

# Current Concepts in Histocompatibility During Heart Transplant

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## Abstract

Sensitized candidates for heart transplant usually end up on a long waiting list and have an increased risk of rejection, graft loss, and incidence of cardiac allograft vasculopathy. An increasing number of studies have demonstrated the negative effect of preformed and posttransplant antibodies on graft survival. Thus, in sensitized patients, the combination of new, appropriate, desensitization protocols, and monitoring of posttransplant development of donor-specific antibodies may improve short-term and long-term outcomes. Introduction of more-sensitive and more-specific techniques for antibody detection provides a valid tool for assessing the degree of pretransplant HLA histocompatibility, and, therefore, predicting the results of crossmatch in sensitized patients, which are difficult to transplant. Currently, there are no accurate and standard methods to determine the functional characteristics of antibodies detected by

solid-phase assay and, therefore, to predict their clinical relevance. Therefore, the future of heart transplantation requires a better understanding of tissue typing techniques and the effect of anti-HLA antibodies on clinical outcome to prevent discrimination against sensitized patients at the time of organ allocation.

**Key words:** Heart, Human leukocyte antigen, Sensitized patients, Transplant, Anti-HLA antibodies

## Introduction

Advances in diagnosing and treating humoral rejection of transplanted organs, as well as human leukocyte antigen (HLA) antibody detection, have significantly increased the success rate of solid-organ transplant.<sup>1,2</sup> In a heart transplant, survival depends on the presence/absence of risk factors and is about 90% and 70% at 1 and 5 years.<sup>3-5</sup> Today, the goal of heart transplant is not only to improve the long-term outcome, but also, to enhance the quality life for patients.

Several studies have clarified the relation between immunosuppressive treatment and immunologic and nonimmunologic complications observed mostly in heart-transplanted sensitized patients (Figure 1).<sup>6-8</sup> Heart transplant in immunized patients continues to be a risk factor owing to immunosuppression-related complications, such as infection, rejection, and graft coronary vasculopathy.<sup>9,10</sup> In the last few years, loads of evidence have indicated that preallosensitization and postallosensitization have a negative clinical effect on the outcome and long-term survival of the heart graft.<sup>11</sup> For this reason, great emphasis has been placed on systems to detect allosensitization and donor-specific anti-HLA antibodies (DSA) before and after heart transplant.<sup>12</sup>

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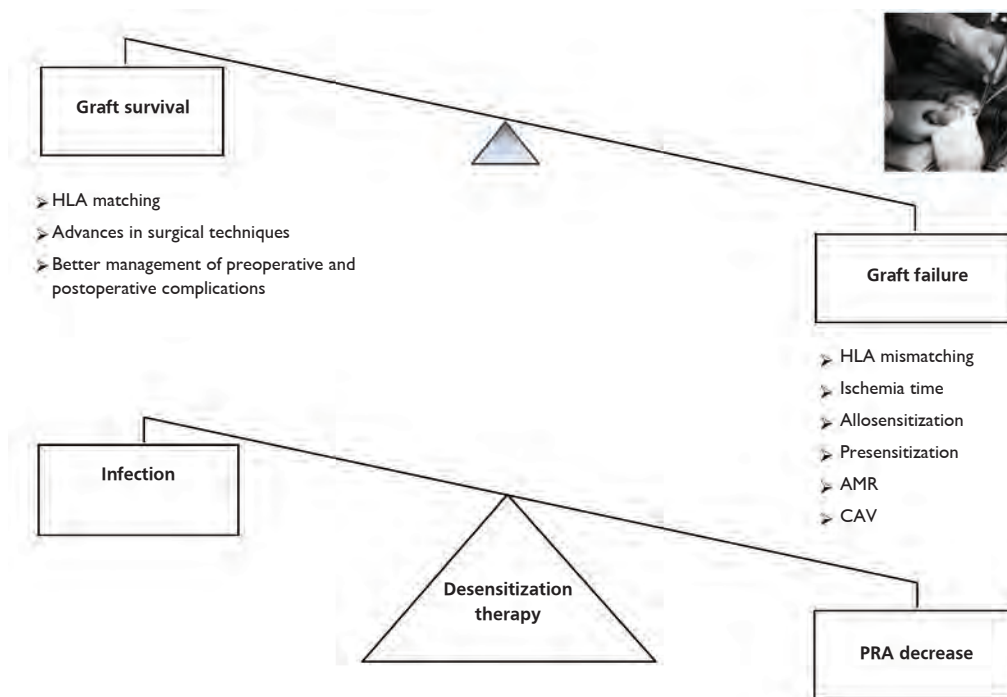
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**Figure 1.** Balances between graft survival and failure and between panel-reactive antibodies (PRA) and the occurrence of infections. In the upper balance, factors that induce either of the graft fates are reported. In the lower balance, several therapeutic options that decrease PRA, but increase the risk of infections, are reported. *Abbreviations:* AMR, antibody-mediated rejection; CAV, cardiac allograft vasculopathy; HLA, human leukocyte antigen; PRA, panel-reactive antibodies

