, and/or karyorrhexis and marked edema and immunopathologic findings are present. These cases may be associated with profound hemodynamic dysfunction and poor clinical outcomes

AMR, antibody-mediated rejection; pAMR, pathological AMR.

Table 1.
Recent novel diagnosis criteria in immunopathologic features.

2.1.2 Acute antibody-mediated rejection in HTx

Symptoms of acute AMR are those of right and left ventricular systolic and diastolic dysfunction and include exertion dyspnea, orthopnea, paroxysmal nocturnal dyspnea, high jugular venous pressure, edema, and abdominal distention. In infants, those can include feeding intolerance, irritability, and poor body weight gain. Acute AMR is associated with hemodynamic compromise in 10–47% of cases [2, 4, 9, 18]. The symptoms and signs of hemodynamic compromise have been highly variable, and the spectrum of cardiac graft dysfunction may range from decreased ejection fraction to cardiogenic shock requiring inotropic support and/or MCS [18].

2.2 Pathological diagnosis of AMR

Although multiple imaging tools have been developed in the detection of AMR as well as cellular rejection, the best diagnostic strategies for AMR have not been established. Therefore, endomyocardial biopsy (EMB) remains the "gold standard" for establishing the diagnosis of AMR. Recent novel diagnosis criteria for AMR consist of immunopathologic criteria (**Table 1**) in addition to the clinical manifestation of AMR [3, 19].

3. Management of AMR

3.1 Preventive method related to AMR

3.1.1 Desensitization strategies

Specific preventive strategies are needed to enable successful HTx in highly sensitized patients, because the presence of DSAs reduces the chance to obtain compatible donors, extends waiting times to HTx, increases the risk of mortality during awaiting HTx, and raises the risks of acute AMR and cardiac allograft vasculopathy after HTx [22]. Despite the emergent application of the promising agents such as bortezomib which is a 26S proteasome inhibitor and eculizumab which is

a recombinant anti-C5 monoclonal antibody, a significant knowledge discrepancy remains with the current data for desensitization, investigated mostly from non-heart organ living donor transplants [23–28] and small observational studies in HTx [29–32]. The ideal desensitization strategy remains elusive especially in the HTx field. Moreover, clinical modalities to evaluate the efficacy of desensitization therapy are limited. Importantly, long-term outcomes and cost-effectiveness of desensitization strategies in HTx have not been well evaluated [4, 9].

3.1.2 Recipient selection in patients with preformed DSAs

As CDC assay is most clinically relevant methods for preventing accelerated early AMR as well as HAR in HTx [4, 9] but needs the technical expertise and experience, in many countries, before transplantation, the potential recipient is screened for circulating anti-HLA antibodies by using SAB assay or PRA test. If the percentage of PRA test is greater than 5–15%, most cardiac transplant centers require a negative prospective crossmatch between donor and recipient sera. However, the requirement for prospective crossmatch, which delays organ harvesting, may remarkably prolong a recipient's waiting time. On the other hand, in Japan, prospective CDC crossmatching with T lymphocytes has been routinely performed and completely avoided HAR since HTx program was started in 1999 [6]. But HTx must avoid false negatives because graft failure due to HAR may be directly life-threatening, so might need more sensitive tests.

3.2 Therapeutic method of AMR

3.2.1 Treatment of hyperacute rejection

According to the ISTH guideline 2016 [18], treatment for HAR should be initiated as soon as the diagnosis is defined, even when the patient is still in the operating room. Treatments for HAR include (1) high-dose intravenous (IV) corticosteroid (CS); (2) plasmapheresis; (3) IV immunoglobulin (IVIg); (4) cytolytic immunosuppressive therapy, such as antithymocyte or lymphocyte globulin; (5) calcineurin inhibitor (CNI) [cyclosporine (CYA) and tacrolimus (Tac)] and metabolic cycle inhibitors (i.e., mycophenolate mofetil; MMF); (6) IV inotropes and vasopressors; and (7) MCS.

Intraoperative myocardial biopsy is strongly recommended to confirm the diagnosis of HAR. Urgent re-HTx may be considered if the above therapies do not restore adequate cardiac graft function, but re-HTx for HAR has a considerably high mortality rate.

3.2.2 Treatment of acute antibody rejection (AMR)

Guidelines for treatment have recently been recommended by the ISHLT 2016 [18]. *Class II a* recommendations are followed.

