Immunologic Risk Assessment and Approach to Immunosuppression Regimen in Kidney Transplantation

John Choi, MD, Anil Chandraker, MD, FASN*

KEYWORDS

• Kidney transplant • Immunologic risk assessments • Immunosuppression regimen
• Transplant immunology

KEY POINTS

- Careful evaluation of immunologic risk is the key to success in kidney transplantation.
- Clinicians should understand the benefit and adverse profile of therapeutic agents to tailor the best immunosuppression regimen for each patient.
- Contemporary diagnostic tools and therapeutic agents in kidney transplantation are limited; collaborative efforts are needed to advance patient care in the era of precision medicine.

INTRODUCTION

Along with an increasing number of patients with end-stage renal disease in the United States,[1] we are also witnessing a record breaking number of kidney transplantations over the past several years.[1,2] In 2018, the United Network for Organ Sharing reported more than 21,000 kidney transplantations across the country. That is nearly 5000 more cases per year compared with 2008. In addition, the government has recently announced a policy change to incentivize kidney transplant in the care of patients with end-stage renal disease.[3] These achievements are the result of multiple efforts in the transplant community, facilitated by a better understanding of alloimmunity and effective immunosuppression practices.[4]

Immunosuppression therapy in transplantation can be categorized into 2 groups: induction therapy that is provided perioperatively and maintenance therapy for long-term allograft protection. The practice of immunosuppression in United States

Transplantation Research Center, Renal Division, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA
* Corresponding author. Transplant Research Center, Brigham and Women's Hospital, 221 Longwood Avenue, Boston, MA 02115.
E-mail address: achandraker@bwh.harvard.edu

Clin Lab Med 39 (2019) 643–656
https://doi.org/10.1016/j.cll.2019.07.010
labmed.theclinics.com

varies among centers,[5,6] and each center maintains a specific protocol considering the immunologic risks of individual patients as well as patient demographics referred to the center. In general, there has been an uptrend in the strength and dose of immunosuppressive medications in the United States.[7] This trend has translated into an overall decrease in rejection events and a higher allograft survival rate, as mentioned elsewhere in this article. However, concern among the community is increasing, because stronger immunosuppression is linked to debilitating adverse effects: opportunistic infection, malignancy, and potential harm to the kidney allograft itself. This highlights the limitations in the current practice of immunosuppression and underpins the importance of developing accurate biomarkers along with targeted therapies tailored to patient-specific immune rejection pathways.

TYPES OF REJECTION

Rejection can be defined by the timing of event. Hyperacute rejection occurs within minutes after transplantation and was most feared complication in early ages of solid organ transplantation. Hyperacute rejection is mediated by preformed antibody against the donor tissue and subsequent complement activation. Owing to improved preformed antibody screening, crossmatching strategy, and treatment options (described elsewhere in this article) this type of rejection is rare in contemporary practice. Acute rejection occurs within weeks to 1 year after transplantation, although it can occur further out. Both acute and chronic rejection are characterized by either or both lymphocyte infiltration and antibodies against the allograft.

Historically, the main goal in transplantation was to improve 1-year graft survival. Therefore, risk assessment tools were developed with a focus on avoiding hyperacute and acute rejection episodes. Despite excellent 1-year graft survival, an increasing incidence of the long-term complications of immunosuppression and chronic rejection makes it imperative to modify outcome goals and update the risk assessment process.

TRENDS IN ACUTE REJECTION AND THE PRACTICE OF IMMUNOSUPPRESSION IN UNITED STATES

The incidence of acute rejection in kidney transplantation has markedly declined over the recent decades. According to the Organ Procurement and Transplantation Network/Scientific Registry of Transplant Recipients annual outcome report from 2015 to 2016, the first year post-transplant rejection rate was only 8% in adult recipients in contemporary practice, compared with 60% and 35% in 1980 and mid 1990s, respectively.[8] This achievement, along with improvements in allograft outcomes, was a result of the introduction of induction therapy agents, better HLA matching (including availability of virtual crossmatching), and stronger maintenance immunosuppression.[7,9,10] In particular, the dramatic improvement in 1-year graft survival and the decrease in composite 1-year rejection events correlates with the introduction of calcineurin inhibitors (CNIs) and lymphodepleting agents as an induction therapy.[9] Therefore, it is not surprising that more transplantation centers in United States are incorporating induction therapy into their protocols—increasing from 45% in 1999% to 70% in 2008.[11] In addition, most centers have adopted triple therapy, composed of tacrolimus, mycophenolic acid, and steroids as an initial choice of maintenance therapy rather than single or double therapy.[6]

