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Desensitization and Induction Immunosuppressive Therapy in Highly HLA-Sensitized Patients Receiving Cadaveric Renal Allograft

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1. Introduction

Approximately 15-20% of patients on the waiting list for cadaveric renal transplant are highly HLA-sensitized. It is well known that the presence of alloantibodies against human class I (A, B, C) and class II (DR, DQ) HLA antigens in transplant recipients waiting for a renal transplant has a significant increase in acute and chronic rejection rates and a poor graft outcome. There are interesting options for minimizing these immunological problems such as national paired kidney exchange program or acceptable mismatch program similar to the one developed by Eurotransplant program. However, despite these efforts, these patients can wait up to 5 years for a kidney graft and just get it finally only 30% of them. It is therefore vital to develop strategies to reduce waiting time and decrease the risk of transplant rejection, through the elimination or reduction of circulating lymphocytotoxic antibodies.

There have been several retrospective and prospective studies that have used immunoabsorption or plasmapheresis together with immunosuppressants and intravenous immune globulins with highly variable success rates that, while not providing a high level of evidence, constitutes a promising therapeutic alternative for these patients.

In addition, the use of rituximab in living donor transplantation ABO incompatible, hypersensitive patients with positive crossmatch, acute rejection resistant to standard treatments and pretransplant desensitization are running with very interesting results. Moreover, newer approaches for treating acute humoral rejection such as the proteasome inhibitor (bortezomib) or eculizumab (an anticomplement monoclonal antibody), are emerging as successful therapeutic options (Gloor J& Stegall, 2010).

Long-term follow-up of these patients and the application on a wider scale of these treatments, will provide the definitive answers about their real efficacy (Nocera, 2009). Nevertheless, acute antibody-mediated rejection (AMR), occurring in 20-50% of patients, and subclinical AMR even in the absence of allograft dysfunction, may decrease allograft survival by chronic histological abnormalities.

The aim of this chapter is to review the current data about approach therapy in highly HLA-sensitized patients receiving deceased renal allograft.

2. Definition of highly HLA-sensitized patients

Patients with PRA (panel reactive antibodies) permanently $> 75\%$, plus HLA-polyspecific reactivity by complement dependent cytotoxicity (CDC) or flow cytometry and multiple previous positive crossmatch, should be considered highly HLA-sensitized patients. HLA antibodies, are present in hyperimmunized patients as a result of pregnancies, blood transfusions and previous failed grafts, and make up an important barrier to renal transplantation.

There are different assays that can be used to determine the PRA, but it is necessary to ensure efficiency and reliability of these tests, so that each laboratory must continuously monitor its processes and results (Ercilla MG, 2010). Monitoring is guaranteed by the accreditation of processes as well as quality control of results, evaluated by external organisations of experts on histocompatibility (for example, the accreditation programme of the European Federation for Immunogenetics and the American Society for Histocompatibility and Immunogenetics).

2.1 The complement dependent cytotoxicity (CDC) assay

A complement-mediated cytotoxicity reaction occurs when an IgM or IgG reacts against an antigen present on the cell surface of specific tissues. After this reaction, the complement system activation leads to cell membrane damage and, secondarily, cell lysis. In addition, the cells covered by the antibody (opsonized) are susceptible to be ingested by the monocyte-macrophage system, as it reduces the ionic charge of the cell surface directly through immune adherence or by binding to C3.

The complement dependent cytotoxicity (CDC) assay estimates PRA by adding potential recipient serum to microtiter plates that contain a pool of lymphocytes with defined HLA antigens. Rabbit complement is added and the plates are viewed after addition of a vital stain. The PRA can then be determined based upon the number of cytotoxic reactions that are observed. The classic assay CDC crossmatch has a high positive predictive value for graft loss in the first 48 hours if positive, which therefore contraindicates transplantation. Alloantibody testing should be performed every three months in all candidate patients for renal transplantation and 15 days after each sensitising event (transfusion, graft loss and pregnancy). This sequential study helps to reveal antibodies that may have been identified in the past but that may not have been detected at the time of transplantation. If the crossmatch is positive by CDC, the process is repeated with the addition of Dithiothreitol (DTT). This step reduces the disulfide bonds present when the antibody is IgM.

A test that is CDC positive/DTT negative (presence of an IgM antibody only) should not preclude transplantation: in this case, the determination of solid-phase anti-HLA alloantibody screening (by immunoabsorption ELISA or flow cytometry, Luminex) is negative in serum that was CDC-PRA positive. By comparison, the presence of a CDC positive/DTT positive test is an indication of IgG anti-donor antibody and is a contraindication to transplantation without the use of a desensitization procedure, especially if a donor specific antibody has been defined. (Klein, 2010; Ercilla MG, 2010)

2.2 The enzyme-linked immunoabsorption (ELISA)

This assay uses microtest trays containing known HLA antigens to which potential recipient serum is added. This test is faster than the CDC assay and the HLA antigens used for screening can be adjusted as necessary to reflect the presumed potential donor pool. As in

the cytotoxicity technique, the mix of antigens should be representative of the general population. In case of anti-HLA antibodies, a colorimetric reaction enzyme occurs. This reaction is quantified by spectrophotometer.

By ELISA assay, we detect all anti-HLA antibodies, including complement fixing and non-fixers. Once it has detected the presence of anti-HLA antibodies (class I or II), specificity against which these antibodies are directed can also be determined by a high definition ELISA plates or unique antigens with the same methodology. Solid-phase anti-HLA alloantibody screening is useful when autoantibodies are suspected and there is a need to rule out them in a patient with positive CDC-PRA. This indicates presence or absence of type IgG anti-HLA antibodies against anti-HLA-I and anti-HLA-II and, in some kits, anti-MICA. By using purified HLA antigens, non-anti-HLA antibodies are not identified. If anti-HLA antibodies are not revealed by cytotoxicity but are detected by solid phase, it is highly recommended that more sensitive crossmatch techniques should be used, such as flow cytometry or virtual crossmatch (VCM) in order to better define the risk for these patients. (Klein, 2010; Ercilla MG, 2010).

2.3 Flow cytometry. Single-antigen bead flow cytometry (SAB-FC)

Flow cytometry measures the fluorescence after patient serum has been added to a defined set of HLA antigen flow beads. A positive test is determined by the mean channel shift in intensity, that is, mean intensity of fluorescence (MFI). This assay allows to identify specific HLA antigens to which the patients are sensitized and constitutes what we know as "virtual lymphocyte crossmatch (VCM)." VCM is indicated in patients who are candidates for retransplantation, women who have previously been multiple pregnant and those with positive results in the solid-phase screening but negative for CDC as well as also is recommended for all living-donor transplants.

In the event that the only positive result is that of the positive VCM, this will indicate a 55% probability of an antibody-mediated rejection episode in the first year versus a 5% probability in the case of a negative VCM and the graft survival at one year is slightly lower. However, a positive VCM, by itself, does not imply that a transplant is necessarily contraindicated, but a careful monitoring and immunosuppression aimed to controlling alloantibody production are needed for a thorough treatment.

A positive B-cell negative T-cell crossmatch usually occurs in presence of anti-HLA-II antibodies, presence of low-titre anti-HLA-I antibodies detectable only in B lymphocytes and presence of specific B lymphocytes autoantibodies. In these cases, the decision for transplantation must be individualised. In others words, T cell negative/B cell positive reactions may be secondary to either class I or class II antibodies, while a T cell positive/B cell negative reaction most likely results from a non-HLA antibody, as class I antigen is expressed on both T and B cells. For living donor recipients, perform a monocyte crossmatch should be useful as may help to detect anti-endothelial antibodies.