Anti-HLA antibodies can be detected with different techniques. Alongside complement-dependent cytotoxicity (CDC), novel, more-sensitive and more-specific solid-phase techniques have produced detailed information regarding antibody specificity, allowing prediction of donor-recipient compatibility by virtual crossmatch (VXM) before a heart transplant.<sup>13</sup>

Although several treatments (such as combination of plasmapheresis with immunomodulators) are used to decrease allosensitization, new strategies are required to prevent or reduce anti-HLA antibodies from developing in patients on waiting list for a heart transplant.<sup>14, 15</sup>

### Controversial issues in human leukocyte antigen matching in a heart transplant

The importance of HLA matching to improve outcome in kidney and bone marrow transplant has been widely accepted, whereas it is still debated in heart transplants.<sup>16-18</sup> There is a paucity of research on the clinical relevance of HLA matching in heart transplanting, because the HLA is not a selection criterion of the recipient's choice. Furthermore, heart disease patients have a severe short-term prognosis, and they cannot wait for a heart transplant with a high degree of HLA histocompatibility.<sup>19</sup> Unlike the

kidney, the ability to match a potential donor with a recipient for a heart transplant is strongly limited by the scarcity of donors, organ maintenance and preservation techniques, and the short time before graft ischemia. Consequently, the donor-recipient HLA matching in a heart transplant is rare, and the low number of patients receiving a histocompatible organ makes it difficult to assess the HLA matching effects on graft outcome.

Few studies highlight conflicting the views on the role of HLA matching in cardiac transplant. Opelz and Wujciak have shown the independent effect of HLA matching on survival after a heart transplant.<sup>20</sup> Two HLA-A, -B, or -DR donor-recipient mismatches have been associated with a 3-year graft survival of 76%, compared with 83% in transplants with 0 or 1 mismatch.<sup>20</sup> To date, introduction of more-sensitive methods of HLA-antibody detection in clinical laboratories have determined an increase of sensitized recipients. Thereafter, an extreme change in desensitization protocols has determined an increase in long-term survival of heart transplant patients. These data are confirmed by other studies that have shown a significant association between HLA-DR mismatch and the severity and earliness of the first rejection episode. In contrast, HLA-DR matching is associated with less rejection and

improved survival at 1, 5, and 10 years after transplant.<sup>21-23</sup> Human leukocyte antigen-DR has a significant role on long-term outcome, survival, and incidence of cardiac allograft vasculopathy (CAV), while no statistically significant influence for HLA-A and -B mismatches has been revealed.<sup>24</sup> Recipients mismatched for 0, 1, or 2 HLA-DR antigens has a survival ratio of 90%, 79%, and 68.1%. Although there was no significant difference between HLA-DR mismatches and the incidence of CAV, a trend toward fewer CAV occurrences at 5 years after transplant has been observed.