1. To restore the immune-mediated cardiac graft injury in AMR: (1) high-dose IV CS and (2) cytolytic immunosuppressive therapy

2. To reduce circulating DSAs or their reactivity: (1) plasmapheresis, (2) immune apheresis (immunoadsorption), and (3) IVIg

3. To keep adequate hemodynamics: (1) IV inotropes and vasopressors and (2) MCS

4. When AMR is suspected, immunohistochemistry stains for complement split products (i.e., C4d) and possibly antibody should be added to standard histologic examination for EMB

5. The presence, quantity, and specificity of DSAs in the recipient serum should be screened

6. Follow-up EMB including immunohistochemistry staining should be performed 1–4 weeks after initiation of therapy

7. Maintenance immunosuppressive therapy after AMR treatment may be adjusted: (1) increase in the dose of current immunosuppressive agent(s), (2) addition of new agent(s), and (3) conversion to different agent(s) as shown below

Class II b recommendations are followed.

1. Systemic anticoagulation may reduce intravascular thrombosis in the cardiac allograft

2. Emergent re-HTx may be considered if the above therapies do not restore adequate cardiac graft function, but prognosis in this situation is poor

The benefit of treating subclinical AMR has not been elucidated. AMR might be a clinical-pathological continuation which starts with a latent immunological response of circulating DSAs with C4d deposition without clinical or histological changes, to a subclinical AMR, and finally to symptomatic AMR. A recent consensus recommends treating AMR in the presence of graft dysfunction regardless of histopathological finding, pAMR 2 in the absence of graft dysfunction if DSAs possibly relevant to AMR are present, and pAMR 3 regardless of the clinical findings [18].

3.2.3 Maintenance immunosuppressive strategies after treating AMR

The principles for the post-AMR management consist of reducing circulating DSAs and suppressing production of additional DSAs and T- and B-lymphocyte responses. However, currently there are only recommendations based on consensus [18].

The current available therapies are as follows: (1) suppression of the T-lymphocyte response (i.e., CS, MMF, cytolytic immunosuppressive therapy, photopheresis, or total lymphoid irradiation), (2) depletion of circulating DSAs (i.e., plasmapheresis), (3) suppression of residual DSAs (i.e., IVIg), (4) depletion of B lymphocytes (i.e., CS, rituximab, or splenectomy), (5) depletion of plasma cells (i.e., bortezomib), and (6) suppression of complement (i.e., eculizumab, IVIg).

4. What are the methods of detecting DSAs most relevant to clinical outcomes?

AMR in HTx is caused by the complex pathogenesis and immunopathologic pathway [2–4, 9, 18]. AMR develops when recipient serum contains DSAs against the endothelial layer of the cardiac allograft. Antibodies bind complement and activate the complement cascade, resulting in endothelial and myocardial injury. Complements, its fragments, and immunoglobulin are deposited on the

endothelium of the cardiac graft microvasculature and proceed inflammatory responses, such as release of cytokines, infiltration of macrophages, increased vascular permeability, and microvascular thrombosis, which results in cardiac graft dysfunction [2, 3].

The presence of circulating DSAs in HTx negatively impacts clinical outcome after HTx. Due to different clinical implications, DSA can be divided into preformed and de novo DSA by the time detected. Preformed antibodies can reduce the possibility to obtain a compatible donor heart and may increase the risk of AMR after HTx. With regard to post-HTx setting, considerable evidences about the impacts of DSAs on outcomes such as rejection, cardiac allograft vasculopathy, and survival have been reported [2–4, 9].

4.1 Currently used methods to assess anti-HLA antibodies

There are several anti-HLA antibody screening methods, each with varying sensitivities, specificities, and clinical usefulness [5–7].

4.1.1 Crossmatching

4.1.1.1 Complement-dependent cytotoxicity (CDC) test

Patel and Terasaki reported a significant correlation between a positive CDC crossmatch (or lymphocyte cytotoxicity test methods) and hyperacute and accelerated acute kidney graft dysfunction in 1969 [5]. Recipient serum is mixed with T and B lymphocytes from the donor and complement source is added. A cytotoxic reaction suggests the presence of complement fixing DSAs. The advantage of the test is its high positive predictive value for HAR or early acute AMR, making it well-defined that a patient should not undergo transplant with a particular donor. The disadvantage of this test is that it requires donor leukocytes and recipient serum prior to transplantation and that it is based on in vitro complement-mediated lysis which may not be of physiological relevance. This method using T lymphocytes has been used for prospective crossmatching in Japan to select the recipient in heart, lung, pancreas, and kidney transplantation. And that using B-lymphocytes is used for reference crossmatching to select the recipient transplanted with these organs according to each transplant center protocol [6, 9].