Clearly identifying the reactivity of antibodies with the donor (DSAs) is logistically difficult because it requires donor cells which may be stored frozen in liquid nitrogen, or determined of the living donor who has to be present for each determination. Therefore, in these cases, when the crossmatch cannot be performed due to lack of donor cells, where this assay plays an important role as it allows identify specific HLA antigens to which the patient is sensitized (virtual lymphocyte crossmatch).

Singh N et al showed the impact that produces pre-Tx DSAs detected by SAB-FC on early clinical outcomes. They tested pre-Tx sera from all consecutive deceased-donor kidney

transplants performed between January 2005 and July 2006 (n=237), 66% had a high-immunologic risk. MFI more than or equal to 100 for class I and more than or equal to 200 for class II were the lowest DSA thresholds associated with inferior antibody-mediated rejection-free graft survival (Singh N et al., as cited in Ercilla MG, 2010). The presence of class I-anti-HLA antibodies post-transplant precedes, even by years, the development of glomerulopathy. In addition, the presence of anti-class II antibodies is strongly associated with chronic rejection in living-donor kidney recipients, but it appears that the worst prognosis is associated with the simultaneous detection of anti-HLA-I and anti-HLA-II antibodies.

There is evidence that it is possible to reduce pre-existing circulating alloantibodies in some patients to levels where the antibodies are unable to trigger hyperacute rejections. This does not imply that there are no B lymphocytes with the capacity to restart alloantibody production, but the short-term survival of grafts transplanted in some centres under these conditions is acceptable. (Klein, 2010; Ercilla MG, 2010).

Compared with the cell-based method, the fluorometric bead system is not as susceptible to drug interference, such as antithymocyte globulin, intravenously administered immunoglobulin (IVIg), and rituximab. IVIg may interfere with the bead assay for a few days after administration. Nonetheless, these new techniques allow for greater identification of HLA antibody specificities and a more accurate interpretation of cross-match results. Patients with high DSA and donor-specific cross matches SFI units are considered at high risk for AMR and warranted more frequent antibody-level monitoring posttransplantation, may need desensitization treatments, or plan biopsies, in order to reduce DSA levels or detect early AMR respectively; sometimes, increase in DSA imply retreatment as can result in reduction in DSA, levels to $\leq 10^5$ SFI units is usually associated with a low risk of AMR.

3. Immunosuppressive therapy in high immunologic risk patients receiving cadaveric renal allograft

3.1 Immunosuppressive agents

3.1.1 Anti-lymphocyte antibodies

Thymoglobulin is a polyclonal immunosuppressive agent that is generated in rabbits, containing antibodies to a wide variety of human T-cell surface antigens, including the major histocompatibility complex (MHC) antigens. These antibodies have the ability to block a number of adhesion molecules, cytokines, chemokines, among others. Anti-lymphocyte antibodies have long been an integral part of induction regimens and, nowadays, continue to be used in the management of patients at risk of early rejection. They are used in combination with steroids, mycophenolate and calcineurin inhibitors or, less frequently, proliferation signal inhibitors and are treatment of choice for acute graft rejection grade II and III of Banff or unresponsive to steroid boluses. Among the available polyclonal globulin, thymoglobulin, has shown a great efficacy and typically requires between 7 and 10 doses.

The reaction of these globulins with some lymphocyte antigens can trigger activation of these cells to release cytokines, which may present with chills, fever and systemic symptoms, mainly with the first dose. Steroids, antihistamines and antipyretics intravenous infusion may prevent these early reactions; polyclonal antibodies will be made through a central venous catheter in at least 6 hours.

Side effects in the medium and long term are related to its immunosuppressive effect. Polyclonal antibodies can increase the risk of infection (herpes simplex virus, varicella-

zoster virus, cytomegalovirus or Epstein-Barr virus) and patients may benefit from prophylactic antiviral strategies. Other opportunistic infections may be due to pneumocystis jiroveci and aspergillus, but are related to immunosuppression accumulated by the patient rather than the administration of polyclonal antibodies. Treatment with antilymphocyte globulin and a serum test for Epstein-Barr virus receptor are associated with the risk of lymphoproliferative disorders in renal transplant population. (Gaber ,AO et al., 2010; Oppenheimer, F et al., 2010) .

Currently almost 70% of renal transplant patients in the United States receive antibody induction, either antithymocyte globulin (Thymoglobulin) or inhibitors of IL-2. Brennan, D et al. (Brennan, C et al., 2006) compared the efficacy and safety of randomized use of basiliximab versus thymoglobulin in patients at high risk of rejection (retransplantation, PRA greater than 20% black, one or more HLA incompatibilities) or delayed graft function who received a cadaveric renal transplantation. After 12 months of follow-up, incidence of biopsy proven acute rejection was 15.6% for Thymoglobulin, and 25.2% for basiliximab ($p = 0.02$) and the antibody-treated acute rejection was lower in patients with thymoglobulin (1.4% vs 8.0%, $p = 0.005$). The overall rate of adverse events was 99.3% to 98.5%, was similar between both induction treatment. The overall incidence of infection was 85.8% to 75.2% with thymoglobulin and basiliximab ($p = 0.03$). This difference appears to be attributable to a higher frequency of urinary tract infection and no CMV viral infections. CMV infection was lower with Thymoglobulin (7.8% vs 17.5%, $p = 0.02$), probably due that a prophylactic antibiotics were used less in patients with thymoglobulin (18.9% vs 30.9%, $p = 0.03$).

3.1.2 Alemtuzumab

Alemtuzumab is a humanized IgG1 kappa monoclonal antibody, genetically engineered, specific for a surface glycoprotein of lymphocytes, 21-28 kD (CD52) present on T and B lymphocytes, natural killer cells and to a lesser extent on monocytes and macrophages. It is indicated for the treatment of patients with chronic lymphocytic leukemia who have been treated with alkylating agents and who have not achieved a complete or partial response. Alemtuzumab causes lysis of lymphocytes by binding to CD52, the antibody mediates lysis of lymphocytes by complement fixation and cell-mediated cytotoxicity, antibody-dependent. The antigen has been detected in a small percentage (<5%) of granulocytes, but not detected in erythrocytes or platelets. Alemtuzumab does not appear to damage the hematopoietic stem cells or progenitor cells. Side effects include first-dose reactions, less severe than those due to OKT3, as well as anemia, leukopenia and pancytopenia. Further long-term controlled studies are needed to establish the potential benefit in terms of efficacy and safety after kidney transplantation.

3.1.3 OKT3

OKT3 is a murine monoclonal antibody against the T3 antigen of human lymphocytes which acts as an immunosuppressant by blocking a molecule (CD3) located in the membrane of human T cells. This molecule appears to be associated with the structure of antigen recognition of T cells. It is indicated to treat acute rejection reaction in renal transplant patients. It must be only used in intravenous form and the dose of other immunosuppressive agents used concomitantly with OKT3 should be decreased to minimal levels and restarted about three days before the end of treatment with OKT3. It produces a rapid and concomitant decrease in the number of circulating T cells (CD3, CD4 and CD8) after the administration. After discontinuation of treatment, CD3 cells reappear rapidly and

reach pretreatment levels within a week. OKT3 should not be used in patients with hypersensitivity to this or any other product of murine origin and in patients with fluid overload or with a history of seizures or a predisposition to allergies. After the first dose of OKT3 patients may experience fever, chills, malaise, encephalopathy, aseptic meningitis, dysnea and with minor frequency, fatal severe pulmonary edema. The most common infections were cytomegalovirus (19%) and herpes simplex (27%). To summarize, OKT3 was the first monoclonal antibody against CD3 used but adverse effect profile makes little used today.