These studies suggest improvement of long-term survival by HLA histocompatibility in a heart transplant, suggesting the importance of considering HLA matching as additional criteria for recipient selection. Conversely, other studies have shown that HLA mismatching is not an independent risk factor of long-term survival in heart transplant patients because a high degree of HLA-A, -B, and -DR matching had no effect on survival, rejection episodes, or infection.<sup>25, 26</sup> These conflicting results may be due partly to nonimmunologic causes, immunosuppressive therapies, or differences in minor histocompatibility antigens could induce graft dysfunction. Anyway, prospective matching should be implemented whenever clinically appropriate, such as in alloimmunized patients, and it would be convenient to restrict matching to only HLA-DR locus, although this approach requires a better understanding of the role of HLA antigens and of the immunologic mechanisms of immune response. Notably, the potential benefits of a prospective HLA matching, in terms of survival and rejection, may be overbalanced by increased mortality owing to the consequent longer transplant waiting time.

#### **Allosensitization and heart transplant: An update**

Patients sensitized for human HLA antigens pose critical problems for a heart transplant.<sup>9, 26</sup> Sensitization occurs from the complex interaction of the host immune system with nonself HLA antigens after transfusion, pregnancy, previous transplant, and ventricular assist device implantation.<sup>9</sup> Recently, improvement of techniques used for antibody detection has increased the number of immunized recipients on the waiting lists for transplant.<sup>6, 9</sup>

Several new studies have shown that the presence of circulating donor-specific anti-HLA antibodies is associated with several effects that influence the

transplant outcome both in the short term and the long term.<sup>5, 27-29</sup> Indeed, allosensitized cardiac transplant candidates are at high risk of developing a hyperacute rejection when antibodies are directed against HLA antigens of the donor.

The presence of pretransplant anti-HLA antibodies is strongly associated with reduced survival, increased rejection, and CAV development after a heart transplant.<sup>30</sup> Furthermore, several studies conducted on transplanted patients have reported the effect of panel-reactive antibodies (PRA) on the shorter-term survival and the high probability of rejection after a heart transplant.<sup>31, 32</sup> Ho and associates found that HLA class I and/or class II antigen presensitization was positively associated with antibody-mediated rejection (AMR) and with a lower rate of graft survival after 1 year, but showed no effect on survival at 10 years after heart transplant.<sup>31</sup> In addition, patients were monitored after the transplant for HLA class I and II antibody production, and statistical analyses showed that posttransplant alloantibody development was a risk factor associated not only with the AMR onset but also with a low survival rate.<sup>33</sup>

Several recent reports have emphasized the clinical effect not only of preformed, but also especially of de novo anti-HLA alloantibodies on graft outcomes in heart transplant recipients.<sup>33-35</sup> In particular, de novo anti-HLA donor-specific antibody development was associated with increased rejection, CAV, and poor survival.<sup>30, 31, 36</sup> Indeed, antibodies against class I or class II antigens seem to have negative effects. Alloantibodies bind class I and II HLA molecules to the endothelial cell surface and transmit proinflammatory and proliferative intracellular signals. These transmittals are influenced by antibody specificity and concentration.<sup>37</sup> The high concentration of class I anti-HLA antibodies transmits inflammatory, and proliferative signals promoting chronic or hyperacute rejection.<sup>38, 39</sup> Instead, a low concentration of the same antibodies up-regulates expression of antiapoptotic proteins conferring resistance to damage.<sup>39</sup>

Recent studies have demonstrated that most de novo donor-specific antibodies were directed against class II antigens, while an analysis of all de novo antibodies produced after transplant, showed an equal proportion of class I and II antibodies.<sup>35, 40</sup>

Furthermore, inflammatory events, caused by class II HLA up-regulation on endothelial cells, make

graft endothelium vulnerable to donor-specific anti-HLA antibodies.<sup>41</sup> It has been shown that class I anti-HLA antibodies, binding to endothelial cells, induce tyrosine kinase phosphorylation, playing a pivotal role in regulating cell survival through activation of PI3K and protein kinase B (PKB) pathways and expression of anti-apoptotic proteins such as Bcl-2.<sup>39, 41</sup> Indeed, endothelial cells exposed to class I anti-HLA antibodies induce an increased PKB and Bcl-2 expression that reach the highest levels when endothelial cells are exposed to a low antibody concentration conferring resistance to endothelial injury.<sup>42</sup> The previously mentioned proliferative mechanisms, induced by high levels of class I anti-HLA antibodies, are due to up-regulation of fibroblast growth factor (FGF) receptors and FGF binding.<sup>43</sup>

