Management of allosensitized cardiac transplant candidates

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Abstract

Cardiac transplantation remains the best treatment in patients with advanced heart failure with a high risk of death. However, an inadequate supply of donor hearts decreases the likelihood of transplantation for many patients. Ventricular assist devices (VADs) are being increasingly used as a bridge to transplantation in patients who may not survive long enough to receive a heart. This expansion in VAD use has been associated with increasing rates of allosensitization in cardiac transplant candidates. Anti-HLA antibodies can be detected before transplantation using different techniques. Complement-dependent lymphocytotoxicity assays are widely used for measurement of panel-reactive antibody (PRA) and for crossmatch purposes. Newer assays using solid-phase flow techniques feature improved specificity and offer detailed information concerning antibody specificities, which may lead to improvements in donor-recipient matching. Allosensitization prolongs the wait time for transplantation and increases the risk of post-transplantation complications and death; therefore, decreasing anti-HLA antibodies in sensitized transplant candidates is of vital importance. Plasmapheresis, intravenous immunoglobulin, and rituximab have been used to decrease the PRA before transplantation, with varying degrees of success. The most significant post-transplantation complications seen in allosensitized recipients are antibody-mediated rejection (AMR) and cardiac allograft vasculopathy (CAV). Often, AMR manifests with severe allograft dysfunction and hemodynamic compromise. The underlying pathophysiology is not fully understood but appears to involve complement-mediated activation of endothelial cells resulting in ischemic injury. The treatment of AMR in cardiac recipients is largely empirical and includes high-dose corticosteroids, plasmapheresis, intravenous immunoglobulin, and rituximab. Diffuse concentric stenosis of allograft coronary arteries due to intimal expansion is a characteristic of CAV. Its pathophysiology is unclear but may involve chronic complement-mediated endothelial injury. Sirolimus and everolimus can delay the progression of CAV. In some nonsensitized cardiac transplant recipients, the de novo formation of anti-HLA antibodies after transplantation may increase the likelihood of adverse clinical outcomes. Serial post-transplantation PRAs may be advisable in patients at high risk of de novo allosensitization.

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1. Background

Cardiac transplantation has evolved over the last several decades to become the best available therapy in select patients with advanced heart failure with a high probability of death. The evolution in the field has been propelled by the development of newer, more effective immunosuppressive agents that decrease the likelihood of acute cellular rejection and increase post-transplantation survival while having modest effects on the incidence of infection and malignancy after transplantation. However, despite encouraging progress, the availability of donor hearts remains rate-limiting in the provision of transplantation to those in need [1]. An inadequate number of available hearts means longer wait-list times for many transplant candidates, with a potential for higher wait-list mortality for the sickest patients.

Recognizing the limitations of the donor pool, pioneer cardiothoracic surgeons in the late 1960s ushered in an alternative for cardiac transplant candidates who would not live long enough to obtain a new heart. This technology involved mechanical circulatory support with a total artificial heart or ventricular assist devices (VADs). Mechanical circulatory support as a bridge to transplantation was introduced in 1969 when the first total artificial heart was implanted as a bridge to transplantation. Initially, the technology had major disadvantages that limited its widespread applicability, but for the last 40 years, tremendous progress has been achieved. In the mid-1990s, wearable implantable VADs began to be used widely as a bridge to transplantation [2]. By the end of the last decade, the mechanical performance and clinical benefits of VADs had
noticeably outweighed their drawbacks. With broader utilization of VADs, higher rates of allosensitization were increasingly recognized in supported transplant candidates [3-5], complicating the ability to obtain an appropriate donor organ.

In view of the inadequate supply of donor hearts and the growing prevalence of heart failure in developed countries, it is expected that the number of patients with advanced heart failure requiring bridging to transplantation with VADs will increase. Recently published data show that the mean survival of United Network for Organ Sharing status 2 patients on the cardiac transplant waiting list has improved since 1990 and currently matches mean post-transplantation survival at 1 year. This observation suggests that the risk-benefit ratio may not favor transplantation in patients listed as status 2 [6]. In the coming years, primarily those patients who are eligible for status 1 will be likely to receive a heart transplant. Currently, the status 1 category on the heart transplant wait-list is largely populated by VAD-supported patients, and this phenomenon is expected to grow in the future. Understanding this trend in cardiac “transplantability” is fundamental in recognizing the increasing challenge that allosensitization represents for the ever-growing number of cardiac transplant candidates that are bridged to transplantation with VADs. Pretransplantation and posttransplantation allosensitization has been associated with outcomes that impact allograft survival negatively; therefore, effective strategies to prevent and decrease allosensitization in this population are necessary.

This review will focus on the clinical aspects of allosensitized cardiac transplant recipients. We will discuss methods for determining allosensitization, risk factors for allosensitization, the impact of allosensitization before and after cardiac transplantation, and available strategies to decrease sensitization in patients awaiting heart transplantation and to treat antibody-mediated rejection (AMR) after transplantation.

