Desensitization across the HLA barrier in kidney transplantaion

Maha Abdalla Mohamed[1], David Lorentzen[3], Jim Kim[2], Arjang Djamali[1, 2]

[1]Department of Medicine, University of Wisconsin School of Medicine and Public Health, Wisconsin, [2]Department of Surgery, University of Wisconsin School of Medicine and Public Health, Wisconsin, [3]Department of Pathology , University of Wisconsin School of Medicine and Public Health, Wisconsin

TABLE OF CONTENTS

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
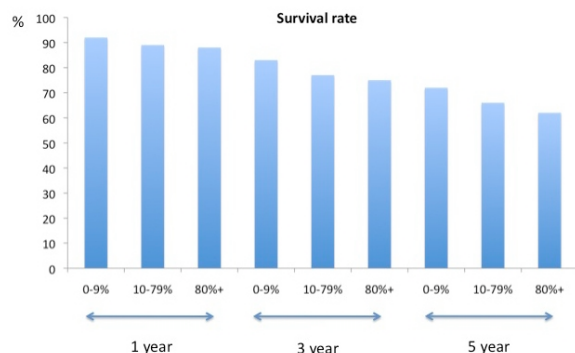
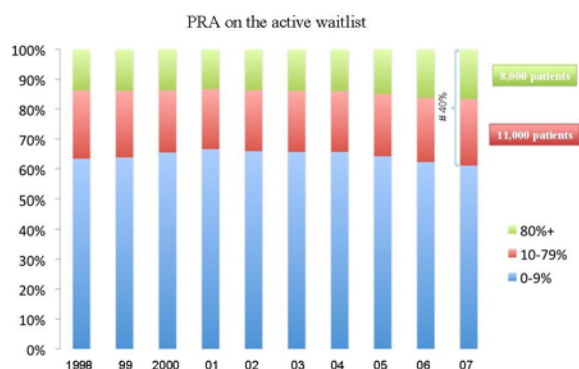**Figure 1.** Survival Rates based on PRA. UNOS 2008.

Figure 2. PRA on the active waitlist. Source: OPTN/SRTR Data as of May 1, 2008.
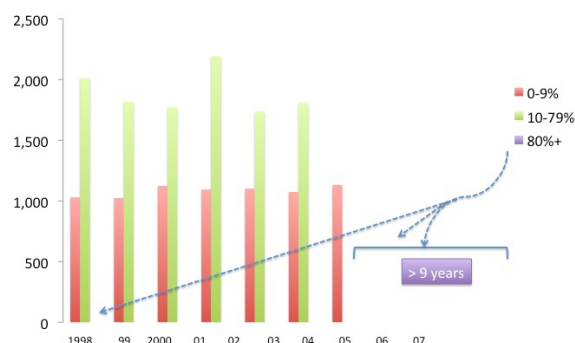

Figure 3. Median time to transplant and PRA. Source: OPTN/SRTR Data as of May 1, 2008.

death-censored graft survival following renal transplantation (1). It has been shown repeatedly that kidney transplantation improves survival when compared with maintenance dialysis for end-stage renal disease (ESRD) patients (2-4). As the demand for organs surpasses the supply, the number of patients in the waiting list and their waiting time continue to increase. Furthermore, transplants are significantly reduced in patients with high levels of preformed anti-Human Leukocyte Antigen (HLA) antibodies.

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).
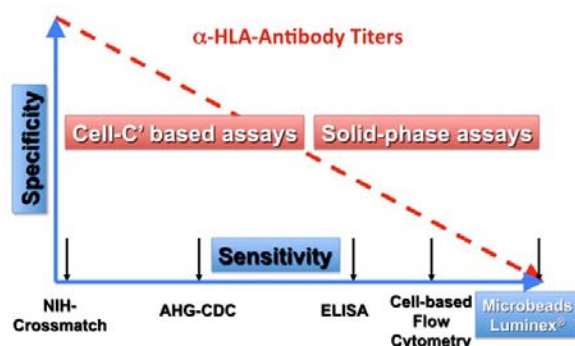
Figure 4. Histocompatibility techniques: Role in risk assessment.

The CDC test is a cell-based assay, which relies on incubating recipient serum with the donor lymphocytes. Subsequently, rabbit complement is added in an effort to improve the test sensitivity. CDC test detects complement-fixed IgG

(IgG1 and IgG3) (14). Although it facilitates detecting pre-transplant HLA antibodies and the initial post-transplant rejection risk stratification, it has been criticized for its low sensitivity (18). Also, CDC test initially relied on manual reading which carried the disadvantage of reader bias (14).