ADVERSE EFFECTS FROM THE TRENDS IN IMMUNOSUPPRESSION PRACTICES

Although implementing stronger immunosuppression did achieve the primary goal of improving 1-year graft survival and decreasing the rejection rate, it came with a significant cost. A prospective international study comparing antithymocyte globulin and anti–IL-2R antibody regimens showed a significant increase in overall infections with antithymocyte globulin, especially infections with bacteria and non-cytomegalovirus viruses (86% vs 75%, *P*-value: 0.03).[12] Notably, some studies also observed an increased association of cytomegalovirus infection with lymphodepleting agents[13–15] and CNIs.[16] In addition, the incidence of BK nephropathy has been reported to be uptrending with lymphodepleting agents and the increased popularity of CNI over mammalian target of rapamycin inhibitors in immunosuppression regimens.[17–19]

In 2003, a group analyzed the United Network for Organ Sharing database to parse out the risk factors for post-transplant lymphoproliferative disorder associated with different immunosuppressants. This study showed that exposure to monoclonal lymphodepleting agents, but not anti–IL-2R antibody had an increased tendency to develop post-transplant lymphoproliferative disorder.[20–22] Skin cancer, namely squamous cell cancer followed by basal cell cancer, is common in kidney transplantation and exposure to CNIs is a well-known risk factor.[23]

Finally, the incidence of chronic allograft nephropathy, which is highly associated with tacrolimus-induced chronic vasoconstriction, has increased proportionally with overall allograft survival, suggesting that the cumulative exposure time to tacrolimus has increased. Despite this finding, clinicians are reluctant to actively withdraw CNI when allograft function is stable, because a prior study showed an association with increased rejection rate and further prospective studies are needed.[24,25]

THE SENSITIZATION IN TRANSPLANTATION WORKING GROUP

In response to contemporary developments in molecular diagnostics, the transplant community has put forth a framework to standardize the immunologic risk assessment process.[26] The group first acknowledged current limitations in coming up with uniform guidelines owing to heterogeneity in laboratory reports (ie, mean fluorescence intensity cutoffs) and terminology, especially in published literature. Then the group suggested that alloimmune risk can be stratified based on 6 assessment criteria: complement-dependent cytotoxicity (CDC) crossmatch, flow crossmatch, single antigen bead test, history of sensitization, HLA molecular mismatch, and HLA identical. This stratification allows for the classification of patients by risk categories ranging from a low risk of de novo alloimmune response to active memory and a high risk for hyperacute rejection. In the following sections, we discuss when and how these data are collected and analyzed in contemporary practice for immunologic risk stratification. Finally, the working group concluded the importance of creating a centralized registry of patients suffering from rejection for effective research.

CURRENT FRAMEWORK FOR IMMUNOLOGIC RISK EVALUATION

In following paragraphs, we review the basic approach to immunologic risk assessment in kidney transplantation. In general, initial evaluation focuses on (1) the recipients' preexisting risk factors, (2) the use of this information to find the best matching kidney, and (3) planning ahead for induction therapy given the unpredictive nature of organ availability (**Fig. 1**A). Once the organ is offered, additional crossmatching is performed as a final checkpoint and based on variables identified during

