1. ABSTRACT

The number of highly sensitized patients on the transplant waiting list continues to rise. HLA allosensitization has profound effects on the wait time to transplant, rejection rates, and long-term outcomes. While technological advances with high sensitivity and specificity have facilitated the detection of donor specific antibodies, there is no consensus on diagnostic and prognostic values of these tests. Recently, multiple desensitization protocols have been developed that comprise high dose IVIG, low dose IVIG with plasmapheresis, rituximab and more novel agents including Bortezomib. Although these preconditioning protocols have led to successful transplantation of sensitized patients, long-term outcomes are limited and suboptimal. Randomized clinical trials are needed to determine optimal treatment and monitoring strategies in patients that are highly sensitized across the HLA barrier.

2. INTRODUCTION

The first documented kidney transplantation in the United States was performed June 17, 1950, on Ruth Tucker, a 44-year-old woman with polycystic kidney disease. However, the donated kidney was eventually rejected due to the absence of immunosuppressive therapy at the time. Thereafter, successful kidney transplantation was done in 1954 in Boston involving identical twin to avoid the risk of rejection secondary to immune reaction. Until the introduction of immunosuppressant to prevent and treat acute rejection, introduced in 1964, deceased donor transplantation was not an option. Subsequent studies demonstrated that meticulous tissue typing was essential to the success. In a data analysis of 73,103 adult renal transplants registered at the United States Renal Data System Registry, Meier-Kriesche et al demonstrated that waiting time on dialysis is a strong and independent risk factor for decreased patient survival as well as decreased
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3. DEFINITION

Large body of evidence demonstrated that presence of antibodies against class I and class II antigens jeopardize transplant outcome. The HLA-sensitized state is defined by the presence of antibodies against Class I (HLA-A, HLA-B) and/or Class II HLA-DR molecules. Although the role of DP and DQ antigens remained unclear for a period of time, recent studies demonstrated that anti DP and DQ antibodies are also associated with hyperacute and accelerated rejections (5). The level of anti-HLA antibodies detected is expressed as Panel or Percent Reactive Antibodies (PRA) (5). These antibodies result from exposure to non-self HLA antigens, usually from previous pregnancies, blood transfusions, and transplant (6). Therefore, females are more likely to be sensitized than males. High sensitization due to pre-transplant elevated PRA (higher than 10 percent) increases rejection risk (6). Therefore, the highly sensitized patients (PRA more than 80 percent) are destined to remain on the waiting list for extended periods on dialysis, which culminate in an added risk factor for decreased patient as well as graft survival (7-8) (Figure 1). Data from the Organ Procurement Transplantation Network/Scientific Registry of Transplant Recipients: 2008 Annual Report illustrated that the numbers of highly sensitized patients who were on the transplant waiting list continued to increase from 4,447 in 1998 to 7,908 in 2007 (Figure 2). In 2007, 38 percent of the transplant waiting list was considered sensitized to HLA antigens, with 16.2 percent having PRA 80 percent (9-10).

Recent data obtained from the United Network for Organ Sharing (2001–2008) shows that the rates of transplantation of patients analyzed by PRA status are less than 16 percent per year for those with PRAs 10 - 80 percent and less than 8 percent for patients with PRA more than 80 percent (11-13). Furthermore, data reviewed from Organ Procurement Transplantation Network/Scientific Registry of Transplant Recipients (OPTN/SRTR) (1998-2004) demonstrated that median time to transplant of patients receiving living donor (LD) and deceased donor (DD) transplants by panel reactive antibody is more than 9 years for patients with PRA more than 80 percent (9) (Figure 3).

4. ADVANCES IN HISTOCOMPATIBILITY TECHNIQUES

Tools for HLA typing and antibody detection have been evolving over the last five decades. Historically, HLA antibody testing was described by Dusset in1954 (14-15). Subsequently, its value to organ transplant was further developed by Patel and Terasaki in 1960s (16). Terasaki et al demonstrated that kidney transplant outcome across a positive cross-match (CMX) barrier associated with poor outcome, probably due to the previously documented high risk of antibody mediated rejection (AMR) (16). Patel and Terasaki effort instituted the basis for modern CMX testing as a mean of allocating kidneys (6, 16). Furthermore, their work evolved the previously used agglutination assay to the complement-dependent lymphocytotoxicity (CDC) test (14; 17).
Desensitization across the HLA barrier in kidney transplantation continued to progress (23-26). Antibody (DSA) detection, particularly class I and class II, different studies focused on improving Donor Specific low level antibodies in highly sensitized patients. As AMR (21-22), sensitivity which incurred a high risk of post transplant while other transplant centers adopted methods with low sensitivity and specificity: as the specificity increases the test sensitivity decreases (20), (Figure 4). Some centers sensitivity and specificity; as the specificity increases the test sensitivity decreases (20), (Figure 4). Some centers considered a positive flow crossmatch one of several risk factors for increased likelihood of rejection episodes, while not a contraindication to transplant (19).