3.1.4 Intravenous immunoglobulin (IVIG)

Intravenous immunoglobulin (IVIG) products: are derived from pooled human plasma and have been used for the treatment of primary immunodeficiency disorders, autoimmune and inflammatory disorders and recently in the treatment of sensitized patients. Mechanism of action: neutralization of circulating antibodies through idiotype-idiotype interactions to inhibit the binding of Ac antiHLA to their target cells in a dose-dependent form; inhibition of secretion of cytokines and other soluble mediators; stimulation of cytokine receptor antagonists; interaction with antigen-presenting cells by blocking T cell activation through the Fc receptor; inhibition of binding of the fractions of complement to target cells because they have a high affinity for activated complement components C3b and C4b resulting in decreased formation of the potent anaphylatoxin C5a and the C5b-C9 membrane attack complex, inhibition of proliferation of T and B cells and "down regulation" of the synthesis of antibodies and others.

Plasmapheresis, in combination with intravenous immunoglobulin, produces durable, donor-specific antibody suppression as effect of plasmapheresis is short-lived, and a rebound occurs usually when plasmapheresis is discontinued and half-life of IVIg is about 3 weeks; therefore, in most protocols its administration is repeated every 4 weeks. In addition, IVIG are useful in the treatment and prevention of posttransplant infectious complications including cytomegalovirus, parvovirus B19 and polyoma BK virus. Adverse effects are minor: headaches, fever, fatigue, myalgia, hypotension, sweating, dizziness, chills, chest tightness, wheezing. They all probably are secondary to increased levels of inflammatory cytokines and vasoactive substances and with high velocity perfusion. Anaphylactic reaction and shock can occur in patients with total or partial deficiency of IgA. In these cases, epinephrine, and diazepam for muscle spasms are indicated. Another adverse effect is renal dysfunction, because of the content of sucrose or sorbitol which can cause osmotic nephrosis in the proximal tubule. This can be avoided by reducing the osmolality of immunoglobulin products containing sucrose, using restorative with sterile water instead of saline and lowers the concentration of Igs and sucrose to <9%. Other life-threatening side effects are thrombotic events. Very slow infusion using IVIG at a concentration of 5%, with low or no sodium content and low osmolality, and using molecular weight heparin reduces the incidence.

3.1.5 Rituximab

It is a chimeric antibody mouse / human directed against the CD20 antigen on B lymphocytes (expressed in mature B lymphocytes and preinfocito B). Directly inhibits B cell proliferation, induces apoptosis and reduces the production of antibodies. It has been used for the treatment of non-Hodgkin's lymphoma B cells, rheumatoid arthritis, autoimmune diseases such lupus, idiopathic thrombopenic purpura, cryoglobulinemia and organ

transplantation. It produces rapid reduction of B cells with subsequent recovery at 12 months. Recent clinical data suggest that the beneficial effects of rituximab may be due to depriving T cells of antigen-presenting cell activity provided by antigen-specific B cells, thus altering effect or functions and inducing a regulatory profile. These data suggest that the beneficial effects of rituximab on autoimmune disease are more likely related to modification of dysfunctional cellular immunity rather than simply a reduction in antibody. A single dose of 375 mg/m² in renal transplantation produces depletion of CD19 and CD20 cells for long periods in peripheral blood and tissue level.

Rituximab can be administered in a peripheral vein and, although rare, can cause anaphylactic reactions, which suggests his administration under close monitoring. The use of rituximab, which is directed against the CD20 antigen, would seem to be a logical strategy, since reduction or elimination of B cells that express CD20 and make anti-HLA antibodies, should have a beneficial effect. However, there are problems with this concept. First, anti-CD20 activity has no effect on plasma cells, which are the primary source of acute antibody production and second, rituximab has no immediate effect on circulating antibody levels. These problems might limit the benefit of rituximab if were used as the sole treatment, however, it appears that the use of rituximab in combination with other treatments, e.g., plasmapheresis which eliminates circulating antibodies, and intravenous immune globulin, that acts neutralizing circulating antibodies through idiotype-idiotype interactions to inhibit the binding of Ac antiHLA, or both, might constitute an improved approach for the management of allosensitization.

There are concerns regarding the use of rituximab, because it has been reported to induce reactivation of polyomavirus JC virus, resulting in progressive multifocal leukoencephalopathy. Optimal treatment of AMR probably requires a combination of rituximab with PP and low-dose IVIG or with high-dose IVIG (1–2 gm/kg) due to the inability of rituximab to deplete CD20-negative plasma cells that continue to produce DSA and mediate graft injury.

3.1.6 Calcineurin-inhibitors

Cyclosporine A binds to an intracellular receptor (cyclophilin) and form an active complex that binds and inhibits the phosphatase activity of calcineurin. Calcineurin participates in the transcriptional control of ribonucleic acid for the synthesis of cytokines (IL-2, IFN- γ , IL-4 and TNF- α). Thus, inhibits the proliferation of T cells preventing clonal expansion of helper and cytotoxic T cells; suppressor T cells are not affected.

Tacrolimus is a macrolide that forms a complex intracytoplasmic with a specific immunophilin (FKBP) capable of blocking the phosphatase activity of calcineurin, and thereby inhibit transcription of different genes (IL-2 and others). It inhibits the activation and proliferation of T cells and the synthesis of cytotoxic T lymphocytes. It also slows the growth and differentiation of B cells by interfering with the expression of IL-4 receptor and IL-5 synthesis. Unlike cyclosporin, tacrolimus does not interact with the receptor of transforming growth factor beta (TGF- β) type 2, which would provide more efficacy in preventing chronic graft rejection. Tacrolimus is used to prevent acute graft rejection and for treatment of corticosteroids-resistant acute rejection. It is administered in combination with steroids and derivatives of mycophenolic acid and mTOR inhibitors.

Adverse effects with greater clinical significance are nephrotoxicity, similar to that produced by cyclosporine A, carbohydrate intolerance and diabetes mellitus, neurological disorders: tremor, headache, dizziness, and severe neurological (seizures, encephalopathy, etc.) and

also increased susceptibility to development of infections and malignancies. (Oppenheimer, F et al., 2010).