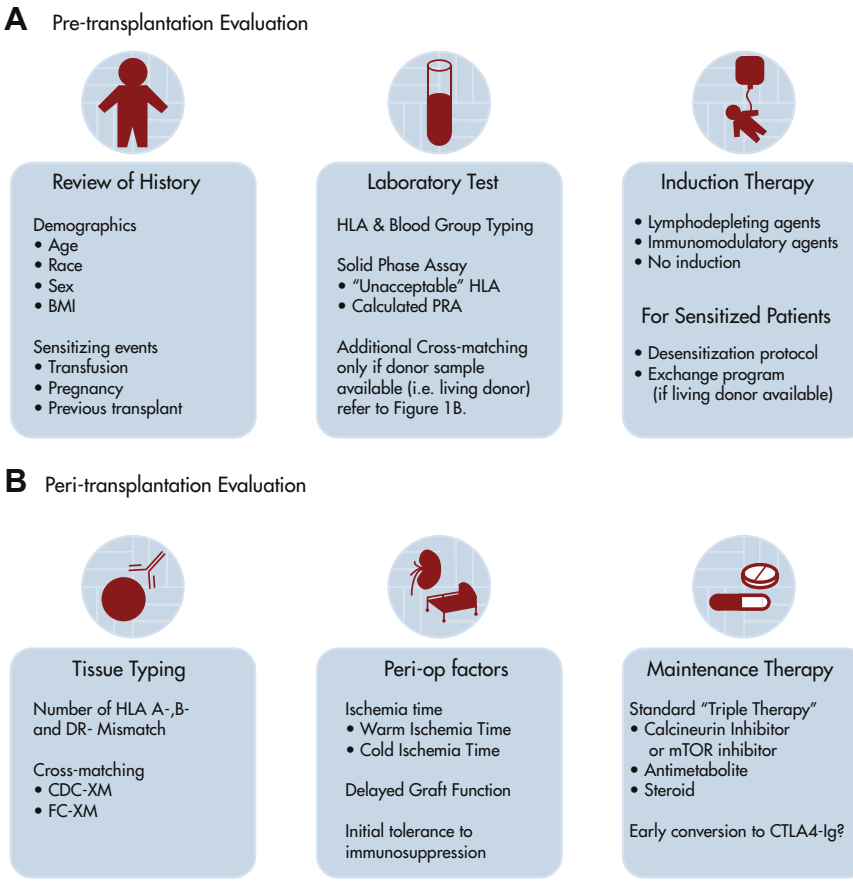

Fig. 1. Immunologic risk assessment and therapeutic approach in kidney transplantation. (*A*) Pre-transplantation immunologic risk evaluation and induction therapy planning. (*B*) Peri-transplantation immunologic risks and management of maintenance immunosuppression regimen. BMI, body mass index; CDC-XM, complement-dependent cytotoxicity crossmatch; CTLA4, cytotoxic T-lymphocyte–associated protein 4; FC-XM, flow cytometry crossmatch; mTOR, mammalian target of rapamycin; PRA, panel reactive antibody.

perioperative period each center determines the appropriate choice of postoperative immunosuppression regimen (Fig. 1B).

Pretransplant Evaluation

Demographic factors

Basic demographic factors such as age, sex, and race can predict the of robustness of the alloimmune response. The incidence of acute rejection episodes shows a linear drop as the recipient gets older.[27] This phenomenon seems to be the result of a decreasing capacity for T-cell proliferation and B-cell activation that occurs with aging.[16] In terms of sex, male recipients who received renal allograft from a female donor were at increased risk of acute rejection compared with those who received allograft from a male donor.[28] In addition, animal studies have shown a protective effect of estradiol in chronic rejection, whereas testosterone induces an opposite effect.[29]

Racial disparity in kidney transplant outcomes[30] has been a long-standing issue in kidney transplantation. Although recent trends show overall improvement in allograft survival, African Americans are at the highest risk for acute rejection episodes.[31] Studies have yet to elucidate qualitative differences in immune mechanisms between races, and for now it is thought to be related to a combination of socioeconomic inequalities[32] as well as faster metabolism of CNI in the African American population (via CYP3A5 genotype).[33] Coding variants in APOL1 gene have been strongly linked to CKD risk and poor allograft survival in African Americans, but whether these high risk alleles have a specific immunomodulatory effect needs to be further examined. Finally, it is also noteworthy that recipient obesity is associated with a higher risk of rejection,[34,35] likely reflecting the chronic inflammatory status associated with obesity.