Crossmatching by means of flow cytometry detection has filled the deficiencies of the CDC crossmatch by providing a more sensitive and more objective method of detection of HLA antibodies. The flow crossmatch (FXM) typically utilizes three-color fluorescence to characterize the CD3, CD19, and indirect binding of the patient antibody to the donor target cells, which allows for characterization of HLA class I (found on both CD3 and CD19 positive cells) and HLA class II (found on CD19 positive cells only) donor-directed antibodies. It also has the ability to detect complement-fixing and non-complement-fixing antibodies, in contrast with the CDC crossmatch which detects only complement-fixing antibodies. The clinical application of the flow crossmatch varies from center to center, with many centers choosing to consider a positive flow crossmatch one of several risk factors for increased likelihood of rejection episodes, while not a contraindication to transplant (19).

It has been recognized that different histocompatibility methods have different levels of sensitivity and specificity: as the specificity increases the test sensitivity decreases (20), (Figure 4). Some centers chose tests with high sensitivity which resulted in delaying transplantation among recipients who are highly sensitized, while other transplant centers adopted methods with low sensitivity which incurred a high risk of post transplant AMR (21-22).

Cell-based assays may lack the ability to detect low level antibodies in highly sensitized patients. As different studies focused on improving Donor Specific Antibody (DSA) detection, particularly class I and class II, donor cross-matching tools continued to progress (23-26). Compounding CDC testing limitations are non-HLA auto-antibodies such as in patients with systemic lupus erythematosus (SLE) and Type I diabetes which can not be distinguished from antibodies to HLA (24, 27-28). Furthermore, the presence of HLA I and II antigens on B cells makes it difficult to distinguish between classes I and II antibodies with anti-human globulin (AHG) CDC histocompatibility testing.

Recently, solid-phase antibody detection methods have been developed. Solid-phase immunoassays (SPI) include flow cytometric tests and x-MAP multiple bead technology (Luminex). These assays are based on specified and purified HLA antigens absorbed on magnetic beads or purified HLA antigen-coated microbeads. The assay detects HLA antibodies by flow cytometry or Enzyme-linked immunosorbent assay (ELISA) (24, 29-30).

Multiple studies demonstrated that single antigen test eliminates the interference from other HLAs and, therefore, improved test resolution (23-25). However, Prei et al speculated that while the produced HLA class I antigens by recombinant DNA technology (alleles) could be negative; the antibody might react with another native antigen (23). It has been recognized that during HLA extraction and binding to the carrier subtle tertiary protein changes may occur (31). These structural molecular changes may lead to false-positive as well as false negative results (31). Single-phase immunoassay (SPI) studies showed that it significantly improved histocompatibility tests sensitivity and specificity (25, 32). These tests advanced improved CMX predictability, virtual crossmatching, as well as DSA strength monitoring (25). The noted limitation of the bead-based assays is the yet unidentified background substance in the sera which may interfere with HLA-specific antibodies binding (25). In an effort to eliminate the suspected IgM effect on the Luminex test result Zachary et al tested the reducing agent Dithiothreitol (DDT) vs. Hypotonic Dialysis (HD). The investigators demonstrated that HD significantly increases the strength of positive control and reduced the strength of negative control compared to DDT. However, the study was limited by small sample size.

In conclusion, histocompatibility tools evolved over the last five decades from cell-based CDC technique to SPI. This development improved histocompatibility assays sensitivity and specificity as well as CMX outcome. However, SPI has to be carefully interpreted and clinically correlated, particularly when interference is suspected.

5. PROTOCOLS

The goal of current desensitization protocols are prevention of immediate hyperacute rejection, reduction of DSA and AMR rates, as well as prolongation of allograft survival in highly sensitized patients. Despite the overall success of transplant programs in achieving these goals, data on long-term outcomes are limited and suggest suboptimal graft survival in patients with a positive crossmatch at the time of surgery.
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**Figure 5.** The three pathways of complement activation are shown, each leading to generation of activated C3b. The classical pathway is triggered by antibody interacting with antigen, and the lectin pathway is activated by a lectin binding to a sugar. The alternative pathway turns over continuously and becomes engaged only in the setting of foreign material. The generation of C3b leads to inflammation (with release of anaphylatoxins C3a and C5a), lysis via the membrane attack complex (C5b-9), and opsonization (C3b and C4b).