4.1.1.2 Flow cytometry crossmatching

Flow cytometry crossmatching method consists of reacting recipient serum with donor lymphocytes and adding a fluorescent-labeled anti-human immunoglobulin secondary antibody [7, 8]. The shifts in the distribution of fluorescence signals are detected in this assay. In flow cytometric crossmatching, complement sources are not added. As this assay is more sensitive at detecting physiological reactions than CDC, this assay is widely used for crossmatching in many countries in solid organ transplant. However, not all DSAs determined by this method are cytotoxic to the cardiac graft because this assay cannot evaluate complement fixation ability of DSAs [7, 8]. The false positivity of this method may decrease the likelihood of obtaining a compatible donor heart. Therefore, due to extremely more severe organ shortage in Japan than other developed countries, flow cytometric crossmatching is used only as a reference to select the heart recipient to increase a chance to obtain a compatible donor heart [9].

4.1.1.3 Virtual crossmatching

The virtual crossmatch protocol was introduced on October 2006 at Texas Transplant Institute for all sensitized patients waiting for deceased donor kidney transplantation [33]. Briefly, HLA typing antibody screening is performed using flow PRA screen beads (One Lambda) for the presence or absence of HLA class I and II antibodies. HLA class I and II single antigens (SA) include A, B, and Cw loci and DR, DQ, DRw, and DP loci, respectively. All final crossmatches are carried out by flow cytometry.

The rationale for recommending virtual crossmatching is double staged: (1) use methods having the sensitivity of DSA detecting assay nearly equal to the CDC crossmatch test and (2) reduce the time and cost for choosing a compatible deceased donor by no longer performing prospective CDC crossmatch test. By undoing prospective CDC crossmatch test, the average turnaround time is reduced by 3 hours which allows quicker organ allocation with reasonable assurance that the sensitized patients at the top waiting list will have an adequate deceased donor with negative final crossmatch [33].

4.1.2 Screening for the presence or absence of DSAs

4.1.2.1 CDC panel reactive antibody screening

The method to detect the anti-HLA antibodies had historically been the CDC assay [5]. The sera were analyzed using a manufactured frozen lymphocyte panels, which consist of mononucleated cells isolated from 60 or 72 healthy individuals of known HLA typing of A and B locus antigens [34]. However, as these CDC PRA cell panel are currently less available, and its technique is more complicated than flow PRA screening, more sensitive assays shown below are widely used. However, CDC PRA remains an alternative to define the level of patient desensitization, in cross-matching with a specific donor to avoid HAR [4, 9].

4.1.2.2 Flow PRA screening

Flow PRA screening uses panels of beads coated with the equivalent of whole cell's HLA class 1 or 2 [35]. Often used for initial screening, it gives a qualitative result on an incomplete panel. Luminex PRA uses panels of beads also coated with the equivalent of whole cell's HLA class 1 or 2. PRAs are more sensitive than CDC but less sensitive than SAB assay. These PRA methods require expert interpretation and there is a possibility to miss antibodies [9].

4.1.2.3 Single antigen Luminex bead assay

SAB assay uses microbeads coated with unique HLA antigen/allele on each bead [35] and detects a specific anti-HLA IgG antibody using a single HLA antigen/allele being interested. SAB assays are the most sensitive, specific, and definitive of the bead assays, but are often considered overreactive, with ambiguous clinical significance. SAB assays are now commonly applied for determining specificities and quantities of antibodies against antigen of interest. SAB assay for preformed HLA antibodies is useful as a reference to establish the protocol of desensitization strategies before and at the time of HTx, to select an adequate compatible donor heart, and to decide posttransplant immunosuppressive regimen [4, 9, 18].