2. Detection of anti-HLA antibodies

Histocompatibility testing identifies appropriate donor-recipient pairs to achieve successful transplantation. Pretransplantation crossmatching identifies recipient serum antibodies that react with donor antigens, a condition that defines the concept of allosensitization. It is critically important to determine whether these antibodies may increase the risk of post-transplantation adverse outcomes, as is the case with anti-HLA immunoglobulins [7].

Screening for allosensitization through the detection of anti-HLA antibodies is at the core of compatible donor selection in solid organ transplantation. One of the major limitations of our current understanding of histocompatibility testing is the lack of complete knowledge regarding which antibody specificities are likely to increase the risk of post-transplantation complications. The limited specificity of certain crossmatch techniques has confounded this issue further.

Anti-HLA antibodies are directed to donor major histocompatibility complex class I and II HLA antigens that are expressed on allograft endothelial cells. The risk of early graft failure after transplantation is higher in the presence of a positive crossmatch with donor HLA antigens due to circulating recipient antidonor antibodies. To detect allosensitization, transplant candidates undergo testing that exposes HLA antigens from random individuals to the recipient’s serum through a variety of different techniques, collectively referred to as a panel-reactive antibody (PRA) test [8,9]. The rationale for PRA testing in cardiac transplant candidates comes from previous experience in kidney transplantation, showing an inverse relationship between PRA level and allograft survival [10-12]. Most early studies identified HLA class I and II antibodies with complement-dependent lymphocytotoxic techniques; however, progress over the years has made other analytical methods available, including flow cytometry and solid-phase flow methods. Over the last 4 decades, technological progress has led to the introduction of different methods for antibody detection [13]. Table 1 includes a summary of the characteristics of currently used antibody-detection techniques in recipients awaiting heart transplantation.

2.1. Clinical relevance of detected antibodies

The histocompatibility testing techniques used vary among centers. The complement-dependent lymphocytotoxicity assay (CDC) continues to be broadly used in all cardiac transplant candidates as an initial screening method to rule out an increased PRA and as a rapid crossmatch technique. Considering the limited specificity of this

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Table 1

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<thead>
<tr>
<th>Characteristic</th>
<th>Cell-based techniques</th>
<th>Solid-phase techniques</th>
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<tbody>
<tr>
<td>Sensitivity</td>
<td>CDC &lt; CDC + DTT</td>
<td>ELISA &lt; flow</td>
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<tr>
<td>HLA antigens</td>
<td>Natural configuration on cell surface, unable to detect specificities</td>
<td>Isolated proteins bound to artificial surface, may detect specificities if single antigens are used</td>
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<tr>
<td>False-positive reactions</td>
<td>Non-HLA specific antibodies</td>
<td>Reactions with cryptic epitopes on denatured HLA molecules</td>
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<tr>
<td>False-negative reactions</td>
<td>Antibody levels below detection threshold</td>
<td>Loss of epitope expression on isolated molecules</td>
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technique, patients who have a PRA of 10% or higher undergo further testing with more specific methods, typically flow cytometry or antibody detection using single antigen beads in a Luminex template. These tests can identify high-risk class I or class II anti-HLA antibodies and commonly offer perspective regarding the likelihood that a particular candidate will receive a new heart within a reasonable time frame. Unacceptable donor antigens can also be identified, making possible the use of virtual crossmatching in some cases [14].

Complement-dependent lymphocytotoxicity has been studied specifically in the context of heart transplantation outcomes. In the early 1990s, Lavee and colleagues [8] described how a CDC PRA of 10% or higher was associated with an increased incidence of acute cellular rejection and cardiac allograft vasculopathy (CAV) in the early post-transplantation period. Smith and colleagues [15] also showed that heart transplant candidates transplanted against a positive crossmatch had drastically reduced allograft survival during the first year when compared with patients with a negative crossmatch. In a retrospective study of heart transplant recipients, Kobashigawa and colleagues [16] found that transplant candidates with a PRA of 11% or higher detected by CDC had higher post-transplantation mortality when compared with those with a lower PRA. Eighty-eight percent of deaths in allosensitized patients occurred within 3 months after transplantation and were mostly due to immune-related causes (allograft rejection and CAV) [16].

Although flow PRA results correlate well with post-transplantation clinical outcomes [17], data are lacking on the clinical use of newer solid-phase assays such as antibody detection using single antigen beads in a Luminex template to estimate the likelihood of AMR, CAV, or allograft survival. In addition, contemporary solid-phase techniques based on the Luminex template use recombinant HLA antigens, which may not be identical in shape to the HLA antigens found on donor cell surfaces. This fact may raise questions concerning the validity of the information obtained. It is also clear that besides offering information on known specificities, more specific techniques offer a wealth of data concerning less known antibodies, which have uncertain clinical significance. While transplantation centers that use contemporary antibody-detection technologies rely on their results to make clinical decisions, evidence-based data on the clinical utility and prognostic significance of newer methods for histocompatibility testing are limited.