Different protocols have been developed to enable successful transplantation in LD and DD recipients who are highly sensitized due to presence of anti-HLA antibodies reactive against their donors. These protocols provide satisfactory early to intermediate-term allograft survival, and constitute an important advances in transplantation (33). Nevertheless, AMR remains a significant challenge, occurring in 20–50 percent of antibody-incompatible kidney transplantations (33). These protocols were developed in an effort to lower the DSA activity and eventually reduce the risk of immediate renal allograft injury. In addition, desensitization results in maintaining low DSA level during the first weeks to months after transplantation. During this time, some renal allograft seem to develop a degree of relative resistance to antibody mediated injury, a condition referred to as accommodation, which is partly thought to be due to up-regulation of protective genes (33-35).

Splenectomy has been explored pretransplant in patients with and without splenomegaly, as well as in transplant recipients with resistant AMR. Splenectomy reduces plasma cells, B-cells precursor cells and surveillance function (36). The effects of splenectomy on posttransplant rejection rates remain inconsistent. In a study by Opelz and Terasaki on 522 patients received splenectomy vs. 1,131 who did not, the study showed no beneficial effect from pre-transplant splenectomy (37-38). In contrast, Kaufman et al. demonstrated that pre-transplant splenectomy significantly reduced post-transplant rejection (39). However, his study was compromised by selection bias. To date, there is no enough clinical data to recommend addition of splenectomy to the current available desensitization protocols. Furthermore, splenectomy has been abandoned by most of transplant community as other modalities proved to be less invasive and probably more efficient (40-41).

### 5.1. Intravenous immunoglobulin

Immune globulin preparations have been long recognized for its immunomodulatory effect in autoimmune disorders. IVIG consist of intact IgG molecules with a distribution of IgG subclasses similar to that in normal human serum (42). IVIG products are prepared from thousands of donors’ plasma thereby ensuring wide range of antibodies representation (6). Most preparations contain traces of IgA which may sensitize deficient patients after long term administration, soluble CD4, CD8, and HLA molecules in addition to certain cytokines. The half-life of infused immunoglobulin in immuno-competent persons is approximately 21 days. IVIG has broad range of activities. IVIG immunomodulate neonatal Fc receptor, FcRn, and HLA class I molecule, which are found on endothelial cells (33). While immunoglobulin products interact with Fc present in endothelial cells, the Fc portion may have the beneficial effect of decreasing B cell activation by preventing signal induction through it’s interactions with Fc (gamma)IIB (an inhibitory receptor on B cells) (6, 43). Furthermore, IVIG interferes with complement activation through blockade of C3 and subsequently it decreases the formation of C5a and C5b-9. Therefore, IVIG interfere with complement activation and cytokines production by decreasing immune inflammatory response and membrane attack complex formation (33, 42, 44) (Figure 5). In addition, the IVIG immunomodulatory effect is believed to be due to its supply of anti-idiotype antibodies, which interact with DSAs (33, 42, 44).

In the National Institute of Health study IGO2, which was a double-blind placebo controlled trial compared 4 monthly doses of IVIG vs. placebo. The study demonstrated IVIG superior to placebo in reducing anti-HLA antibody level (P = 0.004). Furthermore, the investigator concluded IVIG improved deceased donor transplant rate (39 percent vs. 17 percent). In addition, the study demonstrated improved mean time to transplantation (4.8 yr in IVIG group vs. 10.3 yr in placebo group) (6, 46-48).

In a study of 89 highly sensitized patients with positive CMX, transplant candidates underwent an initial in vitro IVIG-PRA evaluation, then received high dose monthly IVIG (2g/kg) x total of 4 doses, 89 percent were transplanted after in vitro test showed some inhibition effect. The investigators reported a 28% incidence of rejection, and patient and allograft 3 year survival of 97.5 and 87.1 percent respectively (6, 45).

Multiple studies demonstrated IVIG treatment is safe and well tolerated among non uremic as well as ESRD patients who are on Hemodialysis, particularly sucrose free products (49). Indeed, IVIG preconditioning treatment in highly sensitized recipients reduces allosensitization, ischemia-reperfusion injury, and risk of AHR and AMR (7). However, no single dose strategy has been accepted thus far, due to the lack of large controlled clinical trials comparing current available protocols efficacy and cost.
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effectiveness. Current desensitization protocols’ used cohort size, induction variable immunosuppressant utilized, needs to be addressed.