It should be emphasized that the pretransplant and posttransplant monitoring of anti-HLA antibodies is important for diagnosing and treating AMR early and could provide a better and earlier identification of patients at risk. However, the traditional endomyocardial biopsy remains an essential tool for rejection diagnosis.<sup>4</sup> Indeed, in a heart transplant, presensitization to HLA class I and/or class II antigens is positively associated with the development of AMR. Moreover, development of de-novo anti-HLA-antibodies after a transplant shows a strong correlation with AMR and a low long-term survival rate.<sup>31</sup> In solid-organ transplant, inflammatory events such as rejection and/or infection cause up-regulation of HLA class II membrane expression, making graft endothelial cells exposed to the effect of anti-class II donor-specific antibodies. Antibody-mediated rejection is complemented by cell-mediated immune responses, which involve direct and indirect recognition pathways.<sup>31, 35</sup> Furthermore, a better understanding of the interaction between cellular and antibody-mediated responses against the graft could allow early modification and customization of the immunosuppressive therapy in transplant.

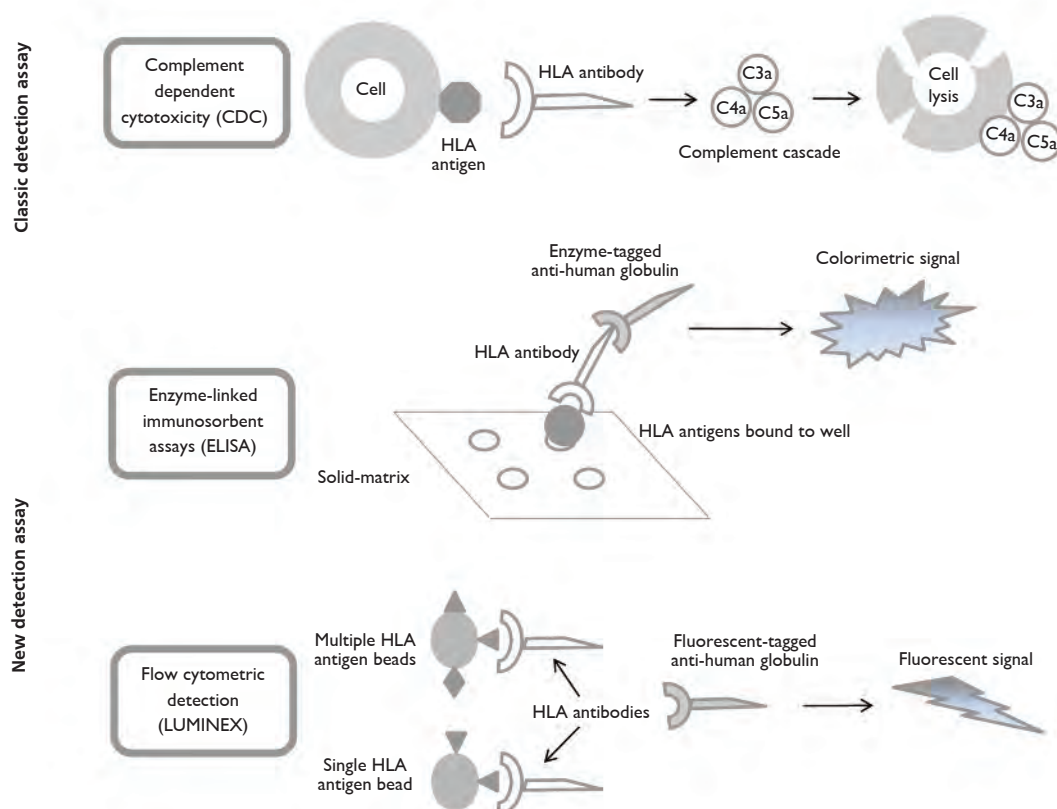
### **The current status of anti-human leukocyte antigen antibody detection**