Donor type

Deceased donor grafts have been associated with a higher risk of acute rejection when compared with living donor grafts. Fortunately, the gap between deceased donor and living donor grafts has become significantly lower in contemporary practice; in 2014 and 2015, acute rejection episodes in 1 year post-transplant recipients of deceased donor and living donor grafts was 8% and 7%, respectively.[36] However, there remains a significant difference in 5- and 10-year death censored allograft survival rate between living and deceased donor kidney transplant (10% vs 15% and 20 vs 30%, respectively, in 2016). Given that the majority of graft failures after 5 years are related to antibody-mediated rejection,[37] it is possible that deceased donor grafts are more prone to chronic antibody-mediated rejection, but further larger scale studies are needed to confirm this. The Kidney Donor Profile Index (KDPI) system was introduced in 2009 to classify the risk of allograft failure based on donor profile.[38] For example, a KDPI of 80% indicates that an allograft from such a donor has a risk of graft failure of more than 80% of all kidney donors recovered in the previous year. A high KDPI score is associated with increased acute rejection.[39]

Previous sensitizing events and solid phase immunoassays

Prior sensitizing events should be carefully reviewed. The Sensitization in Transplantation working group has defined HLA sensitizing events as pregnancies, transfusions, previous transplant, and implants.[26] The Luminex solid phase immunoassay (Luminex, Austin, TX) is one of the most convenient and sensitive methods to check for circulating preformed antibodies before and after transplantation.[40,41] Owing to option of reporting so-called unacceptable antigens, it offers an efficient way of ruling out incompatible donors through a virtual crossmatch.[42] However, clinicians should be aware that there are many factors that go into determining what constitutes an unacceptable antigen.[43,44] The onus is on the individual center to determine the list of unacceptable antigens and the center may strategically set a different mean fluorescence intensity cutoff for positivity to support patients. Furthermore, technical variation between solid phase assays needs to be taken into account, as summarized in a recent review article.[45] Finally, the solid phase assay should not be used as a single tool for decision making, and it needs to be followed up with a functional assay such as cytotoxicity crossmatching as discussed elsewhere in this article.

HLA typing

HLA type identified during pretransplant evaluation helps find the best matching allograft. Traditionally, HLA-A, -B (class I) and HLA-DR (class II) were considered for determining a mismatch. The number of HLA mismatches shows a linear correlation with the incidence of acute rejection.[46,47] A couple of outcome studies using European

transplant databases have suggested that class II mismatches are more important than a class I mismatch,[48,49] especially showing the link between HLA-DR mismatch and antibody-mediated rejection. In recent years, HLA-DQ has been under the spotlight.[50] A series of reports showed an association with de novo anti-DQ antibody with increased acute rejection rate.[51,52] Nowadays, HLA can be characterized at the level of an eplet, the functional portion of epitope. The transplant community is actively working to refine the algorithms for immune risk assessment to incorporate eplet mismatches,[53] which may soon change the paradigm for approaching pretransplant evaluation.

Induction therapy

By reviewing the information collected as outlined, clinicians can obtain a general idea of which induction therapy to provide. There are 2 main categories of induction therapy: lymphodepleting agents (eg, antithymocyte globulin or anti-CD52 antibody) and immunomodulatory agents (eg, anti–IL-2R antibody). Studies have demonstrated the superiority of antithymocyte globulin in preventing acute rejection among high risk patients although this did not translate into improved 1-year allograft survival.[12] In addition, there was a higher incidence of infection with antithymocyte globulin.[12,13,54] Therefore, clinicians should identify lower risk patients—old, non-black, living donor, low panel reactive antibody, and no sensitization events—and provide anti–IL-2R antibody as an induction therapy to avoid exaggerated immunosuppression. In contrast, young, African American, deceased donor transplant recipients with prior sensitization events should be considered for antithymoglobulin antibody induction. There are no specific tools to guide therapy for patients who are at intermediate risk, and clinicians need to titrate the intensity of induction therapy and maintenance therapy to avoid overimmunosuppression or underimmunosuppression. Finally, the question arises if there are certain individuals who may not require induction therapy at all. The ratio of patients who do not receive any type of induction therapy has decreased to near 10% by 2017.[2] A study done with the patients on triple therapy for maintenance showed that low-risk recipients without induction therapy did as well as a group who received anti–IL-2R antibody, a standard choice for low-risk patients.[55] This finding highlights the need for further clinical trials to guide the precise use of induction therapy.