Post-transplantation outcomes in VAD recipients are also affected by allosensitization, as evidenced by slightly higher rates of allograft rejection. However, overall allograft survival seems to be unaffected [18,19]. Recent small studies of heart transplant candidates supported with VADs have offered limited evidence into the relevance of pretransplantation allosensitization in this specific group in the contemporary era. Schmid and colleagues [20] followed 41 patients bridged with VADs and found that post-transplantation survival and the incidence of allograft rejection were comparable with those of controls without VAD support. Pamboukian and colleagues [21] studied 98 patients with and without VAD support and reported on their rates of allosensitization and post-transplantation outcomes. Even though VAD patients had a higher likelihood of having a PRA of 10% or higher (19% vs 2%), this was not associated with higher rates of allograft rejection or vasculopathy. Post-transplantation survival was unaffected in VAD-supported patients. Other studies have found similar rates of allosensitization in VAD recipients; however, this factor does not seem to affect the likelihood of unfavorable immune outcomes or allograft survival [22,23].

The apparent lack of impact of allosensitization on post-transplantation outcomes in VAD recipients may be related to more aggressive immunosuppression used in this group. Because of their higher PRAs, VAD-supported heart transplant candidates are more likely to receive desensitization therapies before transplantation, and allograft rejection episodes may be treated more aggressively. Post-transplantation survival rate in patients supported with VADs is similar to that of nonsupported patients, yet the causes of death are different. Up to 75% of post-transplantation mortality in VAD-supported heart recipients is related to infectious complications, perhaps related to the more aggressive immunosuppression they receive, or direct effects of some VADs on the immune system, whereas rejection may account for 20%. Nonsupported transplant recipients commonly die of rejection (38%), ischemic complications (31%), and respiratory failure (23%) [24].

3. Mechanisms of allosensitization

3.1. Allosensitization by exposure to foreign antigens

Commonly recognized risk factors for allosensitization in all transplant candidates include previous allografts, blood product transfusions, and pregnancy [5]. As explained above, the use of VADs as a bridge to transplantation has also been recognized as a risk factor for allosensitization resulting in an increased PRA [25,26]. The most frequent cause of allosensitization before cardiac transplantation is previous blood transfusions. With the increasing number of older patients who have undergone previous cardiac surgery being listed for transplantation, the number of sensitized candidates is likely to increase. Although cardiac retransplantation represents less than 3% of all cardiac transplants [1], patients with prior allografts are also more likely to be sensitized. Finally, women with a history of pregnancy may have become sensitized to paternal antigens [27,28].

3.2. Ventricular assist devices as risk factors for allosensitization in cardiac transplant candidates

Ventricular assist devices consist of a combination of nonbiological and bioprosthetic materials, in continuous
contact with circulating blood (Fig. 1). Before VAD introduction, it was known that common inert materials trigger host responses that include inflammation, fibrosis, and coagulation, and, not unexpectedly, similar responses were seen in VAD recipients [9,29]. These responses contribute to the pathogenesis of complications seen in
VAD recipients, such as thromboembolism and systemic infection. The incidence of thromboembolism has decreased significantly in VADs that feature a textured interior surface that promotes the growth of a neointimal layer. However, the development of a neointima is associated with the deposition of cytokine-releasing macrophages and activated helper T lymphocytes on the VAD surface [9]. These helper T lymphocytes show a heightened level of activation when compared with those of controls with advanced heart failure. Their activation profile is marked by enhanced spontaneous proliferation after interleukin-2 (IL-2) stimulation but contrasted by a susceptibility to premature cell death, as evidenced by surface expression of CD95, a marker of activation-induced apoptosis. In addition to a susceptibility to early cell death, T lymphocytes of VAD recipients exhibit impaired proliferative responses to T-cell receptor–mediated activation [30]. The combination of these observations may result in an impairment of cellular immunity in VAD recipients, with vulnerability to systemic Candida infections [30]. Activated T lymphocytes in VAD patients selectively express Th2-type cytokines, such as IL-4 and transformation growth factor β. It has been postulated that an excessive load of circulating apoptotic waste and the predominant expression of Th2-type cytokines are responsible for B-lymphocyte hyperreactivity, evolution into plasma cells, and autoimmune body production in VAD recipients. These patients show a 3- to 4-fold frequency of anti-HLA class I and II immunoglobulin G (IgG) levels (Fig. 2) and also significantly higher levels of antiphospholipid antibody when compared with controls with advanced heart failure [9]. Although anti-HLA IgM antibodies that may cause a positive crossmatch are also produced under these circumstances, there is no definite association between their presence and deleterious effects after cardiac transplantation (Table 2) [15,31].