Complexity of the HLA system has prompted development of more-sensitive and more-specific methods to analyze anti-HLA antibodies in transplant candidates, thus allowing a better strategy to determine donor-recipient histocompatibility.<sup>44, 45</sup>

In recent years, techniques to detect anti-HLA antibodies have evolved from cell-based assays to solid-phase and flow-based assays exploiting HLA recombinant antigens (Figure 2).<sup>46</sup> These new methods can reveal anti-HLA antibodies, also at low levels, not previously detectable in cell-based assays.<sup>47</sup> Cell-based methods, such as CDC, are based on interaction of recipient serum with HLA antigens expressed on lymphocyte cell membranes.<sup>13</sup> In solid-phase methods, HLA antigens are immobilized on a solid matrix as in enzyme-linked immunosorbent assay (ELISA), or on beads as in flow-assays (Luminex and Flow-PRA).<sup>48</sup> Solid-phase methods are more specific than cell-based ones and are not influenced by lymphocytotoxic immunosuppressive drugs.<sup>45</sup> On the other hand, cell-based assays mimic physiological conditions but are less sensitive and specific, and they can cause false reactivity.<sup>37</sup> Furthermore, the CDC cannot distinguish between class I and II anti-HLA antibodies and requires a large cell panel to provide coverage for detecting both the most common and rare HLA antigens.<sup>13</sup> Complement-dependent cytotoxicity assay detects both IgG and IgM anti-HLA antibodies, both activating and nonactivating the complement. Solid-phase methods detect IgG, are specific for anti-HLA antibodies, and can distinguish between IgM or IgG class I and II anti-HLA antibodies.

Currently, new single-antigen-coated assays, carrying only 1 antigen per bead, allow unique identification of HLA specificities.<sup>13</sup> This new technology may be the best approach in patients with multiple antibody specificities.<sup>48</sup> However, these new tests have generated considerable debate about the clinical significance and specificity of the anti-HLA antibody titer. In fact, antigens coated on the surface of beads may be structurally different from membrane antigens.<sup>49</sup> Increasing evidence has shown that the antibody titer does not necessarily determine the antibody clinical significance; indeed, a low titer of antibodies can dramatically increase within a few days of antigen restimulation.<sup>49</sup> Currently, there are no accurate and standardized methods to determine functional characteristics of antibodies detected by solid-phase assay and, therefore, to predict their clinical relevance.

Rose and associates have shown that complement-activating antibodies were responsible for the poor outcome of heart transplants, but also



**Figure 2.** Schematic representation of anti-HLA antibodies detection assays. In CDC assay, antibody presence is detected by its ability to lyse the cells in the presence of the complement. In the solid-phase assay, HLA antigens are bound to a solid-matrix (ELISA) or to microbeads (flow-assays), and the antibodies are detected by an enzymatic chromogenic reaction or by excitation of a fluorogenic dye.

**Abbreviations:** HLA, human leukocyte antigen

complement-fixing non-HLA IgM antibodies can have deleterious effects.<sup>50</sup> Many solid-phase assays were unable to distinguish between activating- and nonactivating-complement antibodies. Although the frequency of nonactivating-complement antibodies in patients awaiting a second renal transplant has been shown to be approximately 40%, there are no convincing data to show the clinical significance or otherwise of nonactivating-complement HLA antibodies. In the routine clinical setting, using bead technology for antibody detection, no distinction is made between activating-complement and nonactivating-complement when matching donors and recipients for transplant. It is certain, therefore, that some patients are being denied transplants based on detecting nonactivating-complement HLA antibodies for which we have no data indicating whether or not they have damaged grafts.<sup>13</sup> The role of nonactivating-complement antibodies in clinical transplants remain controversial, although a new assay with C1q recently has been developed to define the complement-activating antibodies using the