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 factor for increased likelihood of rejection episodes, while not a contraindication to transplant (19).

It has been recognized that different histocompatibilty 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 immunosorbtion 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 cross-matching, 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 CXM 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
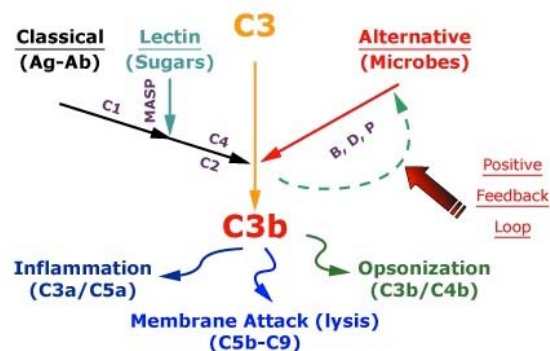
Figure 5. The three pathways of complement activation are shown, each leading to generation of activated C3b. The classical pathway is triggered by antibody interacting with antigen, and the lectin pathway is activated by a lectin binding to a sugar. The alternative pathway turns over continuously and 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, Kauffman et al investigative work in living and deceased donor recipients concluded 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 immunmodulatory 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 immunemodulate 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-C9. 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-iodiotypic antibodies, which interact with DSAs (33, 42, 45).

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

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 a 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 cytogam 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 high DSA 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 (79.7 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.

6. REFERENCES

1. Herwig-Ulf Meier-Kriesche, Friedrich K Port, Akinlolu O Ojo, Steven M Rudich, Julie A Hanson, Diane M Cibrik, Alan B Leichtman and Bruce Kaplan: Effect of waiting time on renal transplant outcome: *Kidney International* 58, 1311–1317, (2000)

2. Vollmer, WM, Wahl, PW, Blagg, CR: Survival with dialysis and transplantation in patients with end-stage renal disease: N *Engl J Med* 308, 1553–1558, (1983)

3. Krakauer, H, Grauman, JS, McMullan, MR, Creede, CC: The recent U.S. experience in the treatment of end-stage renal disease by dialysis and transplantation: *N Engl J* Med 308, 1558–1563, (1983)

4. Wolfe, RA, Ashby, VB, Milford, EL, Ojo, AO, Ettenger, RE, Agodoa, LY, Held, PJ, Port, FK: Comparison of mortality in all patients on dialysis, patients on dialysis

awaiting transplantation, and recipients of a first cadaveric transplant: *N Engl J Med* 341,1725–1730, (1999)

5. J Michael Cecka, Raja Rajalingam, Jennifer Zhang, and Elaine F. Reed: Histocombatibility Testing, Crossmatching, and Immune Monitoring: *Handbook of Kidney Transplantation, Fifth Edition,* 36-60, (2010)

6. Stanley C. Jordan, and Mark D. Pescovitz˙ Presensitization: The Problem and Its Management: *Clin J Am Soc Nephrol* 1, 421-432, (2006)

7. Meier-Kriesche HU, Port FK, Ojo AO, Rudich SM, Hanson JA, Cibrik DM, Leichtman AB, Kaplan B: Effect of waiting time on renal transplant outcome: *Kidney Int* 58, 1311 –1317, (2000)

8. Kaplan B, Schold J, Meier-Kriesche HU: Overview of large database analysis in renal transplantation: *Am J Transplant* 3, 1052 –1056, (2003)

9. Organ Procurement Transplantation Network/Scientific Registry of Transplant Recipients: 2008 Annual Report: *Transplant Data* 1998–2007.

10. Department of Health and Human Services, Health Resources and Services Administration, Healthcare Systems Bureau, Division of Transplantation; United Network for Organ Sharing: *University Renal Research and Education Association* (2008)

11. Ashley A. Vo., Alice Peng, Mieko Toyoda, Joseph Kahwaji, Kai Cao, Chih-Hung Lai, Nancy L. Reinsmoen3 Rafael Villicana, and Stanley C. Jordan1: Use of Intravenous Immune Globulin and Rituximab for Desensitization of Highly HLA-Sensitized Patients Awaiting Kidney Transplantation: *Transplantation* 89, 9, 1095–1102, (2010)

12. Organ Procurement Transplantation Network/Scientific Registry of Transplant Recipients. 2009. Available at: http://www.optn.org/data/. Accessed August 7, 2010.