Polyclonal expansion of B lymphocytes and their subsequent hyperreactivity may also be associated with increased levels of CD40 ligand (CD40L) derived from inappropriately activated T lymphocytes. CD40 is a member of the TNF receptor superfamily that is expressed in B lymphocytes and has an important role in stimulatory pathways resulting in B-cell survival and proliferation. Its ligand, CD40L, is expressed by activated T lymphocytes. Its circulating form in serum has been found to be biologically active in terms of B-lymphocyte activation and is predictive of autoantibody formation and autoimmune disease activity [32]. In a study of 111 patients supported with textured pulsatile left VADs (LVADs) as bridge to transplantation, Schuster and colleagues [32] showed that increased serum

<table>
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<tr>
<th>Cells</th>
<th>Found on LVAD surface; stimulate T-lymphocyte activation via IL-2 receptor pathways</th>
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<tr>
<td>Activated macrophages/monocytes (CD14⁺, CD68⁺, NFκB+)</td>
<td>Found in circulation, on LVAD surface; in heightened activation state, prone to apoptosis, poor response to TCR-mediated activation</td>
</tr>
<tr>
<td>CD95(Fas)+ T lymphocytes</td>
<td></td>
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<tr>
<td>Hyperreactive B lymphocytes</td>
<td>Found in circulation; release anti-HLA class I and II IgG, antiphospholipid antibody</td>
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<tr>
<td>Cytokines</td>
<td></td>
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<tr>
<td>IL-2</td>
<td>Promotes T-cell activation, downregulated by selective loss of Th1 T-cells</td>
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<tr>
<td>IFN-γ</td>
<td>Promotes T-cell activation, downregulated by selective loss of Th1 T-cells</td>
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<tr>
<td>IL-10</td>
<td>Stimulates B-cell hyperreactivity</td>
</tr>
<tr>
<td>sCD40L</td>
<td>Stimulates B-cell hyperreactivity</td>
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IFN indicates interferon; sCD40L, soluble CD40 ligand; TCR, T-cell receptor.

![Fig. 2. PRA levels in VAD-supported vs unsupported cardiac transplant candidates. Patients who underwent VAD support before transplantation demonstrate an increase in 90th percentile (taller bar) and mean (the horizontal line within each bar) PRA levels when compared with non-VAD controls (P < .0001). Reprinted from J Heart Lung Transplant, volume 25, Joyce et al, Impact of left ventricular assist device (LVAD)–mediated humoral sensitization on post-transplant outcomes, pages 2054-9, copyright 2005, with permission from Elsevier.](image)

![Fig. 3. Increased levels of soluble CD40L in the circulation of allosensitized LVAD recipients. Sensitized LVAD recipients had more than 8-fold higher levels of serum CD40L when compared with either nonsensitized LVAD recipients, heart failure controls, or healthy volunteers. Results are expressed as the mean ± SEM of experiments using sera from 12 sensitized LVAD recipients, 10 nonsensitized LVAD recipients, 8 New York Heart Association class IV heart failure controls, and 6 healthy volunteers. Reprinted from Hum Immunol volume 63, Schuster et al, B-cell activation and allosensitization after left ventricular assist device implantation is due to T-cell activation and CD40 ligand expression, pages 211-20, copyright 2002, with permission from Elsevier.](image)
levels of CD40L are associated with clinical allosensitization detected with a CDC in VAD recipients (Fig. 3).

Perioperative platelet transfusions also result in the development of anti-HLA class I antibodies in VAD recipients [4,33]. Red blood cell transfusions seem to have a less significant impact on the level of circulating anti-HLA antibodies, especially when leukocyte-reduced products are used [34,35]. Anti-HLA class II antibody levels do not seem to be affected by blood product transfusions. HLA-DR3 may be associated with a higher likelihood of developing anti-HLA class II antibodies in VAD recipients [9].

There is some evidence to suggest that the degree of sensitization may vary between different VAD types, being lower for devices without a textured interior surface and axial flow devices owing to a smaller area of contact between the device and bloodstream, with a lesser degree of immune activation [36,37]. Some early data on patients with axial flow devices (MicroMed DeBakey LVAD) showed increased production of C5a and IL-6 during the first 12 weeks after implantation when compared with patients who received a nontextured pulsatile device (Novacor LVAD). This finding created concern for the possibility of increased B-cell activation and subsequent sensitization mediated by IL-6 [38]. However, investigators in that study did not look at anti-HLA antibodies or post-transplantation outcomes in those patients.