4.2 Complement binding antibodies

Since complement activation plays a major role in antibody-mediated immune response on transplanted graft, detection of ability of antibodies to bind specific complement components seems to provide further clinical benefit for the diagnosis of AMR. Conventional solid-phase assays, such as SAB assay, cannot distinguish between complement binding and non-complement binding antibodies, and the intensity of antibodies by mean fluorescence intensity (MFI) may not be the best index of cytotoxic ability of antibodies because not all antibodies with high MFI may be prejudicial to graft function [9, 36]. Detection of antibodies capable of binding the component of classical pathway, such as C1q, C4d, or C3d, may indicate the potential for antibody-mediated cell injury. The complement binding antibody assay may be potentially more specific than conventional solid-phase assays to predict immune response to donor antigen and more sensitive than CDC assay. Although the diagnostic approach using complement binding DSAs seems to be supportive for the more adequate donor matching with sensitized recipients than the CDC assay or other conventional indirect methods such as SAB assay, the usefulness of complement-fixing DSA assay for desensitization or posttransplant monitoring in HTx remains unclear.

4.2.1 Cascade of complement activation

Complemental cascade is a multifunctional system of receptors and regulators as well as effector molecules [37]. The classical complement pathway can be initiated by the binding of antigen–antibody complexes to the C1q protein. The binding of C1q changes conformation and activates serine protease C1r which then cleaves and activates the serine protease C1s. The activated C1s cleaves C4, yielding C4a and C4b, and C2, yielding C2a and C2b. The larger fragments C4b and C2a generate the classical pathway C3 convertase. This convertase then cleaves C3 into a small C3a fragment and a larger C3b fragment. While the anaphylatoxin C3a interacts with its C3a receptor to recruit leukocytes, C3b contributes to further downstream complement activation. A larger C3b binds to the cell surface. C4b can be regulated by decay accelerating factor dissociating C4b and C2a. Successful regulation of C4 by factor 1 leaves C4d as an end product that truncates the complement cascade. C3b is more versatile than C4b. Factor I also can cleave C3b to ultimately produce C3d, which is a ligand for complement receptor type 2 on B lymphocytes. Moreover, C3b can generate an alternative C3 convertase with factor B, which allows C3b to start an amplification loop that can greatly increase the amount of C3b deposited on a cell surface. Finally, C3b binds to the C3 convertase, to generate C5 convertase, which cleaves C5 into C5a and C5b. Subsequent interactions between C5b and other terminal components C6, C7, C8, and C9 generate the membrane attack complex (MAC) or the C5b-9 complex which makes pores on the target cell membranes to lysing (**Figure 1**).

4.2.2 Principles of C1q, C3d, and C4d binding assays

The C1q binding SAB (C1q SAB) assay (One Lambda, Canoga, USA) aims at defining HLA antibodies capable to bind C1q. Serum samples are first heat-inactivated to eliminate interference of endogenous complement components. Sera are incubated together with recombinant C1q. An antibody capable to bind C1q is detected by a phycoerythrin (PE)-conjugated anti-C1q antibody (**Figure 2**) [38]. As IgM or IgG cannot be identified by this anti-C1q antibody, C1q binding anti-HLA antibodies detected can be either IgM or IgG isotype.

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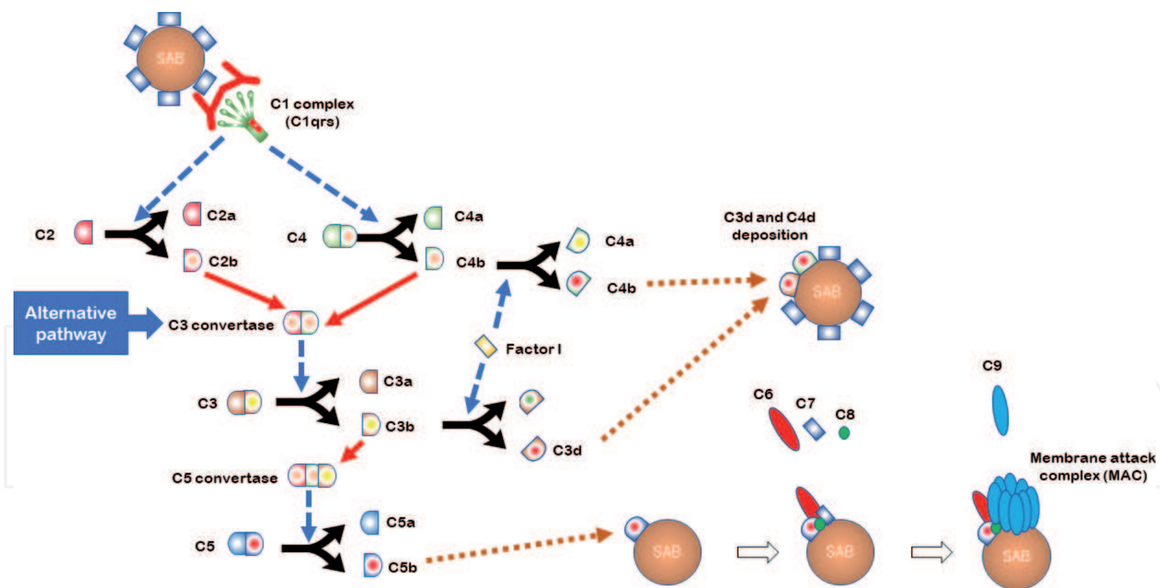
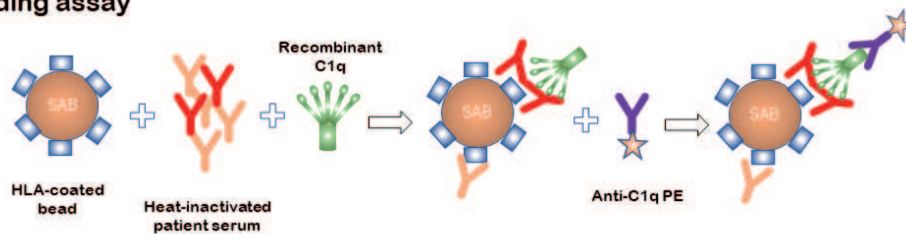