Special Considerations in Highly Sensitized Patients

The number of transplants in highly sensitized patients has increased over the past 5 years,[2] driven by changes in the allocation system that advocates for these patients.[56] The management of highly sensitized patients is a complex topic and the details are outside the scope of this article. However, we briefly discuss the current approach to highly sensitized patients, where availability of a potential living donor is crucial. If a living donor is available and compatible, the patient can proceed with a transplant; in cases with a concern for incompatible HLA, the team can seek a paired donor exchange. If a patient is offered a deceased donor allograft, most transplant centers in the United States activate a desensitization protocol to avoid hyperacute rejection. The protocol typically involves multiple rounds of intravenous immunoglobulin with plasmapheresis and, depending on the center, it includes rituximab or bortezomib to further suppress B cells and plasma cells. Once a patient has undergone a desensitization protocol, serum should be retested for the effective removal of antibodies. There are multiple ongoing clinical trials to find effective treatment approaches for sensitized patients

and it will require a multi-institutional effort to recruit patients and follow their outcomes.[26,57]

Perioperative Assessment

Crossmatching

In the case of a deceased donor transplant, only virtual crossmatching is available until the donor blood sample becomes available. Once the deceased donor sample has arrived at the transplant center, or if a patient has a known living donor, functional capacity of preformed donor-specific antibodies can be performed as a final check point. CDC crossmatch (CDC-XM), one of the oldest tests invented for transplant compatibility,[58] remains a critical step in assessment. Donor B and T lymphocytes are incubated separately with recipient serum, and the presence of anti-HLA antibodies in the recipient serum is determined using CDC. Transplantation is aborted when T-cell CDC-XM shows a positive result. This result indicates that the recipient serum has strong reactivity against HLA class I antigens, which are expressed on all nucleated allograft cells. In contrast, if only the B-cell CDC-XM result is positive, this may be due to a low level of HLA class I antibody selectively targeting B cells or the presence of antibodies against HLA class II, and clinicians will weigh the risk and benefit of proceeding with the transplant.[59] It is also important to determine if the positivity is due to IgG antibody rather than IgM, and this can be tested by adding dithiothreitol to selectively inactivate IgM in the assay. A negative CDC-XM result should not completely obliviate the consideration of immunologic risks in further management decisions. There could be situations where the preformed donor-specific antibody titer is too low to fix complement or the antibody does not effectively bind complement. Although CDC-XM picks up the main pathogenic complement-fixing antibodies such as IgM, IgG1, and IgG3, the presence of other antibody isotypes can also contribute to rejection. This is where more sensitive assays, such as flow cytometry crossmatching, which detects donor-specific antibodies can be useful.[59] Positive flow cytometry crossmatching does increase the likelihood of antibody-mediated rejection[60] and should be taken into account for the long-term management of immunosuppressants.

Cold ischemia time

Recipients with an allograft that had a cold ischemia time of more than 24 hours were at an increased risk of acute rejection compared with those with a cold ischemia time of less than 12 hours (relative risk, 1.13). This effect was especially pronounced in patients who underwent repeat transplantation (relative risk, 1.66).[61]

One of the challenges in the new kidney allocation system is related to cold ischemia time. The high organ discard rate[62] is in part related to increased organ harvests from donors with a high kidney donor prolife index (KDPI), also known as a marginal kidney. These marginal kidneys are then shared at the regional level, which makes the matching process longer, and by the time of transplant, centers are worried about the association between long cold ischemia time and allograft outcome.[63] Along with revising the allocation system, technological improvements in organ preservation and perfusion techniques may be able to salvage more than 1000 kidneys per year.[62,64]

Warm ischemia time and anastomosis time

There are limited studies that have specifically investigated the association between warm ischemia time and the risk of acute rejection. Heylan and colleagues[65] conducted a single-center study in Belgium and concluded that, in patients who received kidney from a brain dead donor, there was no association with acute rejection in the first 3 months after transplantation and warm ischemia time. Now the technologies have

improved and the interest has shifted to understanding the differences in the outcome of allograft survival between the traditional static cold storage preserved organs versus machine-perfused organs as well the use of a normothermic or hypothermic perfusion. More studies are required to understand the risk for immune response in the context of particular modes of organ preservation and guide the management strategies.