More recent data have shown that the initial immune system abnormalities noted after the implantation of axial flow pumps, including CD95-mediated T-lymphocyte activation and apoptosis, seem to resolve after 7 weeks [39]. This improvement in immune activation is thought to account for the lower degree of sensitization more commonly seen in patients with axial flow devices. A small study in patients supported with an axial flow pump who had no anti-HLA antibodies before implantation found that there were no detectable levels of anti-HLA class I or class II IgG after a mean follow-up period of 87 days after implantation. Furthermore, acute cellular rejection episodes and post-transplantation survival within the first year were comparable to published statistics in patients without mechanical circulatory support [40].

4. Management of allosensitized cardiac transplant candidates

The management of allosensitized cardiac transplant candidates presents steep challenges for transplantation cardiologists and surgeons. The differences in specificity of different antibody-detection techniques, the uncertainty about which antibody specificities are relevant, and the incomplete understanding of B-cell immunity in allotransplantation make solid progress in this area difficult. There is limited available literature on strategies to treat allosensitization in cardiac transplant candidates, and much of the current therapeutic practices are derived from experience with the transplantation of other solid organs. It is clear, however, that allosensitized patients experience delays to transplantation because of the relatively limited acceptable donor pool imposed by their anti-HLA antibody specificities [9,35] (Fig. 4).

The initial step in managing allosensitized transplant candidates is to avoid further exposure to foreign human antigens by minimizing the transfusion of blood products as much as possible. Once a cardiac transplant candidate has become sensitized, traditionally indicated by a PRA of 10% or higher, the time required to wait for a donor that is crossmatch-negative may be prohibitive. Therefore, measures to decrease the likelihood of having a positive prospective crossmatch, which could result in transplantation delays and a higher chance of dying on the wait-list, should be considered.

In patients ill enough to require VAD support, decreasing the levels of circulating alloantibodies is of particular importance. Contemporary VADs have limited durability and continuously expose patients to serious complications, including systemic infection, hemolysis, and thromboembolism. Some of these complications can jeopardize the candidate’s eligibility for transplantation and also can profoundly affect quality of life and survival. Until further improvements are made to mechanical circulatory support devices, the goal at most centers is to transplant VAD recipients as expeditiously as possible after an initial period of postoperative recovery, to allow improvement in end-organ function and physical rehabilitation. High degrees of allosensitization pose a great threat for this patient subset, and broader regional sharing for status 1A and 1B heart recipients makes crossmatching challenging.
4.1. Methods to decrease levels of allosensitization

4.1.1. Plasmapheresis

Mechanical removal of circulating antibodies with plasmapheresis has been used in highly allosensitized cardiac transplant candidates to decrease the likelihood of allograft rejection. It can be implemented in patients with a high PRA, either en route to the operating room on the day they will receive their transplant or while on the wait-list to decrease their PRA and increase the likelihood of finding a negative crossmatch donor. It has been often combined with varying doses of intravenous immunoglobulin (IVIG). There are data that support the preoperative use of plasmapheresis and IVIG, showing post-transplantation outcomes similar to those of nonallosensitized patients. In a study by Pisani et al [41], 16 of 118 cardiac transplant candidates were found to have a PRA of 10% or higher, with a high rate of positive crossmatch. All sensitized patients underwent plasmapheresis in combination with IVIG immediately before transplantation. The frequency of rejection and allograft survival was similar between sensitized patients who underwent plasmapheresis and IVIG and nonsensitized controls [41]. Similar results have been reported by other investigators with small case series [42,43].

4.1.2. Intravenous immunoglobulin

Intravenous immunoglobulin has also been used alone to decrease the level of allosensitization before heart transplantation, especially in VAD recipients. In a series of 4 VAD recipients who developed high levels of allosensitization after device implantation, treatment with IVIG resulted in decreases in their PRA to levels below 10% within 4 months from administration [44]. John et al [45] published a head-to-head comparison of IVIG and plasmapheresis in 55 allosensitized VAD recipients before transplantation. Intravenous immunoglobulin resulted in a mean reduction of 33% in anti-HLA class I reactivity 1 week after treatment, with minimal adverse effects. Plasmapheresis achieved similar results but required longer treatments and was associated with a higher rate of infectious complications (Fig. 5). In patients whose PRA did not respond to low-dose IVIG, higher doses achieved comparable reductions in the degree of allosensitization, with an observed increased incidence of acute renal failure. Notably, in this series, treatment with IVIG significantly reduced wait time to transplantation [45].

4.1.3. Rituximab

In recent years, rituximab, a chimeric monoclonal antibody to CD20, has been used anecdotally to diminish the degree of allosensitization in cardiac transplant candidates. Rituximab depletes B lymphocytes through complement-dependent cytotoxicity, antibody-dependent cytotoxicity, and induction of apoptosis [46]. Animal studies in baboons have shown that this agent effectively depletes CD20+ B lymphocytes, resulting in significant blunting of IgM and IgG responses. Studies in humans have focused mostly on the use of rituximab to treat B-cell lymphomas; however, it has also been used to treat autoimmune disorders and in organ transplantation, recognizing its immunomodulatory properties [47]. A study in allosensitized patients on dialysis awaiting kidney transplantation found that while rituximab was a powerful B-cell ablational agent, some B-cell subpopulations such as CD19+/CD5+ B cells recovered to baseline levels within 6 months. Other subsets, such as B memory cells, may remain suppressed for up to 2 years after treatment [48]. These findings suggest that alloreactivity may not be fully suppressed after rituximab administration.