3.1.7 Derivatives of mycophenolic acid, mycophenolate mofetil or enteric-coated mycophenolic acid

Mycophenolate is an ethyl ester of mycophenolic acid that selectively inhibits de novo synthesis of purines, the proliferation of T and B lymphocytes, the expression of adhesion molecules and proliferation of smooth muscle cells of the vascular wall. The enteric-coated mycophenolic acid sodium salt is designed to try to improve gastrointestinal tolerance. The efficacy and safety of both drugs are similar. Its main indication is the prevention of acute graft rejection and may play an important role in preventing chronic rejection. Commonly used with cyclosporine A or tacrolimus to prevent acute graft rejection and have also been proposed for the treatment of corticosteroid-resistant acute rejection or refractory to treatment. May appear blood disorders (anemia, leukopenia and thrombocytopenia), which are not severe. Viral infections, especially cytomegalovirus are more frequent than when used in place of mTOR inhibitors. (Oppenheimer, F et al., 2010)

3.1.8 mTOR inhibitors, sirolimus and everolimus

Anti-mTOR drugs, sirolimus and everolimus are potent immunosuppressants with antiproliferative and anti-migratory capacity that act by blocking the intracellular signalling that regulates the growth and proliferation of T2 cells. mTOR inhibitors are macrolide acting in a late stage cell proliferation by inhibiting cytokine-specific signals. To act it requires form a complex with an immunophilin, but unlike the tacrolimus, do not inhibit calcineurin. Everolimus is a derivative of sirolimus with a shorter elimination half-life and greater oral bioavailability.

In primary immunosuppression, associated with cyclosporine A, have a synergistic immunosuppressive effect, and the incidence of acute rejection varies between 10 and 20%. While competing for the same tacrolimus cyclophilin, the association of mTOR inhibitor-tacrolimus is, at least, as effective as tacrolimus, mycophenolic acid association. Its main advantage is a reduction in the appearance of de novo tumours and the absence of nephrotoxicity, although significant proteinuria has been reported, especially after late use in grafts with impaired function. In cases of nephrotoxicity may be useful in association with mycophenolate, after discontinuation of calcineurin. An additional advantage is the lower rate of cytomegalovirus infection. Its side effects are: hypercholesterolemia, hypertriglyceridemia and thrombocytopenia, which are related to the administered dose. These side effects may offset their benefits in the longer term in highly renal transplant considering that are patients with high immunological risk whose should remain on full-dose triple therapy.

3.1.9 Proteasome inhibitor (bortezomib)

Bortezomib, a proteasome inhibitor approved for the treatment of multiple myeloma, induces plasma cell apoptosis. Its role in desensitization protocols and treatment of humoral rejection may offer promise results in transplant recipients. The pharmacokinetics of bortezomib can be characterized by rapid and wide distribution, a prolonged elimination half life, and hepatic cytochrome P-450 (CYP) isoenzyme metabolism. Side effects more frequent are a low-grade gastrointestinal side effect, mild to moderate anemia, neutropenia,

and thrombocytopenia, and primarily mild cases of peripheral neuropathy. Despite the mild decrease in PRA levels, bortezomib therapy led to more than 50% decrease in the levels of anti-HLA antibodies triggering C4d deposition on single antigen Luminex beads as measured in MFI with single-antigen bead flow cytometry.

Results in desensitization of patients with this agent before transplantation are less consistent. Wahrmann et al. could not observe a significant decrease of circulating HLA antibodies in two highly sensitized dialysis patients who were treated with two cycles of bortezomib, indicating that this agent is not able to eliminate long-lived plasma cells. Furthermore, *in vitro* studies indicate that contact with alloantigen enhances the susceptibility of plasma cells to proteasome inhibition-mediated apoptosis, which might also serve as an explanation for the observed differences in the effectivity of bortezomib in the pre- and posttransplant phases.

In the study by Walsh et al., two patients undergoing acute AMR with high DSA and positive C4d staining on biopsy two weeks after kidney transplantation were treated with a multiday regimen consisting of plasmapheresis, methylprednisolone and bortezomib along with a single dose of rituximab. By nearly 14 days after treatment, DSA levels had dropped significantly as well as repeat biopsy showed faint peritubular capillary C4d labeling and decreased glomerular C4d deposition.

Trivedi et al. (Trivedi et al., 2009, 2010) reported thirteen living donor renal transplant patients treated with bortezomib one to two cycles and plasmapheresis to remove HLA antibodies posttransplant. All patients treated with bortezomib/plasmapheresis resulted in a primary DSA reduction of more than 50% measured by means of single antigen bead on Luminex. In 10 of 13 patients, complete DSA removal, below than 1000 mean fluorescent intensity occurred. At 1 year posttreatment, antibody intensity remains significantly depressed in the group as a whole, despite tetanus toxoid and measles IgG levels remained unchanged and above the level of protection. These data suggest that proteasome inhibitors plus plasmapheresis results in prolonged reduction of HLA antibodies while leaving protective immunity intact. Some patients had reappearance of anti-HLA antibodies despite initial effective reduction, and the authors suggested that certain patients may need more than one cycle of treatment to decrease DSA levels.

3.1.10 Eculizumab (anti-C5, anticomplement monoclonal antibody)

The monoclonal antibody eculizumab, which binds to complement factor C5 and prevents formation of the membrane attack complex C5b-9, is currently in clinical use for the treatment of paroxysmal nocturnal hemoglobinuria and being tested for the treatment of atypical hemolytic uremic syndrome. Its use combined with plasmapheresis or IVIg decrease C5b-C9 complex deposition in the kidney. It is very important to be immunized against meningitis (*Neisseria meningitidis*) to all patients two weeks before the administration of eculizumab as due to its mechanism of action, the use of this drug increases the patient's sensitivity to meningococcal infection.

Eculizumab selectively inhibits the human complement protein C5, preventing its division into C5a and C5b, thus annulling the formation of C5b-9 terminal complement, which is behind the formation of transmembrane channels that cause cell lysis. The adverse reactions most frequently reported are headache, nasopharyngitis, nausea, pyrexia, myalgia, fatigue and herpes simplex, observed in at least 5 of every 100 patients. The most serious side effect was meningococcal septicemia.

Complement activation plays a critical role in mediating AMR after kidney transplantation. As eculizumab has the ability to inhibit C5b-C9 MAC and C5a generation, it should act as a strong accommodation promoter and prevent AMR. Fortunately, recent data presented by Stegall et al. (Stegall et al., 2010, as cited in Jordan, S, 2010) supports this contention. These investigators treated ten patients who underwent desensitization with plasmapheresis + IVIG with eculizumab after transplantation. After nearly 12 months of follow-up for all patients, none developed AMR. Several protocol biopsies showed C4d deposits but no evidence of AMR. This finding is suggestive of incomplete complement activation, which is permissive for accommodation. This author says, that a combination of high-dose IVIG with eculizumab maybe act to modify elements of cellular immunity, humoral immunity, and complement effectors. Confirmation of these ideas awaits clinical trials.

3.1.11 Receptor antagonists interleukin-2 (I1-2R): Basiliximab

This anti-CD25 monoclonal antibody, one chimeric (basiliximab) are widely used in renal transplantation patients with low-moderate immunological risk during the induction phase. Usually, induction therapy with basiliximab is used in combination with calcineurin-inhibitors, derivatives of mycophenolic acid and prednisone. This antibody is directed against a chain of IL-2 receptor, whose expression on the cell surface requires activation of the T cell. Basiliximab is used in two doses of 20 mg each, for intravenous injection at time of transplantation and on the fourth day after transplantation, respectively. The first dose should be administered before reperfusion of the organ. Hypersensitivity reactions, including anaphylaxis, have been reported in isolation with the use of these antibodies, which, moreover, are considered safe and an adverse event profile similar to those reported with placebo.