Figure 1.
Complement of classical complement pathway.

A: C1q binding assay



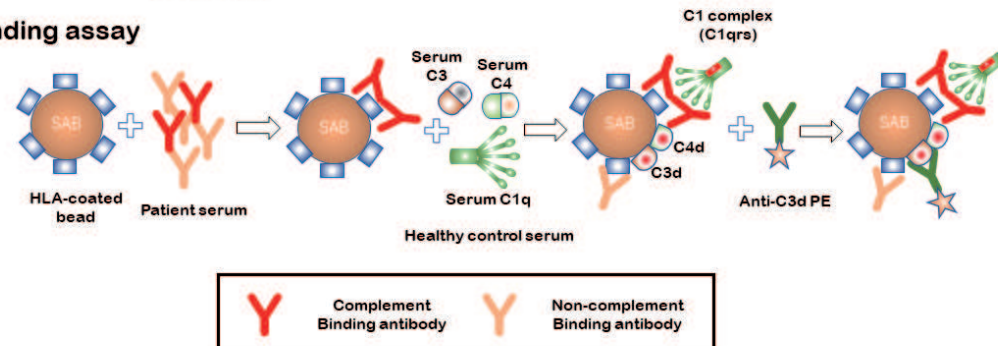


Figure 2.
Principles of C1q and C3d binding assays. (A) C1q binding assay: Heat-inactivated patient serum is incubated with single antigen beads (SABs) and recombinant C1q. Following a wash step, phycoerythrin-conjugated anti-C1q antibody is added to detect C1q binding HLA antibodies. (B) C3d binding assay: Patient serum is first incubated with SABs. Following binding of HLA antibodies to the beads, a healthy control serum as the complement source is added for further incubation. Following a wash step, PE conjugated anti-C3d antibody is added to detect C3d deposition on the beads.

Whereas C1q binding to antigen-bound antibody is the first step in activating classical complement pathway, binding of C1q to antibodies does not necessarily mean that all downstream events in this cascade will occur, as has been shown for human monoclonal HLA antibodies [39]. In this regard, C3d and C4d binding assays might be a more accurate modality to predict in vivo complement activation causing cell injury. As C1q binding to antibody triggers complement cascade once and then complement split products, such as C3d are clustered on beads in turn, the C3d assay may have a higher sensitivity than the C1q assay [40, 41]. As schematized in **Figure 2**, the C3d assay is like the C1q assay in methodology but does not need a recombinant complement product. Following an initial incubation of serum

samples with SABs, healthy control human serum is added as a source of complement, and then an anti-human C3d detection antibody is added.

4.3 Significance of anti-HLA antibodies before and after HTx

4.3.1 Preformed anti-HLA antibody (anti-HLA antibody made before HTx)

Clinical significance of pretransplant sensitization and current modality to detect preformed DSAs are already described above. In summary, as non-CDC crossmatching are more sensitive for detecting DSAs than CDC assay, flow cytometry crossmatching or virtual crossmatching is more prevalent in the world. However, in patients negative for CDC crossmatching, positive for flow cytometry crossmatching is not associated with higher incidence or severity of cardiac graft failure than negative for flow cytometry crossmatching. Its high sensitivity for DSA detection may result in increasing of false positive for predicting HAR or early acute AMR and decreasing the opportunity of sensitized candidates to obtain a compatible donor heart especially in Japan where donor shortage is extremely severe [6]. Therefore, more precise sensitive tests should be added to CDC-based strategies to avoid the false positive. In recent years, there has been a great interest in the detection of DSAs with complement binding capacity to overcome these issues.