Most of the available data on treating allosensitized transplant candidates with rituximab come from the renal transplant literature. Vieira et al [49] selected 9 highly sensitized kidney transplant candidates to be treated with escalating single doses of rituximab. They achieved significant B-lymphocyte depletion with a moderate decrease in PRA in 2 patients and the suggestion of loss of antibody specificities in 5 patients. Two patients showed no
change in their PRA after rituximab. These effects were associated with infectious complications in some patients.

Among very few early reports in the heart transplant literature, Balfour et al [50] reported the use of rituximab in a pediatric transplant candidate who had failed previous treatments with IVIG, plasmapheresis, and mycophenolate mofetil. The patient was successfully transplanted after a crossmatch-negative donor was found [50]. Unfortunately, published experience with rituximab in allosensitized cardiac transplant candidates is scarce at this time. Further data are needed to determine the usefulness of rituximab in allosensitized transplant candidates.

5. Clinical outcomes in allosensitized cardiac transplant candidates

Pretransplantation allosensitization increases the likelihood of AMR and CAV and decreases overall allograft survival (Fig. 6) [3,15,16,51-53]. The relationship between anti-HLA antibodies before transplantation and the development of these conditions is well recognized, emphasizing the importance of proper PRA screening and assignment of appropriate donor-recipient matches.

5.1. Antibody-mediated rejection

Antibody-mediated rejection is not an unusual occurrence [54]. Institutional experience suggests that the incidence of AMR may be approximately 15% [5]; however, this figure varies depending on the patients studied and the diagnostic methods used. In a series where patients were commonly given OKT3, AMR was evident in up to 52% of cases, whereas in a series where C4d deposition associated with allograft dysfunction is used as the diagnostic criterion, the reported incidence has been as low as 3% [53]. Acute AMR typically occurs shortly after transplantation, usually within the first 4 months but more commonly during the first 4 weeks after transplantation [55]. However, it can occasionally present several months or years after transplantation. Up to 68% of patients who develop AMR early on show evidence of significant allograft dysfunction, in contrast with those in whom AMR presents late, in whom the frequency of allograft dysfunction is estimated at 13% [5,53].

The pathophysiology of AMR is not fully understood. It is likely the result of antibody-induced, complement-mediated activation of endothelial cells. The process continues with cytokine release and increased endothelial adherence of leukocytes, culminating in allograft ischemic injury [56].

C4d, an inactive byproduct of complement activation, has been observed in the capillaries of cardiac allografts with AMR, suggesting recent complement activity [57].

The criteria to diagnose AMR have not been uniform. Clinically, it presents with hemodynamic compromise in 29% to 47% of cases, especially when it occurs early after transplantation. The criteria for hemodynamic compromise also vary between centers but generally include a decrease in left ventricular ejection fraction, unexplained elevation in cardiac filling pressures with a simultaneous decrease in cardiac output, and the need for inotropic support. In addition to clinical signs, pathologic markers of AMR have to be present.

In 2005, the International Society for Heart and Lung Transplantation proposed criteria for the immunopathologic diagnosis of AMR [5]. In the presence of clinical evidence of allograft dysfunction, the following diagnostic criteria were suggested:

- Histologic evidence of acute capillary injury (endothelial swelling or denudation with congestion and macrophage infiltration, with possible neutrophil infiltration and interstitial edema and/or hemorrhage in more severe cases),
- Immunopathologic evidence for antibody-mediated injury (tissue immunofluorescence positive for IgM or IgG + complement deposition [C3d, C4d, or C1q], or CD68-positive macrophages in endothelium; endovascular fibrin can be seen in more severe cases),
- Serologic evidence of donor-specific anti-HLA class I or class II antibodies, or other antidonor antibodies at the time of biopsy.

Even though immunoglobulin deposits are part of the proposed diagnostic criteria for AMR, immunofluorescence for endothelial immunoglobulin deposits does not correlate well with clinical AMR or with circulating anti-HLA antibodies [58]. Recently, immunofluorescence for C4d has shown a high degree of correlation with serum anti-HLA antibodies [57]. Rodriguez et al [59] studied 665 consecutive endomyocardial biopsies from 165 cardiac transplant recipients with immunofluorescence for the presence of immunoglobulin and complement deposits. The combined detection of C4d and C3d correlated well with acute AMR and clinical evidence of heart failure. The additional

Fig. 6. Survival according to CDC crossmatch results in 636 cardiac transplant recipients between 1982 and 1992. Reprinted from Transpl Immunol volume 1, Smith et al., The effect of panel reactive antibodies and the donor specific crossmatch on graft survival after heart and heart-lung transplantation, pages 60-5, copyright 1993, with permission from Elsevier.
detection of immunoglobulin and C1q did not improve diagnostic accuracy [59]. In addition, some early evidence suggests that immunofluorescence for C4d may be helpful in assessing the response to treatment for AMR in cardiac transplant recipients [57,60].