13. United Network for Organ Sharing Data Base. Available at: http://www. unos.org.

14. Arun Gubta, Paul Sinnot: Clinical Relevance of pretransplant human leukocyte antigen donor-specific antibodies in renal patients waiting for a renal transplant, A risk factor: *Human Immunology* 70, 618-622, (2009)

15. Dusset H: Leukoagglutinins. IV. Leukoagglutinins and blood transfusion: *Vox Sang* 4, 190, (1954)

16. Patel R, Terasaki PI: Significance of the positive cross match test in kidney transplantation: *N Engl J Med* 280, 735 –739, (1969)

17. Terasaki PI, McClelland JD: Microdroplet assay of human serum cytotoxins: *Nature* 204, 998-1000, (1964)

18. Susal C, Pelzel S, Simon T, Opelz G: Advances in pre- and post-transplant immunologic testing in kidney transplantation: *Transplant Proc* 2004; 36: 29-34.

19. Gebel H. M., Bray, R. M., Nickerson, P: Pre-Transplant Assessment of Donor-Reactive, HLA-Specific Antibodies in Renal Transplantation: Contraindication vs. Risk: *American Journal of Transplantation* 3, 1488–1500 (2003)

20. Zachary, A. A., Klingman, L., Thorne, N., Smerglia, A. R. & Teresi, G. A: variations of the lymphocytotoxicity test. An evaluation of sensitivity and specificity: *Transplantation* 60, 498–503, (1995)

21. James Gloor and Mark D. Stegall: Sensitized renal transplant recipients: current protocols and future directions: *Nat Rev Nephrol* 6, 297–306, (2010)

22. Bray, R. A: Flow cytometry in the transplant laboratory: *Ann NY Acad Sci* 677, 138–151, (1993)

23. Pei, R., Lee, J.-H., Shih, N.-J., Chen, M. & Terasaki, P. I: Single human leukocyte antigen flow cytometry beads for accurate identification of human leukocyte antigen antibody specificities: *Transplantation* 75, 43–49, (2003)

24. Pei, R., Lee, J.-H., Chen, T., Rojo, S. & Terasaki, P. I: Flow cytometric detection of HLA antibodies using a spectrum of microbeads: *Hum Immunol* 60, 1293–1302, (1999)

25. Andrea A. Zachary, Donna P. Lucas, Barbara Detrick, Mary S. Leffell: Naturally occurring interference in Luminex_ assays for HLA–specific antibodies: Characteristics and resolution: *Human Immunology* 70, 496–501, (2009)

26. El-Awar, N., Lee, J. & Terasaki, P. I: HLA antibody identification with single antigen beads compared to conventional methods: *Hum Immunol* 66, 989–997, (2005)

27. Terasaki PI, Mottironi VD, Barnett EV: Cytotoxins in disease: Autocytotoxins in lupus: *N Engl J Med* 283, 724, (1970)

28. Guarnotta G, Triolo G: Immune complex-mediated inhibition of lymphocyte Fc-r receptors in plasma of patients with type 1 (insulin-dependent. diabetes mellitus: association with anti-ssDNA antibodies: *Clin Immunol Immunopathol* 54, 228, (1990)

29. Sumitran-Karuppan S, Moller E: The use of magnetic beads coated with soluble HLA class II proteins in antibody screening and for specificity determination of donor reactive antibodies: *Transplantation* 61, 1539, (1996)

30. Pei R, Wang G, Tarsitani C, Rojo S, Chen T, Takemura S, Liu A, Lee JH: Simultaneous HLA class I and class II antibodies screening with flow cytometry: *Hum Immunol* 59, 313, (1998)

31. Arun Gubta and Paul Sinnott: Clinical relevance of pretransplant human leukocyte antigen donor-specific antibodies in renal patients waiting for a transplant: risk factor: *Human Immunology* 70, 618-622, (2009)

32. Zachary AA, Leffell MS: Detecting and monitoring human leukocyte antigen specific antibodies: *Human Immunol* 69, 591–604, (2008)

33. James Gloor and Mark D. Stegall*:* Sensitized renal transplant recipients: current protocols and future directions: *Nat Rev Nephrol* 6, 297–306, (2010)