4.3.2 Donor-specific anti-HLA antibodies posttransplant

The development of anti-DSAs after HTx has been implicated in allograft injury. DSAs which are produced by sensitization after transplantation are called de novo DSA. Both preformed and de novo DSAs should be assessed for monitoring the efficacy of desensitization therapy and posttransplant immunosuppressive regimen. Solid-phase assays, such as the SAB assay, are recommended to detect circulating antibodies. A percent of PRA greater than 10% or preformed DSAs at the time of HTx increases the risk for suboptimal post-HTx outcome. Monitoring for DSAs should be performed at 1, 3, 6, and 12 months post-HTx in accordance with ISHLT guidelines [18]. Patients at low risk should be monitored annually, and sensitized patients should be monitored more frequently. In any patient with symptoms or signs of graft dysfunction, DSA testing should be performed. The presence of DSA with graft dysfunction including restrictive physiology should be considered for AMR treatment.

4.4 Clinical significance of complement binding antibody in AMR

Although DSAs can induce a wide spectrum of graft injuries from no damage to severe myocardial or endothelial injury, not all DSAs are responsible for causing AMR or the poor prognosis post-HTx [41–43]. Since improved analysis is needed to better distinguish DSAs relevant to clinical outcome, SAB assays for detecting complement binding capability of HLA antibodies (C1q, C3d) have been introduced [43–46] with the hypothesis that complement binding antibodies induce more severe graft injury than their non-activating counterparts [41]. Several studies have revealed significant association of C1q or C3d binding DSAs with high incidence of AMR or poor graft or patient survival [44, 45]. Although several studies have compared two complement binding assays, the superiority or difference of these diagnostic utilities remains unclear. Therefore, C1q or C3d binding DSA assay cannot be a definitive method to detect DSAs relevant to AMR. However, these assays can be a supportive method to decide immunotherapy.

4.4.1 Clinical applications of C1q biding assay for preformed DSA

Over the past two decades, sensitization rates in adult HTX candidates (PRA > 10%) have doubled from 7.7 to 13.5% [1]. An increased incidence is expected due to the increased application of left ventricular assist devices (LVADs) as a bridge to HTx strategy, improved congenital heart disease surgery with patients surviving to require HTx, and increased re-HTx. Although development of desensitization strategies is needed to enable successful HTx in these highly sensitized patients, LVAD infections remain the most frequent complication of LVAD care with high morbidity and mortality, and desensitization method should not be over-immunosuppressive. Therefore, clinical tools to evaluate DSAs relevant to desensitization efficacy are more required in HTx than in other solid organ transplantations.

In our institute, we have used C1q SAB assay as an auxiliary method to evaluate the effects of desensitization therapy.

4.4.1.1 Case

A 43-year-old gentleman with dilated cardiomyopathy who had been supported by a NIPRO extracorporeal LVAD (NIPRO Corp, Osaka, Japan) for 1677 days underwent HTx. As he had received a transfusion of packed red blood cells and platelets at the time of LVAD implantation and packed red blood cells for gastrointestinal bleedings several times, he had high intensity anti-HLA antibodies and had not been selected as a recipient of several donor hearts due to positive CRC crossmatching against the particular donor T lymphocytes. Therefore IVIg (5 g/day) was given for 5 days for desensitization treatment. The antibody level of eight anti-HLA antibodies was higher than 15,000 MFI assessed by SAB assay before IVIG treatment. However, in seven of those antibodies, the antibody level assessed by C1q SAB assay was lower than that assessed by SAB assay. Although the antibody levels of anti-HLA antibodies assessed by SAB assay 1 month after IVIG treatment were higher than those before IVIG treatment, the antibody levels of all the anti-HLA antibodies assessed by C1q SAB assay 1 month after IVIG treatment were lower than those before IVIG treatment (**Figure 3**).