Antibody-mediated rejection causes very severe episodes of rejection, often with hemodynamic compromise, that may not respond to intensification of immunosuppressive therapy alone. In addition to hemodynamic support with inotropic agents, current therapeutic strategies center on inactivation of circulating antibodies.

5.1.1. Treatment of AMR

Recognizing the importance of antibody production and complement deposition in allograft endothelium as the underlying pathophysiology in AMR, mechanical removal of circulating antibodies with plasmapheresis was one of the first therapies tested on affected patients. In a series of 328 cardiac transplant recipients in the late 1980s, 3.4% of patients were found to have a positive prospective IgG crossmatch. These patients experienced acute AMR much earlier than did controls with a negative crossmatch. Moreover, AMR was associated with hemodynamic compromise in 73% of cases. In addition to intensification of immunosuppression, plasmapheresis was used in the patients with AMR, with a treatment success rate of 75% [55]. Other smaller series in Europe and Asia have documented similar experiences [61,62]. High-dose intravenous corticosteroids, T-lymphocyte depleting agents such as rabbit anti-thymocyte globulin, and tacrolimus have been used in addition to plasmapheresis, with varying degrees of success. Intravenous immunoglobulin has also been used to provide further immune modulation in some patients with AMR. More than 90% of patients treated aggressively for AMR recover but remain with a high risk of recurrence [53].

A few case series and case reports have also documented successful treatment of AMR with rituximab-based therapy [63-65]. Garrett et al [66] treated 8 patients with pathologic evidence of AMR with weekly doses of rituximab for 4 weeks. All patients recovered their baseline LVEF without associated infectious or other drug-related complications [66].

5.2. Cardiac allograft vasculopathy

Allosensitization also contributes significantly to an increased risk of developing CAV [52,56,59,67,68]. Cardiac allograft vasculopathy is a major cause of cardiac allograft failure and decreased survival after the first post-transplantation year [67]. Although it is possible that both T- and B-lymphocyte–mediated immunity play a role in its pathogenesis, patients who demonstrate circulating donor-specific anti-HLA antibodies, or an immunohistologic pattern of AMR early after transplantation, are more likely to develop CAV [52].

Cardiac allograft vasculopathy causes diffuse concentric stenosis of the coronary arteries because of intimal expansion and adventitial sclerosis. Although detailed human studies on the pathogenesis of CAV are lacking, it is believed that the pathologic process is initiated by antibody- and complement-mediated injury to endothelial cells and may be accelerated by common coronary disease risk factors such as hypertension and hyperlipidemia. The antibodies most frequently associated with CAV are those against donor HLA, in

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Fig. 7. Intravascular ultrasound example of a de novo lesion of transplantation vasculopathy. No lesions are shown at baseline examination (A). When the same site is identified on follow-up examination, significant intimal thickening is seen at that site (B). This lesion is defined as a de novo lesion of transplantation vasculopathy. Note that this lesion is circumferential and noneccentric. Reprinted with permission from Kapadia SR, Nissen SE, Tuzcu EM. Curr Opin Cardiol 1999;14:140-150.
particular to class I antigens, which are richly expressed in human endothelial cells. HLA class II antigens are constitutively expressed in human endothelium, and their synthesis can be stimulated by proinflammatory molecules such as interferon-γ [59]. Anti-HLA IgG can stimulate the proliferation of endothelial and smooth muscle cells, causing intimal expansion. Although lytic levels of complement cause hyperacute rejection, the role of complement in CAV is not well understood. Complement activation can result in the release of tissue growth factors that cause endothelial proliferation and the migration of fibroblasts and smooth muscle cells. In addition to this, sublytic doses of the membrane attack complex of complement can induce endothelial production of tissue factor. This phenomenon has been deemed responsible for the procoagulant characteristics and intimal fibrin formation seen in coronary arteries with CAV. The presence of fibrin has been independently associated with CAV, allograft failure, and death [67,69].

The diagnosis of CAV is challenging because of the often diffuse nature of the disease. Comparative coronary angiography and intravascular ultrasound [70] are widely used to demonstrate progressive narrowing of allograft coronary arteries (Fig. 7). Serial dobutamine stress echocardiography is also used to detect clinically significant CAV, and some data suggest that it may have prognostic value comparable to intravascular ultrasound and coronary angiography in predicting the occurrence of cardiac events [71].