3.1.12 Therapeutic apheresis

Plasmapheresis is a plasma exchange procedure to removal from blood plasma molecules with specific antigen recognition like antibodies or autoantibodies, molecules that alter the physical properties of plasma, immune complexes, toxic molecules and others. The therapeutic goal of plasma is to reduce circulating levels of these molecules to mitigate the underlying disease process. The vast majority of disorders successfully treated by plasmapheresis treatment involving the removal of IgG, as it has a longer half life and low rate of synthesis. Other factors removed as complement, coagulation proteins or inflammatory mediators contribute to a lesser extent the therapeutic benefit of plasmapheresis by its short half-life and high rate of synthesis.

Therapeutic plasma exchange has been used successfully in the treatment of many hematological, neurological, renal, and metabolic disorders, rheumatic and acute humoral rejection. This last is a condition that requires early diagnosis and intervention. Many groups have developed protocols for immunosuppression and immunomodulation that often include therapeutic plasma exchange.

Plasmapheresis therapy is successfully used in the treatment or prevention of rejection in solid organ transplantation. Although the cellular immune response is responsible for mediating most of the rejections of allografts, acute humoral rejection of the transplanted organ refers to a severe dysfunction associated with the presence of antibodies directed against the donor organ. This type of rejection is generally resistant to immunosuppressive and immunomodulatory therapies, occurs more frequently in patients with preexisting

antibodies to the ABO system antigens or HLA expressed by the graft and is associated with a poor prognosis for graft survival. Numerous studies showed evidence of HLA antibodies decreased with plasmapheresis; in addition, patients with refractory acute rejection, the use of plasma exchange schemes and IVIG results in a better renal graft survival. The number of plasmapheresis sessions is greater the higher the antibody titer donor-specific. In addition, as soon as plasmapheresis stops, there is a rebound in the title antibody. Therefore, plasmapheresis is considered an additional technical assistance to other therapeutic procedures, particularly treatment with IVIG.

Extracorporeal immunoadsorption is other technique for the elimination of pathogenic antibodies and circulating immune complexes. Immunoadsorption is capable to eliminate huge amounts of immunoglobulins from the patient's circulation with a minimum of side effects (associated with the substitution of fresh frozen plasma or albumin or removal of other plasmatic factors to above 50%).

Most evidences about immunoadsorption are based on uncontrolled case series and individual observations. Indications for extracorporeal immunoadsorption are presently limited to HLA-pre-sensitized kidney recipients, rapidly progressive glomerulonephritis, haemolytic uraemic syndrome, life-threatening autoimmune diseases among others. Immunoadsorption devices can be subdivided into non-selective, semi-selective and highly selective adsorbers. In patients with acute vascular rejection after renal transplantation, immunoadsorption can be used to remove anti-HLA antibodies in combination with conventional anti-rejection therapy. It seems feasible to apply immunoadsorption instead of plasmapheresis for acute, vascular rejection although a controlled trial should demonstrate whether one or the other is more effective and associated with less adverse effects.

Immunoadsorption could also be successfully used for the reduction of anti-HLA antibody titre before transplantation for obtaining a negative cross match in highly sensitised patients. A median of plasma processed during the pre-transplant immunoadsorption session could be high and may not be achieved with the use of plasmapheresis due to a high likelihood of adverse reactions attributable to the administration of fresh frozen plasma or albumin. By contrast to plasmapheresis, immunoadsorption allows the treatment of higher plasma volumes with a greater reduction of immunoglobulins (immunoadsorption is capable of removing >85% of IgG during one session). In the future, immunoadsorption may replace plasmapheresis in the treatment of some but not all diseases, however, the high costs associated with immunoadsorption therapy must be taken into account. (Schwenger,& Morath,C, 2010).

3.1.13 Others agents in Phase I, II or III clinical trials

ISA247 (voclosporine), a cyclosporine analogue, has the advantage of inducing less postransplantation diabetes and reduced nephrotoxicity (Phase III study).

CP-690550, a specific inhibitor of the JAK3 protein kinase, has an effect comparable to tacrolimus on the acute rejection rate and kidney function. Orally is administered with basiliximab, mycophenolate and steroids. Initial results suggest that co-administration with mycophenolate involves excessive immunosuppression, with increased BK virus infection and cytomegalovirus.

Belatacept, is a humanized antibody that blocks the costimulatory signal by binding to CD80 and CD86 antigen presenting cells, thereby promoting anergy and apoptosis of T cells. Its efficacy is similar to that of cyclosporin A, but with a more favorable toxicity profile. The

need for intravenous injection and a slight increase in the development of lymphoproliferative disease in liver transplantation recipients with negative serology to Epstein-Barr virus could partially limit its use (Multiple Phase II and III trials).

Alefacept and Efalizumab are humanized antibodies that inhibit T-cell adhesion and are in Phase I and II clinical trials.

3.2 Clinical evidences on desensitization therapy strategies in high immunological risk patients

In this section, we review the most relevant publications related to therapies in high immunologic risk patients, making emphasis on aspects such as incidence of acute rejection, long-term allograft survival and function, mortality and others. Highly sensitized transplant recipients, regardless of the desensitization protocol used, are at increased risk for AMR. Both desensitization and AMR are managed with the similar therapeutic arsenal; however protocols are center-specific and there are no consensus guidelines. The two desensitization protocols more frequently used are high-dose IVIG or low-dose IVIG with either plasmapheresis or immunoadsorption. Additionally, some transplant centers may add intravenous steroids, rabbit antithymocyte globulin, or rituximab. For variant of AMR where over 30% of infiltrating cells are mature plasma cells, which do not express CD20, several transplant centers have utilized bortezomib instead of rituximab.

Yuan XP et al (Yuan XP et al., 2010) evaluated the efficacy of plasmapheresis plus low-dose intravenous immunoglobulin in highly sensitized patients waiting for a deceased-donor renal transplant. In 25 patients (group 1), a positive T- and/or B-cell cytotoxicity crossmatch was rendered negative by plasmapheresis plus low-dose intravenous immunoglobulin treatment. During the same time, 32 highly sensitized patients (group 2), without desensitization, had a negative crossmatch and received deceased-donor renal transplants. Group 1 showed a numerically higher rate of acute rejection and antibody-mediated rejection, but the difference was not statistically significant. No differences in Kaplan-Meier graft survival were found between group 1 and group 2 after long-term follow-up. They conclude that desensitization with plasmapheresis, plus low-dose intravenous immunoglobulin enables successful deceased-donor renal transplant in highly sensitized patients with a positive crossmatch and achieve results similar to highly sensitized patients with negative crossmatch. Moreover, antibody-mediated rejection occurred predominantly in recipients with donor-specific antibodies of high titers. They used anti-thymocyte globulin for induction in both groups.

Loupy A, (Loupy A et al., 2010), combined posttransplant prophylactic intravenous immunoglobulin, rituximab and plasmapheresis in kidney recipients with preformed donor-specific antibodies. All patients had a concomitant evaluation of glomerular filtration rate, protocol biopsies, and DSA mean intensity of fluorescence (MFI) at 3 month and 1 year posttransplant. The first strategy combined posttransplant quadritherapy and intravenous immunoglobulin (group 1, n=36) and the second added to the above protocol rituximab and plasmapheresis (group 2, n=18). Peak and day-0 class-I or II DSA max-MFI were similar in both groups. The rate of acute antibody-mediated rejection (AMR) was similar in both groups (about 19.6% vs. 16.6%, respectively). At 1 year posttransplant, group 2 was characterized significantly by lower score microcirculation inflammation lesions, a lower rate of transplant glomerulopathy and a lower rate of chronic AMR. The decline in DSA-MFI from day 0 to 1 year was about 44% in group 1 compared with 80% in group 2 and the 1-year glomerular filtration rate was about 43 vs. 54 ml/min/1.73 m², respectively. The

study raises the possibility that a more prophylactic immunosuppressive strategy at day 0 combining intravenous immunoglobulin/anti-CD20/plasmapheresis in high-risk population, is associated with significant differences in long-term function and chronic AMR rate, although similar rates of early acute clinical humoral rejection may be observed.