solid-phase technique.<sup>51</sup> In any case, there is no single and standardized method to define a positive or negative result in terms of medical consequences, so further study is needed, not only retrospective, but also prospective, to determine the clinical effect of antibodies detected by solid-phase tests.<sup>15, 52</sup> These studies would allow standardized interpretation of fluorescence data starting from a few basic points: The amount of circulating antibodies is critical in mediating the risk of hyperacute rejection, and a prospective negative crossmatch using CDC is associated with lower likelihood of hyperacute rejection. However, the latter claim is undermined by the fact that it is still not clear what the antibody threshold level is in the recipients that distinguishes a positive from a negative crossmatch, although several studies have shown a correlation among the level of the antibodies detected with solid-phase assays, the crossmatch result, and the outcome of transplant.<sup>53, 54</sup> Probably, a better functional characterization of the antibodies should be based on a combination of cell-based and solid-phase assays.

### Virtual crossmatch

Solid-phase assays, with single-antigen detection, have enabled the introduction of virtual crossmatch (VXM) as a screening tool for immunized patients.<sup>47</sup> Several methods, such as cytotoxicity crossmatch (CDCXM) and flow cytometric crossmatch (FCXM), which combine recipient serum with donor cells, are routinely used to assess donor-recipient compatibility.<sup>54-56</sup> Crossmatch tests avoid the transplant of an organ to a recipient who has specific-donor antibodies.

Pretransplant CDCXM and FCXM are required to locate a compatible allograft for immunized patients, but the time it takes to find a negative donor-recipient crossmatch can be fatal for the recipient. For immunized patients, grafts from local donors are generally well accepted, because it is possible to perform a crossmatch. And VXM uses the results of specific antibody screening to predict acute incompatibility. This method compares the HLA genotype of the donor (using the results from low-resolution DNA testing) with antibody profiles in the serum of immunized patients.<sup>54, 56</sup> Virtual crossmatch is considered positive if the antibodies detected by the solid-phase assay corresponding to the donor typing (Figure 3). The predictive value of VXM has been investigated in several retrospective studies with conflicting results.

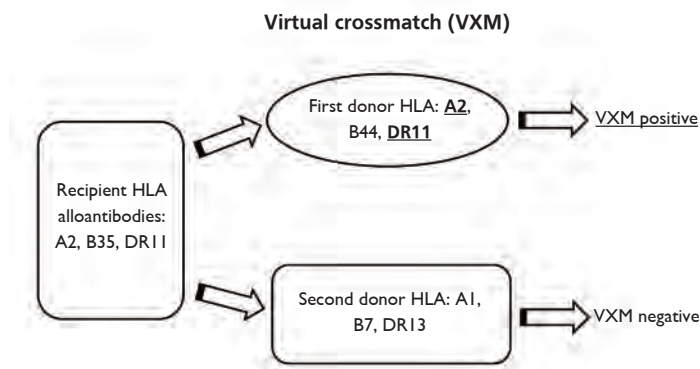
A recent study has examined VXM accuracy in immunized recipients and showed no differences between AMR incidence, cellular rejection, and survival among patients with VXM, and those with a prospective serologic crossmatch.<sup>57</sup> In the same study, the authors estimated the positive predictive value of VXM as 79% and its negative value as 92%.

According to these observations, VXM could be considered an accurate test and allow the following: Increase the opportunity for transplant, shorten waiting times, yield better outcomes, improve the allocation of compatible organs, and better define risk stratification in sensitized recipients of a heart transplant. However, recently, a case has been reported of a patient undergoing a heart-lung transplant with negative FCXM and positive VXM.<sup>58</sup>

Subsequent investigations have clarified that the antibody was probably directed against an epitope of HLA that was evident on the single antigen beads but not in the native form of HLA molecules of the donor lymphocytes.<sup>56</sup> There are also cases where VXM shows compatibility but CDCXM or FCXM are positives. This result could be due to a high threshold of fluorescence intensity, whereas an incompatible VXM and a negative CDCXM or FCXM could be due to a low threshold of fluorescence intensity. For these reasons, the threshold of clinical significance should be accurately standardized, because level and potential biological activity of donor-specific antibodies are likely to affect graft outcome.<sup>59</sup> Given the inherent limitations of retrospective studies, a prospective evaluation of VXM for predicting clinically relevant outcomes is required.<sup>60, 61</sup>