The therapeutic options for CAV are limited. In contrast to native coronary artery disease, percutaneous coronary interventions are not commonly possible because of the diffuse nature of stenotic process. Stents have been used occasionally for palliative purposes in patients with symptomatic ischemia. Coronary artery bypass grafts are of limited applicability for similar reasons. Retransplantation may be an option for select candidates with severe multivessel CAV with evidence of allograft dysfunction. However, patients must be chosen carefully to allow acceptable outcomes of retransplantation to be achieved [72].

Contemporary immunosuppressive agents such as sirolimus and everolimus have demonstrated benefits in delaying the onset of clinically evident CAV [73,74]. When CAV is detected with angiography, intensification of immunosuppression with these agents is an acceptable choice to slow the progression of CAV [75].

6. The importance of de novo anti-HLA antibodies after cardiac transplantation

Despite advances in immunosuppressive therapy, the incidence of acute AMR and CAV continues to limit long-term outcomes [54,76]. A study by Rose et al [77] demonstrated that the presence of anti-HLA antibodies after cardiac transplantation results in lower survival when compared with being antibody-free. At 5 years after transplantation, survival in the antibody-negative group was 90% compared with 53% in the antibody-positive group. Acute rejection and infection, partly related to augmented immunosuppression to treat rejection episodes, were the leading causes of death in antibody-positive patients. Circulating anti-HLA antibodies were also associated with higher rates of CAV. These findings have been corroborated by subsequent studies [12,78,79].

Anti-HLA antibodies can develop in patients who were not allosensitized before transplantation. This suggests that the transplanted heart may release alloantigens responsible for neosensitization of the recipient. Tambur et al [80] showed that up to 35% of nonallosensitized cardiac allograft recipients can develop anti-HLA antibodies within the first year after transplantation. Antibodies against HLA class I antigens were more commonly present than anti-HLA class II antibodies, with PRAs ranging widely from 10% to almost 80% for both classes. Female sex, HLA-A mismatch, and longer ischemic time were identified as risk factors for de novo sensitization after transplantation. Anti-HLA class I and II antibodies showed strong associations with the incidence of early acute cellular rejection. In addition, de novo class II antibodies were associated with more severe CAV and higher mortality due to allograft failure (Fig. 8). Of all anti-HLA class I antibodies, 41% represented donor-specific antibodies. Donor-specific class II antibodies were uncommon (<10%). The relative infrequency of donor-specific antibodies may suggest that most are bound to allograft antigens, therefore being underrepresented in the serum [80].

Although contemporary data in cardiac transplant recipients show that de novo anti-HLA antibodies may have a major impact on post-transplantation outcomes, the available evidence is not enough to recommend serial PRA screening in all cardiac allograft recipients. It may be advisable to conduct serial PRAs during the first year after transplantation in patients with features that will place them in a high risk category for post-transplantation allosensitization. Serial PRAs in this subset could potentially signal the need for more aggressive immunosuppression to prevent acute rejection episodes and delay allograft failure; however, the
clinical utility and cost-effectiveness of such an approach remain in question.

7. Conclusions

Cardiac transplantation has become the best available therapy in select patients with advanced heart failure with a high probability of death. However, an inadequate number of available hearts remains rate-limiting in the provision of transplantation to those in need, leading to longer wait-list times for many transplant candidates, with a potential for higher wait-list mortality.

In view of the limited donor pool, mechanical circulatory support with VADs was introduced as a bridge to transplantation. Unfortunately, broad use of VADs has been associated with higher rates of allosensitization, causing delays to transplantation because of difficulty in finding a crossmatch-negative donor. In addition to VAD-related sensitization, cardiac transplant candidates are frequently exposed to other sources of sensitization such as blood product transfusions.

The consequences of pretransplantation allosensitization on clinical outcomes after transplantation can be serious. Antibody-mediated rejection and CAV are particularly prominent and can result in graft failure and decreased survival. The pathophysiology of these conditions is not fully understood; therefore, available treatments are based on expert opinion and anecdotal clinical data. For this reason, significant emphasis is placed on preventing and decreasing allosensitization before transplantation.

Current strategies to decrease allosensitization focus on the direct removal of circulating antibodies with plasmapheresis, inactivation of antibodies with IVIG, and B-lymphocyte depletion with rituximab. These approaches have also been used to treat AMR after transplantation, with encouraging results. However, most treatments are based on theoretical assumptions and scarce clinical data; therefore, their true efficacy is unknown.

In the future, current strategies to avoid allosensitization will be complemented by improvements in VAD design that will decrease the likelihood of immune activation. Newer generation devices may result in lower degrees of allosensitization by reducing the surface area in direct contact with the bloodstream, limiting the use of textured surfaces and using less immunogenic biocompatible materials.

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