Vo A, et al (Vo AA et al., 2010, 2008) used high-dose intravenous immune globulin (2 g/kg x2 doses) and rituximab (2 doses) for desensitization of highly HLA-sensitized patients awaiting kidney transplantation. All patients received intravenous ganciclovir while staying at the hospital and valganciclovir for 6 months as outpatients, with dose adjustments for renal function. Both fungal and bacterial infection prophylaxis, including *Pneumocystis carinii*, was performed in all patients according to standard clinical practice. From July 2006 to February 2009, seventy-six treated patients (31 living donors, 45 deceased donors) were transplanted. For living donors (LD) and deceased donors (DD) recipients, significant reductions were seen in T-cell flow cytometry crossmatch from pretreatment to time of transplant. Patients received their kidney transplants when the complement-dependent cytotoxicity (CDC) T-cell crossmatch was negative at a 1:2 dilution of serum or the mean flow-channel shift in the T-cell flow-cytometric crossmatch was below 250. Time on wait list for DD recipients was reduced from a mean of 95 to 4.2 months after treatment; 37% experienced acute rejection but patient and graft survival up to 24 months was 95% and 84%, respectively. The mean serum creatinine, at 12 and 24 months were about 1.5 and 1.3 mg/dl, respectively. They concluded that IVIG and rituximab seems to offer significant benefits in reduction of anti-HLA antibodies allowing improved rates of transplantation for highly sensitized patients, especially those awaiting DD, with acceptable antibody-mediated rejection and survival rates at 24 months. All patients had reduced numbers of CD19+ cells after rituximab infusion. No patients developed neurologic symptoms suggestive of progressive multifocal leukoencephalopathy or required further hospitalization, and the rate of urinary tract infection was not greater than that among transplant recipients who were not highly sensitized. No other important infectious complications were noted.

Although 13 of 16 patients who received a kidney transplant had a persisting positive crossmatch at the time of transplantation (below the threshold given above), no hyperacute rejection episodes were noted. The same group developed an in vitro test system to predict whether intravenous immune globulin might reduce PRA or crossmatch positivity in individual patients. For patients who did not respond well in this test system or who had high antibody titers before desensitization, intravenous immune globulin and rituximab were considered not to be sufficient alone, and the patients received in addition plasmapheresis.

Vo AA, Jordan SC, et al (Vo AA; Jordan, SC. et al., 2008, 2009), analyzed the use of subcutaneous alemtuzumab induction therapy with intravenous immune globulin and rituximab in an uncontrolled study in 54 highly HLA-sensitized patients from 3/05 to 4/07. No patient developed acute injection-related reactions after alemtuzumab, however, bone marrow suppression was occasionally seen requiring reduction or elimination of mycophenolate mofetil approximately 1-2 months posttransplant. Patient and graft survival at 12 month was 98%/96%, respectively. Acute rejection episodes occurred in 35% with 20% being C4d+ acute rejection. Mean serum creatinine at 12 month was about 1.4 mg/dl. Infections occurred in eight patients (five with polyoma BK viremia, one CMV/PBK and two with CMV viremia). They concluded that induction therapy with alemtuzumab appears feasible and indeed promising, but awaits more definitive study.

Scemla A et al (Scemla A et al., 2010), revised the incidence of infectious complications in 38 highly sensitized renal transplant recipients treated by rituximab. They compared this population with 26 highly sensitized renal transplant recipients who received comparable

treatment but without rituximab. Mean posttransplant follow-up was 25.5 ± 11.5 and 34.6 ± 16.4 months in the rituximab and control groups, respectively. A total of 84 severe infectious episodes occurred in 39 patients (rituximab 55.3% vs. controls 69.2%, ns). Two patients died in each group. Three of these four deaths were related to infectious complications. Specifically, rituximab was not associated with an increased risk of infection. Kamar N et al (Kamar N et al., 2010), revised the occurrence of infectious disease and its outcome after rituximab therapy (375 mg/m^2 , 2-8 courses) in 77 kidney-transplant patients between April 2004 and August 2008. Their results were compared with a control group ($n=902$) who had received no rituximab. After a median follow-up of 16.5 months for rituximab patients and 60.9 months for control patients, the incidence of infectious disease was 45.45% and 53.9% (ns), respectively. The incidence of bacterial infection was similar between the two groups, whereas the viral-infection rate was significantly lower, and the rate of fungal infection was significantly higher in the rituximab group. Nine out of 77 patients died after rituximab therapy, of which seven deaths were related to an infectious disease, compared to 1.55% in the controls ($p=0.0007$). They concluded that in the whole population, the independent predictive factors for infection-induced death were the combined use of rituximab and antithymocyte-globulin given for induction or anti-rejection therapy, recipient age, and bacterial and fungal infections.

Flechner SM et al (Flechner SM et al., 2010), revised the role of proteasome inhibition with bortezomib in the treatment of antibody-mediated rejection in 20 patients. AMR was diagnosed about 19.8 months posttransplant. De novo class I DSA was detected in 55% and class II DSA in 90% recipients. Patients received intravenous corticosteroids followed by a 2-week cycle on days 1, 4, 8 and 11 of plasmapheresis and 1.3 mg/m^2 bortezomib; then 0.5 mg/kg intravenous immunoglobulin four times. Their results were a significantly decrement in peak-nadir dominant DSA. Patient survival was 100%, and graft survival 85% with a mean follow-up of 9.8 months. The treatment was generally well tolerated but caused fatigue, gastrointestinal complaints, fluid retention, and thrombocytopenia in a number of patients. The last follow-up estimated glomerular filtration rate was $41.9 \pm 16.8 \text{ ml/min}$, however, only 25% returned to their baseline renal function. They concluded that the bortezomib-containing regimen demonstrated activity in AMR but seems to be most effective before the onset of significant renal dysfunction or proteinuria and the use of bortezomib to treat AMR should be evaluated in controlled trials using dosing strategies that include longer courses or retreatment schedules.

Something similar was found by Raghavan R et al (Raghavan R et al, 2010), this author revised the use of bortezomib in kidney transplantation and said that the use of this biological agent in the field of transplantation may seem to show promise in the realm of transplant recipients desensitization and treatment of AMR, and will be defined better as more clinical data and trials become available.

Lonze BE et al (Lonze BE et al., 2010) review a 43-year-old patient with end-stage renal disease and 100% panel reactive antibody who was treated with desensitization protocol using two cycles of bortezomib undertaken after anti-CD20 and intravenous immunoglobulins. A flow-positive, cytotoxic-negative cross-match live-donor kidney at the end of an eight-way multi-institution domino chain became available. The patient received three pretransplant plasmapheresis treatments. Intraoperatively, the superior mesenteric vein was the only identifiable patent target for venous drainage. Eculizumab was administered postoperatively in the setting of antibody-mediated rejection and an inability to perform additional plasmapheresis. Creatinine remains normal at 6 months

posttransplant and flow cross-match remained negative. In this report, they described the combined use of new agents (bortezomib and eculizumab) and modalities (nontraditional vascular access, splanchnic drainage of graft and domino paired donation) in a patient who would have died without transplantation.