34. Koch, C. A., Khalpey, Z. I. & Platt, J. L: Accommodation: preventing injury in transplantation and disease: *J Immunol* 172, 5143–5148, (2004)

35. Salama AD, Delikouras A, Pusey CD, Cook HT, Bhangal G, Lechler RI, Dorling A: Transplant accommodation in highly sensitized patients: a potential role for Bcl-xL and alloantibody: *Am J Transplant* 1, 260–269, (2001)

36. Robert A. Montgomery and Andrea A. Zachary: Transplanting patients with a positive donor-specific crossmatch: A single center's perspective: *Pediatr Transplantation* 8, 535–542, (2004)

37. Biggs R: Splenectomy and Renal Transplantation: *British medical journal* 4, 247, (1974)

38. Opelz, G., and Terasaki, P. I: Role of splenectomy in renal Transplantation: Transplantation 15, 605, (1973)

39. Kauffman, H. M., *et al*: Splenectomy and renal transplantation: *Surgery, Gynecology, and Obstetrics* 193, 33, (1974)

40. Tyden G, Kumlien G, Fehrman I: Successful ABO-incompatible kidney transplantations without splenectomy using antigen-specific immunoadsorption and rituximab: *Transplantation* 76, 730–731, (2003)

41. Locke JE, Zachary AA, Haas M *et al*: The utility of splenectomy as rescue treatment for severe acute antibody mediated rejection. *Am J Transplant* 7, 842–846, (2007)

42. Michel D. Kazatchkine, MD, and Srini V. K Averi, D.V.M., PhD: Immunomodulation of autoimmune and inflammatory diseases with intravenous immuno globulin: *N Engl J Med* 345, 10, 747-755, (2001)

43. Nicole A. Weimert and Rita R. Alloway: Renal Transplantation in High-Risk Patients: *Drugs* 67 (11), 1603-1627, (2007)

44. Jordan, S. C., Toyoda, M. & VO, A. A: Intravenous immunoglobulin a natural regulator of immunity and inflammation: *Transplantation* 88, 1–6, (2008)

45. Rossi, F., Dietrich, G. & Kazatchkine, M. D: Antiidiotypic suppression of autoantibodies with normal polyspecific immunoglobulins: *Res Immunol* 140, 19–31, (1989)

46. Stanley C. Jordan, M.D., and (by invitation. Ashley Vo, Pharm. D., Dolly Tyan PhD, and Mieko Toyota, PhD: Desensitization therapy with intravenous gammaglobulin (IVIG): Applications on solid organ transplantation: *Transactions of the American clinical and climatological association* 117, 199-211, (2006)

47. Jordan SC, Tyan D, Stablein D, McIntosh M, Rose S, Vo A, Toyoda M, Davis C, Shapiro R, Adey D, Milliner D, Graff R, Steiner R, Ciancio G, Sahney S, Light J: Evaluation of intravenous immunoglobulin as an agent to lower allosensitization and improve transplantation in highly sensitized adult patients with end-stage renal disease: Report of the NIH IG02 trial: *J Am Soc Nephrol* 15, 3256–3262, (2004)

48. R. A. Montgomery: Renal Transplantation Across HLA and ABO Antibody Barriers: Integrating Paired Donation into Desensitization Protocols: *American Journal of Transplantation* 10, 449–457, (2010)

49. S. C. Jordana,b, A. A. Voa, A. Penga, M. Toyodab and D. Tyanc: Intravenous Gammaglobulin (IVIG): A Novel Approach to Improve Transplant Rates and Outcomes in Highly HLA-Sensitized Patients. *American Journal of Transplantation* 6, 459–466, (2006)

50. J. H. M. Beimier, C. Susal and M. Zeier: Desensitization strategies enabling successful renal transplantation in highly sensitized patients: *Clin Transplant* 20, (suppl. 17), 7-12, (2006)

51. Lorenz M, Regele H, Schillinger M, Kletzmayr J, Haidbauer B, Derfler K, Druml W, Bohmig GA: Peritranslant immunoadsorption: a strategy enabling transplantation in highly sensitized cross-match positive cadaveric kidney allograft recipients: *Transplantation* 79, 696, (2005)

52. Montgomery RA, Zachary AA, Racusen LC: Plasmapheresis and intravenous immune globulin provides effective rescue therapy for refractory humoral rejection and allows kidneys to be successfully transplanted into cross-match-positive recipients: *Transplantation* 70, 887–895, (2000)