Delayed graft function

The rate of recovery of kidney function after transplantation is predictive of rejection, and is clinically classified into immediate (<1 day), slow (<5 days), and delayed (>7 days) recovery of graft function. A systematic metanalysis in 2009 showed a 38% increased risk of acute rejection within the first year in patients who suffered from delayed graft function.[66] A similar trend was reported again in 2016, when Gill and colleagues[67] looked into the long-term impact of delayed graft function. In this study, the group looked into data from the Scientific Registry of Transplant Recipients and analyzed data on patients where 2 group of patients received kidneys from the same deceased donor and only 1 transplant developed delayed graft function. This study revealed a 5-fold higher risk of acute rejection within 1 year for patients who suffered delayed graft function. In addition, patients who experience slow graft function are at higher risk of acute rejection within the first 6 months (40%) when compared with patients with immediate graft function (30%), but lower than patients who experienced delayed graft function (47%).[68]

Initial Response to the Immunosuppression Regimen

During postoperative care, patients are introduced to a number of new medications,[69] including immunosuppressants and infection prophylaxis. Clinicians should pay close attention if patients are experiencing any side effects. Not only can certain side effects be lethal, but unrecognized side effects can also lead to noncompliance and rejection episodes.[59,70]

The standard regimen in contemporary practice includes tacrolimus, mycophenolate, and prednisone. Mycophenolate, despite the development of an enteric-coated formula, remains a major cause of diarrhea after transplantation.[71] Diarrhea can significantly increase the circulating level of tacrolimus,[72] which leads to allograft injury[73] and overimmunosuppression. In contrast, the resolution of diarrhea can decrease serum tacrolimus level and, if this goes unnoticed, the patient may develop acute rejection. In addition, many antibiotics, antifungals, antiepileptics, or antihypertensive medications can either increase or decrease CNI levels.[74] If a patient who received an allograft with severe vascular disease shows sensitive vasoconstriction to CNI and the estimated glomerular filtration rate contraindicates the use of a mammalian target of rapamycin inhibitor, conversion to CTLA4-Ig can be considered.[75] However, premature conversion has been associated with a higher incidence or rejection[76]; therefore, clinicians should weigh the risk and benefit of the timing of conversion to CTLA4-Ig therapy.

Selective Tests to Guide Maintenance Therapy

There are a few tests for the post-transplant monitoring of cellular immunity to guide clinicians in managing maintenance immunosuppressants. Examples include the enzyme-linked immunosorbent spot for interferon-gamma,[77] a highly sensitive enzyme linked immunosorbent assay-like test used to infer the frequency of donor memory T-cell activity, which uses T-cell–depleted donor or third-party stimulator cells with donor HLA as an antigen. The Immuknow assay,[78,79] which measures the

release of adenosine triphosphate by $CD4^+$ T cells upon Phytohemagglutinin mitogen treatment, provides a global measure of $CD4^+$ T-cell activation in the setting of alloimmune response. Each test has its own strengths and weaknesses but center-to-center variation and process standardization have prevented these tests from being more widely adopted across the country.[80–82] For in-depth information on biomarkers in transplantation, please refer to our recent review in *Clinics in Laboratory Medicine*.[83]

SUMMARY

The first human kidney transplantation in 1954 was performed between identical twins; nowadays, ABO blood group-incompatible organs are being transplanted successfully. The society endeavors to discover strategies of overcoming immunologic hurdles and expand eligible transplant candidates; in the near future, we may be witnessing xenotransplantation, which will significantly relieve the burden of the current organ shortage. In contrast, we ought to acknowledge the unmet needs for advancing the current immunologic risk assessment tools. Although the evaluation process described in this review provides a descriptive idea of immunologic risk, critical decisions such as listing transplant candidates and their unacceptable antigens, proceeding toward surgery with a high-risk organ donor, as well as the choice of immunosuppression is largely dictated by the intuition and experience of gathered by physicians at an individual center. Finally, as graft survival time increases with less acute rejection that is mainly T-cell driven,[84] antibody-mediated rejection has surfaced as a major threat against the long-term outcome of allografts.[37] This finding highlights the critical need for characterizing the immunologic risk of long-term B-cell–driven immunity.[85]

State-of-the-art biomarkers and targeted therapeutics are on the horizon.[83,86]

ACKNOWLEDGMENTS

Funding support was provided by NIH grants to author Dr.Choi T32DK007527; Dr. Chandraker has nothing to reveal. Authors report no conflict of interest.

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