### Classic and novel treatments for desensitization and immunomodulation.

Several studies have clarified the relation between the immunosuppressive drugs and immunologic and nonimmunologic complications observed in a heart transplant.<sup>6</sup> Indeed, the choice of immunosuppressive approach in relation to patient preoperative characteristics is the most appropriate



**Figure 3.** Virtual crossmatch is positive with the first donor because some of the recipient's anti-HLA antibodies (anti-A2 and anti-DR11), detected by a single-phase assay, are directed against donor-specific HLA antigens (A2 and DR11). Instead, virtual crossmatch is negative with the second donor because all of the anti-HLA antibodies detected are not directed against donor-specific HLA antigens.

**Abbreviations:** HLA, human leukocyte antigen

tool in reducing the incidence of early and late complications, and to achieve better results in terms of survival and quality of life of the transplanted patient.<sup>62, 63</sup> The knowledge of antibody-mediated immune response mechanisms has provided insights for potential therapeutic intervention, but complete elimination of donor-specific anti-HLA antibodies during AMR is rarely achieved with traditional anti-humoral therapy.<sup>4</sup>

There are several strategies available to reduce sensitization in patients waiting for a heart transplant and to treat AMR. Indeed, in alloimmunized patients, it is crucial to remove and monitor anti-HLA antibodies.<sup>11, 64</sup> Currently used protocols take different approaches: Removal of anti-HLA antibodies from circulation and drug administration to reduce their production also in combination with other agents, before, perioperatively, or immediately after transplant.<sup>14, 49</sup> For several years, AMR therapy and desensitization regimens have been based on plasmapheresis, intravenous immunoglobulin (IVIG), antilymphocyte antibody preparations, and, more recently, on a B-cell-specific monoclonal antibody (rituximab) and a proteasome inhibitor (bortezomib).

### **Classic approaches on sensitized patients**

Little evidence in the literature supports the use of plasmapheresis as monotherapeutic treatment in sensitized heart transplant recipients, because this therapeutic approach mechanically removes circulating antibodies but does not affect their production. Most studies, instead, have reported a combined treatment of plasmapheresis with other immunomodulators, such as IVIG. Sensitized patients undergoing pretransplant plasmapheresis and IVIG treatments present with a frequency of graft rejection and survival similar to patients negative for anti-HLA antibodies.<sup>65, 66</sup>

Intravenous immunoglobulin was found to be an important component in desensitization protocols and in AMR treatment, especially in kidney transplant.<sup>67</sup> Despite the fact that using high-dose IVIG was beneficial in treating idiopathic and virally induced cardiomyopathy, the advantage for its use in desensitization and in AMR therapy in heart failure is less evident. Recently, Shehata and associates summarized the literature data on using IVIG as a desensitizing agent in patients undergoing cardiac transplant.<sup>68</sup> Because evidence in favor or

against routine use of IVIG as a desensitizing agent and in improving transplant outcome is insufficient, further randomized and standardized clinical trials are required to verify the effectiveness of this approach.<sup>66-68</sup>

Several studies have underscored that the desensitization effect, with IVIG and plasmapheresis, also depends on the polymorphism of genes encoding INF- $\gamma$ , IL-2, and IL-4R $\alpha$  proteins.<sup>69</sup> These findings suggest that cytokine polymorphisms and anti-HLA antibody analyses would be important to identify potential resistance to desensitization treatment and determine the appropriate therapeutic approach.

Other studies have reported a successful decrease of anti-HLA antibodies in the posttransplant period using mycophenolate mofetil. Both clinical and experimental data support the mycophenolate mofetil effect on T- and B-cell proliferation and immunoglobulin production. Mycophenolate mofetil reduced the risk of IgG production during the first year after a heart transplant.<sup>70, 71</sup>