53. Magee CC, Felgueiras J, Tinckam K, Malek S, Mah H, Tullius S: Renal transplantation in patients with positive lymphocytotoxicity crossmatches: one center's experience: *Transplantation* 86, 96–103, (2008)

54. Haririan A, Nogueira J, Kukuruga D, Schweitzer E, Hess J, Gurk-Turner C, Jacobs S, Drachenberg C, Bartlett S, Cooper M: Positive cross-match living donor kidney transplantation: longer-term outcomes: *Am J Transplant* 9, 536–542, (2009)

55. Thielke JJ, West-Thielke PM, Herren HL, Bareato U, Ommert T, Vidanovic V, Campbell-Lee SA, Tzvetanov IG,

Sankary HN, Kaplan B, Benedetti E, Oberholzer J*: Living donor kidney transplantation across positive crossmatch: The University of Illinois at Chicago experience: *Transplantation* 87, 268–273, (2009)

56. Vieira CA, Agarwal A, Book BK, Sidner RA, Bearden CM, Gebel HM, Roggero AL, Fineberg NS, Taber T, Kraus MA, Pescovitz MD: Rituximab for reduction of anti-HLA antibodies in patients awaiting renal transplantation: safety, pharmacodynamics, and pharmacokinetics: *Transplantation* 77, 542-8, (2004)

57. Ahmed AR, Spigelman Z, Cavacini LA, Posner MR: Treatment of pemphigus vulgaris with rituximab and intravenous immune globulin: *N Engl J Med* 355, 1772-9, (2006)

58. Faguer S, Kamar N, Guilbeaud-Frugier C, Fort M, Modesto A, Mari A, Ribes D, Cointault O, Lavayssière L, Guitard J, Durand D, Rostaing L: Rituximab therapy for acute humoral rejection after kidney transplantation: *Transplantation* 83, 1277-80, (2007)

59. Salama AD, Pusey CD: Drug insight: rituximab in renal disease in transplantation: *Nat Clin Pract Nephrol* 2, 221-30, (2006)

60. Ashley A. Vo, Pharm.D., Marina Lukovsky, Pharm.D., Mieko Toyoda, Ph.D., Jennifer Wang, M.D., Nancy L. Reinsmoen, Ph.D., Chih-Hung Lai, Ph.D., Alice Peng, M.D., Rafael Villicana, M.D., and Stanley C. Jordan, M.D: Rituximab and intravenous immune globulin for desensitization during renal transplantation: *N Engl J Med* 3593), 242-251, (2008)

61. Rajkumar, S. v., Richardson, P. G., Hideshima, T. and Anderson, K. C: Proteasome inhibition as a novel therapeutic target in human cancer: *J Clin Oncol* 23, 630–639 (2005)

62. Kyle, R. A. and Rajkumar, S. v: Multiple myeloma: *N Engl J Med* 351, 1860–1873 (2004)

63. Perry DK, Burns JM, Pollinger HS, Amiot BP, Gloor JM, Gores GJ, Stegall MD*: Proteosome inhibition causes apoptosis of normal human plasma cells preventing antibody production: *Am J Transplant* 9, 201–209, (2009)

64. Diwan T, Burns J, Perry D. *et al*: Effect of proteasome inhibition on alloantibody secreting cells *in vivo*: *Am J Transplant* 9 (Suppl. 2), 241 (2009)

65. Everly MJ, Everly JJ, Susskind B, Brailey P, Arend LJ, Alloway RR, Roy-Chaudhury P, Govil A, Mogilishetty G, Rike AH, Cardi M, Wadih G, Tevar A, Woodle ES*: Bortezomib provides effective therapy for antibody- and cell-mediated acute rejection: *Transplantation* 86, 1754–1761 (2008)

Abbreviations: AHG: anti-human globulin, AMR: antibody mediated rejection, CDC: complement-dependent lymphocytotoxicity, CMX: crossmatch, DD: deceased donor, DDT: dithiothreitol, DSA: donor specific antibody, ELISA: enzyme-linked immunosorbet assay, HD: hypotonic dialysis, HLA: human leukocyte antigen, IVIG: intravenous immunoglobulin, LD: living donor, PRA: Panel reactive antibody, SLE: systemic lupus erythematosus, SPI: single-phase immunoassay

Key Words: Kidney Transplantation, Desensitization, Crossmatch, Intravenous Immunoglobulin, Plasmapheresis, Splenectomy, Rituximab, Review

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