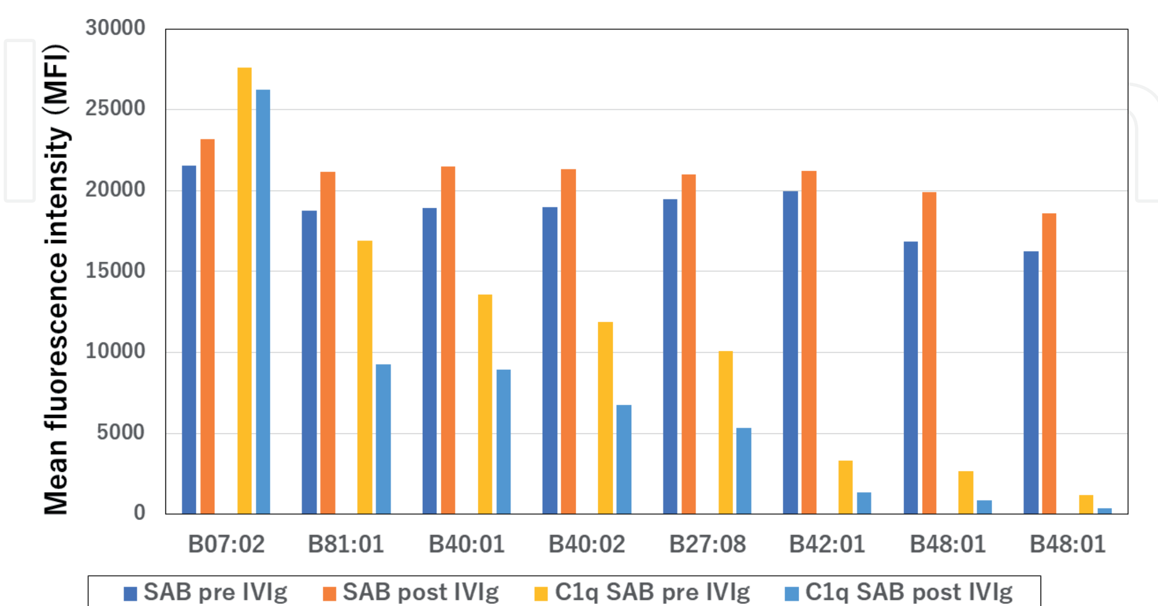


Figure 3.
Changes in mean fluorescence intensity by single antigen Luminex bead assay and C1q binding SAB assay before and 1 month after desensitization treatment. SAB, single antigen Luminex bead assay; C1q SAB, C1q binding SAB assay; IVIg, intravenous immune globulin.

After three courses of IVIg desensitization, he underwent HTx with a donor heart with negative prospective CDC crossmatching using the donor T lymphocytes. Although virtual SAB crossmatch showed preformed DSAs against HLA loci A2, A26, B35, and B65 with high MFI (**Table 1**), C1q SAB revealed that C1q binding abilities of these antibodies were all negative. Then we decided not to perform plasmapheresis or antithymocyte globulin in order to avoid over-immunosuppression. 20 mg of basiliximab was given IV just after discontinuing cardiopulmonary bypass and confirming hemostasis and at the 4th postoperative day. Maintenance immunosuppressive regimen consisted of Tac, MMF, and CS. Although routine EMB revealed pAMR 1 at 1 week after HTx, he experienced no hemodynamic compromise or cardiac graft dysfunction, and EMB revealed no further AMR or acute cellular rejection. He is currently at home with good cardiac graft function 2.5 years after HTx (**Table 2**).

4.4.2 Clinical application of C1q binding assay for maintenance immunosuppressive strategies

It is also known that not all patients with persistent production of DSA suffer loss of their allografts, indicating that DSAs are not equal in terms of their detrimental effects on allograft function. A C1q-positive de novo DSA has been reported to be associated with an increased rate of AMR and transplant glomerulopathy in kidney transplantation [46–49]. However, the prevalence and clinical significance of DSA characterized by C1q binding have not been well investigated in adult HTx patients [50–52].

In our clinical experience of 64 consecutive patients who received a HTx between May 1999 and January 2015, 12 patients had DSAs after HTx, but none had C1q binding antibodies. There were no significant differences in overall or cardiac event-free patient survival between DSA positive and negative patients with the same immunosuppressive regimen post-HTx (**Figure 4**). These data suggested that no reinforcement immunosuppressive regimen is needed, if the patient had no C1q binding DSA midterm after HTx [53].