Wahrmann M et al, (Wahrmann M et al., 2010), analyzed two sensitized hemodialysis patients that were selected to receive two subsequent bortezomib cycles (1.3 mg/m² on days 1, 4, 8, and 11). Dexamethasone was added to the second cycle to enhance treatment efficiency. During a half-year follow-up period, cytotoxic panel reactive antibody decreased from 87% to 80% (patient 1) and 37% to 13% (patient 2). Patient 1 showed a 40% reduction in binding intensities of identified Luminex HLA single antigen reactivities and, in parallel, slight reductions in ABO blood group antibody and total immunoglobulin levels. In patient 2, bortezomib did not affect circulating antibody levels in a meaningful way. Both patients showed a more than 50% reduction in the levels of anti-HLA antibody-triggered C4d deposition to Luminex beads. They concluded that, without additional immunosuppressive measures, bortezomib has modest effects on circulating antibodies against HLA or blood group antigens. However, the reduced levels of antibody-triggered complement fixation, imply potential clinical relevance of proteasome inhibition for recipient desensitization.

Sberro-Soussan R (Sberro-Soussan R, et al., 2010), evaluated the in vivo efficacy of one cycle of bortezomib (1.3 mg/m² x 4 doses), used as the sole desensitization therapy, in four renal transplant recipients experiencing subacute antibody-mediated rejection with persisting DSA (>2000 Mean Fluorescence Intensity, MFI). Bortezomib treatment did not significantly decrease DSA MFI within the 150-day posttreatment period in any patient. In addition, antiviral (HBV, VZV and HSV) antibody levels remained stable following treatment suggesting a lack of efficacy on long-lived plasma cells. They concluded that one cycle of bortezomib alone does not decrease DSA levels in sensitized kidney transplant recipients in the time period studied and need to evaluate this new desensitization agent properly in prospective, randomized and well-controlled studies.

Locke JE et al, (Locke JE et al., 2009) present a single case in which eculizumab was used combined with plasmapheresis and immunoglobulins to salvage a kidney undergoing severe AMR. They showed a marked decrease in C5b-C9 (MAC) complex deposition in the kidney after the administration of eculizumab.

Stegall et al. recently reported on their results obtained in a first series of patients in whom the use of eculizumab was tested for the prevention of AMR in crossmatch-positive kidney transplantation. Sixteen crossmatch-positive kidney transplantations realized under the usage of eculizumab were compared to a historical control group of 51 transplantations where desensitization had been performed without eculizumab. Acute humoral rejection was found in only 6% of patients desensitized with eculizumab as compared to a 40% rate in the historical control group. However, 4 of 16 patients in the eculizumab group with persistent levels of high DSA after transplantation showed signs of chronic allograft injury, including endothelial cell activation and transplant glomerulopathy (Stegall, 2010, as cited in C. Siisal & Morath C, 2011).

Thielke et al. reported that a negative crossmatch was successfully achieved in 51 of 57 positive-crossmatch patients treated with antithymocyte and anti-CD20 antibody induction therapy in addition to plasma exchange and low-dose intravenous immunoglobulin. The rate of allograft survival was 93% at 1 year and 81% at 2 years.

Glantz et al (Glantz et al., 2002) reported 15 patients with either a panel reactive antibody (PRA) of >50 percent or with a positive crossmatch to their potential living donor who were

given 2 g/kg of IVIG monthly for three months. Thirteen of the 15 showed evidence of desensitization (reduction of PRA by at least 50 percent or a repeat negative crossmatch to the living donor) and underwent renal transplantation. The mean decrease in PRA for recipients was 80 percent and a post-IVIG administration NIH cytotoxicity crossmatch was negative prior to transplantation. The IVIG was repeated at the same dose on post-transplant day zero and one. Thymoglobulin was used for induction and maintenance immunosuppression consisted of mycophenolate, corticosteroids and tacrolimus. The IVIG was again repeated at post-transplant day 20 or 21 and 40 or 41. One graft was lost secondary to thrombosis and one graft was lost secondary to rejection. No other episodes of rejection were reported in the remaining allografts during follow-up of over one year.

Kaposztas et al. reported 2-year outcomes in their recent retrospective study looking at 54 patients treated for AMR. Group A had 26 patients who underwent treatment with plasmapheresis and rituximab, and group B had 28 patients who received plasmapheresis without rituximab. Patients who had low serum IgG levels also received IVIG. Two-year graft survival was significantly better in the group that received rituximab (90% vs 60%), with the difference attributed to rituximab. A trend toward improved graft survival was also seen in those who received IVIG. This retrospective study has one of the largest cohorts reported to date and supports the use of rituximab for treating AMR, with good short-term allograft survival; however, many patient variables were not consistent between the groups (Kaposztas et al, 2009, as cited in Jordan, S, 2010).

Gloor et al, (Gloor et al., 2003) described 14 patients with a positive cytotoxicity crossmatch to a potential living donor. Patients underwent plasmapheresis on days four, three, and one pretransplant, on the day of transplantation, and on day one and three post-transplantation. Intravenous immunoglobulin 100 mg/kg was administered after each plasmapheresis session. Rituximab at a dose of 375 mg/m² was given on post-transplant day four. Splenectomy was performed at the time of transplantation in those with an intact spleen (two had previously been splenectomized). Thymoglobulin was used for induction and tacrolimus, mycophenolate and corticosteroids were used for maintenance therapy. Patient survival at a mean follow-up of 15 months was 86 percent. Histologic evidence of AMR occurred in 43 percent. The risk of AMR was related to the baseline anti-HLA antibody titer. All four subclinical episodes responded to treatment and follow-up protocol biopsies showed no histologic evidence of rejection. Both episodes of rejection defined as clinically significant AMR demonstrated evidence of chronic allograft nephropathy on subsequent biopsies.

Stegall et al (Stegall et al, 2006, as cited in C. Siisal & Morath C, 2011) designed one study to compare high dose IVIG (13 patients) with plasmapheresis/low dose IVIG protocols (32 patients) in renal transplant recipients with high DSA levels. Plasmapheresis plus low dose intravenous immunoglobulins (IVIG) received also anti-CD20 antibody (32 patients), and 19 of the 32 patients in this group also underwent splenectomy; post-transplant plasmapheresis and low dose IVIG were continued on post-surgery days one to three for a total of two to three sessions. High single dose IVIG (13 patients), which is the high dose IVIG group. Plasmapheresis plus low dose IVIG plus anti-CD20 antibody plus pretransplant Thymoglobulin combined with post-transplant DSA monitoring (16 patients), was the plasmapheresis/monitoring group. Achieving a negative crossmatch was significantly more likely with both plasmapheresis protocols versus high dose IVIG (84, 88, and 38 percent respectively). Significantly lower humoral rejection rates were also reported with the plasmapheresis protocols (37, 29, and 80 percent, respectively), although none of the patients in the high single dose IVIG group received rituximab or post-transplant administration of

IVIg. Patients with low baseline antibody titers responding to high dose IVIg may do equally as well with further optimization of therapy. However, whether or not the administration of rituximab or the routine post-transplant administration of IVIg would be of benefit in reducing the incidence of acute rejection in a high dose IVIg protocol is unclear at this time as this study not included randomization and only participated a low numbers of patients.