5.2. Plasmapheresis

Multiple trials have shown that immunabsorption (IA) and plasmapheresis (PP) lower HLA-specific antibody levels in different clinical scenarios. While, IVIG is used to modulate immune response, PP is used to mechanically remove alloantibodies. In an 1996 study by Higgins et al, in 13 deceased donor recipients, with positive CMX, the investigator demonstrated that IA converted pretransplant positive CMX to negative crossmatch (50-51). In a retrospective review, by Montgomery et al, in 7 live donor transplant recipients (3 with AMR and 4 with positive CMX), recipients received every other day PP followed by standard IVIG or cytogram 100mg/kg. PP/IVIG were discontinued when CMX negative status is obtained. All preemptive patients developed AHR within the first month post transplant, requiring post transplant continuation of PP/IVIG. Thereafter, no relapses were observed over a mean follow up period of 40 weeks (52). The investigator concluded that combined IVIG and PP for desensitization showed promise, however, with variable rate of success and durability (52). Clinical trials with small cohort (40-110 positive CMX recipients) and follow up duration ranging between 1- 8 years repeatedly concluded that positive pretransplant CMX and highDSA titers are associated with increased risk of AMR, and lower long term allograft survival compared to non-sensitized studies national survival average (33, 53-55). PP with IVIG carries the benefit of faster desensitization compared with the slow effect of IVIG alone. However, it has been proved repeatedly that anti-HLA antibody titer rebound once PP treatment stopped. Whether PP with IVIG improve the long term allograft survival and reduce the prevalence of transplant glomerulopathy in highly sensitized recipients’ remains to be answered by controlled randomized trials.

5.3. Rituximab and novel agents

Rituximab is a chimeric anti-CD20 monoclonal antibody that has been approved for the treatment of lymphoma. A few studies suggest that rituximab might also be effective in treating autoimmune diseases as well as AMR (11; 56-59). The use of this drug as part of a desensitization protocol was examined by Vo et al (60). In their open-label, phase 1-2, single center study, 20 highly sensitized patients received preconditioning IVIG and rituximab combination. The investigators reported significant decrease in pretransplant mean PRA compared to post-transplant one (77 percent pretransplant vs. 44 percent post-transplant, p less than 0.001). In addition, the study showed that mean time to transplantation decreased from 144 months preconditioning to 5 months post desensitization. The investigators concluded that IVIG and rituximab combination suggest promising results for patients waiting transplant from both living and deceased donors. This was followed by a second open label study using larger sample size (11). Ashley et al enrolled seventy-six patients with positive pretransplant T-cell flow cytometry crossmatch (negative B cell crossmatch) and a PRA more than 30 percent. The study group were desensitized with IVIG (2g/kg day 1 and 30) and rituximab (1g day 15). Non-death censored patient and allograft 24-month survivals were 95 and 84 percent respectively. Deceased donor waiting time improved from 95 months to 4.2 months post desensitization. Although, IVIG/Rituximab regimen partially reduced mean pretransplant class I PRA (79 percent desensitization vs. 67.1 percent post desensitization, P= 0.0001), the patients were transplanted. Despite significant reduction of pre-desensitization class I PRA, post-desensitization class I PRA remained significantly elevated. Acute rejection rate was 37 percent of which 29 percent were AMR. The investigators concluded that IVIG plus rituximab offered significant benefit in reducing anti-HLA antibodies, and subsequently improved the rate of transplantation. Will this combination prove effective in allograft long-term survival remains to be answered. Due to lack of well powered, controlled and blinded clinical trial investigating rituximab efficacy with IVIG, rituximab utility in pretransplant desensitization remains to be explored.

Bortezomib is a proteosome inhibitor with effectiveness in treating plasma cell malignancies such as multiple myeloma (61-62). Bortezomib is a candidate drug for reducing DSA through its apoptotic effect on plasma cells (63-64). Evidence from small series suggests that Bortezomib might be used for the treatment of AMR after transplantation (64, 65). However, the results of studies using Bortezomib as part of desensitization protocols have not been published yet.

In conclusion, there is enough literature demonstrating the beneficial effect of high dose IVIG and low dose IVIG with PP. However, most of these studies are limited by sample size, lack of control and randomization. Although transplantation across the HLA donor barrier has become possible, data suggest that long-term outcomes in positive crossmatch patients are suboptimal with current desensitization strategies. Other areas of research include the treatment of subclinical rejection and long-term monitoring of patients.

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Send correspondence to: Maha A. Mohamed, Department of Medicine, University of Wisconsin School of Medicine and Public Health, Wisconsin, Tel: 608-270-5689, Fax: 608-270-5677, E-mail: mmohamed@medicine.wisc.edu