			A		**B**		**Cw**	
HLA class I		**Recipient**	**33**	**—**	**44**	**—**	**14**	**—**
		Donor	**2**	**26**	**35**	**62**	**1**	**9**
DSA (MFI)	Pre-IVIG	SAB assay	11,429	57.3	13,725	13,911	90.4	397
		C1q SAB assay	0	0	0	0	1.35	0
	At HTx	SAB assay	3911	10.7	4629	7631	72.7	77.4
		C1q SAB assay	3.7	0	0	6.4	1.2	0.9
	2 months after HTx	SAB assay	315	37.9	633	3025	1300	12.1
		C1q SAB assay	0	0	0	0	0	0

HLA, human leukocyte antigen; DSA, donor-specific anti-HLA antibody; MFI, mean fluorescent intensity; SAB, single antigen Luminex bead assay; C1q SAB, C1q binding SAB assay; IVIg, intravenous immune globulin.

Table 2.
Human leukocyte antigen class I of the recipient and the donor and the antibody level of the donor-specific HLA antibodies assessed by single antigen Luminex bead assay and C1q binding SAB assay before IVIG therapy, at the time of heart transplantation and 2 months after heart transplantation.

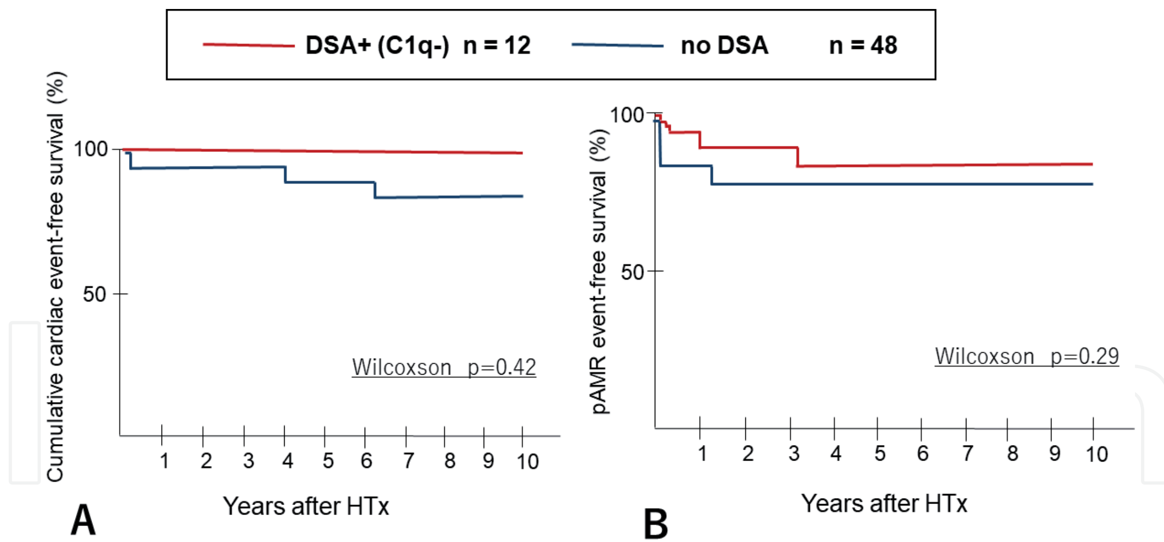


Figure 4.
Cumulative overall (A) and cardiac event-free (B) survival in patients with and without developing DSA. DSA, donor-specific anti-human leukocyte antigen; HTx, heart transplantation.

5. Conclusion

Over the past decades, sensitization rates in adult HTX candidates have doubled patients due to the expanding application of LVAD and prolonged waiting period. Sensitized HTx candidates have extended the waiting times for obtaining a compatible donor heart and increased mortality while waiting. An effective desensitization strategy has the potential to increase access to and success of HTx in sensitized patients, thus improving outcomes for this disadvantaged and growing transplant population. Although CDC PRA screening remains a standard method to define the efficacy of desensitization therapy, CDC PRA cell panels are currently less available, and its technique is complicated. Therefore, more sensitive assays flow PRA screening or SAB are widely used. However, flow cytometry is too sensitive to select the recipient, because not all DSAs determined by this method are cytotoxic to the cardiac graft. Although C1q or C3d binding DSA assay cannot be a definitive method to detect DSAs relevant to AMR, these assays can be a novel supportive method to decide immunotherapy.

Conflict of interest

None.

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