4. Remarks and conclusions

The main goal of monitoring circulating antibodies is to measure PRA and identify specific antibodies in order to evaluate the patient's immunological risk and interpret a crossmatch. The introduction of HLA antibody characterization based on interactions between recipient serum and purified HLA antigens bound to solid-phase substrates has improved detection and quantification of donor-specific antibodies (DSAs).

Currently, few kidney transplant options exist for hypersensitive patients on the waiting list if they do not undergo previously to desensitising treatments or strong induction therapy. In this respect, high doses of intravenous immunoglobulins may reduce the level of circulating antibodies, but, many patients only respond partially, and the efficacy varies among patients. Plasmapheresis can decrease circulating antibodies, but there is normally a significant increase in their titre levels once the sessions have been completed. Therefore, this technique is now considered a complement to the use of immunoglobulins for decreasing antibody levels. Likewise, rituximab has also been shown to have a beneficial effect when combined with immunoglobulins and plasmapheresis to reduce anti-HLA antibodies rate and to treat antibody-mediated rejection. On the other hand, newer interventions aimed at the prevention of DSA-mediated allograft injury using complement blockade, or the inhibition of DSA synthesis using proteasome inhibitor-mediated plasma cell depletion are promising.

In any case, the best therapeutic strategy may be of combining these drugs, particularly when there is early detection of acute antibody-mediated rejection through histological or serological techniques. Whether long-term beneficial outcomes are achieved with these drugs without life-threatening side-effects, remains to be elucidate.

According to our previous results, we tentatively propose the following desensitization and induction protocol:

Recipients with positive cytotoxicity crossmatch or retransplantation recipients with positive cytometry crossmatch and negative cytotoxicity crossmatch are potential candidates for pre-transplant desensitisation. For first transplant recipients with positive cytometry crossmatch but with negative cytotoxicity crossmatch, desensitisation may not be necessary. For patients who are only positive for virtual lymphocyte crossmatch, with negative cytotoxicity and cytometry crossmatches, there are currently insufficient data that support the appropriateness of desensitisation. Patients on the waiting list more than 12 months and at least three studies quarterly permanently with PRA > 50-75% polyspecific, multiple previous positive crossmatch, and multiple HLA-antigens positive reactivity that makes transplantation highly unlikely, if they have absence of IgA deficiency and antibodies antiIgA, they could receive high dose of immunoglobulins, plus plasmapheresis and one or two doses of rituximab.

Requirements for performing kidney transplantation in these patients would be:

- a. Pre-transplant negative cytotoxicity crossmatch, and
- b. Negative virtual crossmatch test prior to the kidney transplant, i.e., absence of all class I or II HLA antigens in the donor that have produced an alloresponse in the recipient at any time.

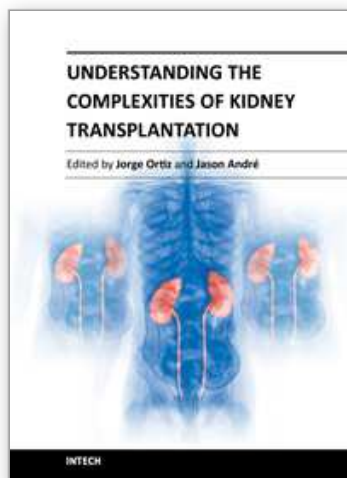
- c. Induction therapy with thymoglobulin tacrolimus, mycophenolate, methylprednisolone.
- d. Desensitisation treatment would consist in rituximab, various plasmapheresis sessions with IV immunoglobulin infusion following each session.
- e. Monitorization of CD19+/CD20+ lymphocyte populations and checking for any appearance of opportunistic infections using a PCR assay for CMV, Epstein-Barr viral serology, B-19 parvovirus and polyomavirus BK are necessary.
- f. Cytomegalovirus infection prophylaxis with gancyclovir/valgancyclovir 6 months, pneumocistis jirovecii prophylaxis with trimethoprim sulfamethoxazole and fungal infection prophylaxis with nystatin or oral fluconazole must be considered.
- g. Monitoring PRA title every 15 days the first 3 months and then monthly during first year and before or after any deterioration of renal function. A rising DSA titer may suggest the need for intensification of therapy with potential modification of maintenance immunosuppression or initiating intensive therapy using IVIG and/or plasmapheresis.
- h. Monitoring of neurological symptoms: progressive multifocal leukoencephalopathy, reactivation of polyoma JC virus also is very important.
- i. In the case of immunoligal-mediated renal dysfunction, it is important perform a graft biopsy and C4d staining. Treatment for apparent AMR is essentially by combining methylprednisolone, plasmapheresis (or immunoadsorption) and IVIG, with a duration that will be dependent upon an improvement in renal function, decrease in the titer of DSA or improvement of biopsy findings. If there is no good response to treatment, individual assess whether repeated rescue therapies, such as rituximab or eculizumab. In the case of appearance of plasma cells in the renal graft biopsy, it should be assessed individually using bortezomib as salvage therapy. Subclinical rejection (as defined by positive C4d staining associated with histologic evidence of antibody mediated rejection) on protocol biopsies may be associated with future AMR or subsequent evidence of chronic allograft injury. Whether or not treatment of subclinical rejection in this setting has a benefit on long-term graft survival is unknown, however, given the high risk of acute rejection, most physicians would favor restarting plasmapheresis/IVIG or other treatment.
- j. An additional critical issue is antibody development against allogeneic antigen systems on graft other than HLA that are not necessarily detected in routine antibody testing, like anti-major histocompatibility complex class I related A (anti-MICA), antiendothelial antibodies, antibody binding to angiotensin type-1 receptor and others. These antibodies have found a strong association with antibody-mediated rejection in recipients whose sera did not contain antibody to donor HLA, indicating that antibodies directed against non-HLA antigens also have a certain impact. These issues are not reasons for this chapter and may be addressed in future. More studies are required in this field to determine the frequency and magnitude of damage caused by non-HLA immunity.

5. References

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Understanding the Complexities of Kidney Transplantation

Edited by Prof. Jorge Ortiz

ISBN 978-953-307-819-9

Hard cover, 564 pages

Publisher InTech

Published online 06, September, 2011

Published in print edition September, 2011

Kidney transplantation is a complex field that incorporates several different specialties to manage the transplant patient. This book was created because of the importance of kidney transplantation. This volume focuses on the complexities of the transplant patient. In particular, there is a focus on the comorbidities and special considerations for a transplant patient and how they affect kidney transplant outcomes. Contributors to this book are from all over the world and are experts in their individual fields. They were all individually approached to add a chapter to this book and with their efforts this book was formed. Understanding the Complexities of Kidney Transplantation gives the reader an excellent foundation to build upon to truly understand kidney transplantation.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Juana Margarita Rufino Hernández, José Manuel González-Posada Delgado and Domingo Hernández Marrero (2011). Desensitization and Induction Immunosuppressive Therapy in Highly HLA-Sensitized Patients Receiving Cadaveric Renal Allograft), Understanding the Complexities of Kidney Transplantation, Prof. Jorge Ortiz (Ed.), ISBN: 978-953-307-819-9, InTech, Available from:

<http://www.intechopen.com/books/understanding-the-complexities-of-kidney-transplantation/desensitization-and-induction-immunosuppressive-therapy-in-highly-hla-sensitized-patients-receiving->

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