Recently, Bućin and associates reported a desensitization protocol for patients who had undergone a successful heart transplant despite having high levels of donor-specific antibodies. The pretransplant desensitization protocol has included protein-A immunoadsorption (IA), intravenous immunoglobulin, and immunosuppressive drug treatment. Posttransplant treatment consisted of tacrolimus, mycophenolate mofetil, prednisolone, IA, and daclizumab.<sup>72</sup> The use of IA, in combination with pretransplant immunosuppressive drug treatment, temporarily reduced antibody levels, although controlled clinical trials are required to determine appropriate doses of these medications, exposure times, protocols, and effect on rejection.<sup>72</sup>

### **Novel approaches on sensitized patients**

Recently, use of rituximab, a chimeric monoclonal antibody directed against CD20, has been introduced in the clinical practice.<sup>73</sup> Treatment with rituximab decreases allosensitization, reducing B lymphocytes and PRA levels, but most of the published material refers to immunized patients with kidney disease. There are few reports on the use of rituximab as pretreatment in pediatric heart transplant and in adult recipients.<sup>73, 74</sup> Patients with a high PRA show a decrease of pretransplant circulating antibody levels from 70% to 30% after plasmapheresis, IVIG,

and high-dose cyclophosphamide and rituximab.<sup>73</sup> Other studies in adult heart recipients have reported the effects of rituximab in treating refractory AMR.<sup>75</sup> Moreover, all conventional immunosuppressive therapies and desensitization regimens, including rituximab, have no effect on depletion of plasma cells.<sup>14, 49</sup> Probably, the immunosuppressive treatments that fail to inhibit the mature plasma cells have little effect on reducing antibody production.

Bortezomib represents a new, effective, anti-humoral treatment strategy for promoting depletion of plasma cells by proteasome inhibition (PI). Proteasome inhibition reduces anti-HLA antibodies levels through immunomodulatory mechanisms and a complex series of biochemical events that result in pleiotropic effects, particularly on plasma cells.<sup>76</sup> Initial reports using PI have demonstrated the ability of bortezomib to reduce significantly anti-HLA antibodies levels and also, to provide effective treatment of antibody- and cell-mediated rejection with minimal toxicity.<sup>77</sup> Initial results with bortezomib in AMR in kidney transplant recipients have been confirmed and extended to pediatric and adult heart transplant recipients. However, the major advantage of PI has been in overcoming humoral barriers.<sup>78, 79</sup>

Eckman and associates demonstrated that a bortezomib-based regimen provided effective therapy for late, refractory AMR in a heart transplant adult recipient, and it was well tolerated. This remarkably positive experience, despite the refractory nature of the AMR episode, argues strongly for continuing to evaluate bortezomib use in these patients.<sup>76</sup> Nevertheless, there remain unresolved issues, and a clinical trial is ongoing to compare the outcomes of sensitized versus nonsensitized pediatric heart transplant recipients (NCT01005316).

### Conclusions and future directions

The potential benefits of a prospective HLA matching in heart transplant are unclear. Several studies have shown that HLA-matched transplant improves survival and reduces the negative effects of immunosuppressive therapy. There are unresolved technical problems that prevent implementing prospective HLA matching as routine procedure in heart allocation.

Pretransplant allosensitization and de novo antibody production increase the probability of acute

and chronic graft rejection resulting in decreased survival. For this reason, anti-HLA antibodies identification and monitoring has a pivotal role before and after transplant and allows safe and reasonable management of these patients. Better detection of anti-HLA class I and II antibodies allows one to identify unacceptable antigen loads or low levels of clinically relevant antibodies. Significant success in averting antibody-mediated acute rejection has been achieved with current strategies to decrease allosensitization based on revolving circulating antibodies, such as plasmapheresis, and treatments with IVIG, rituximab, and more recently, bortezomib.

There are still unresolved issues regarding management of immunized patients awaiting heart transplant. The controversy concerns the optimal follow-up, the most appropriate method for monitoring circulating antibodies, and the degree of antibody-mediated rejection, as well as selection of therapeutic interventions to treat these conditions.<sup>80</sup> One of the major limitations is the limited knowledge regarding the specific antibodies associated with the increased risk of posttransplant complications. A better understanding of B-cell immunobiology, and development of therapies specifically designed to abolish the donor-specific antibody production, are required to find the right balance between immunologic responses and immunosuppressive strategies.

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