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Renal Transplantation in High-Risk Patients

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Contents

Abstract 1603
1. Terminology and Pathophysiology of Acute Rejection 1604
   1.1 Antibody-Mediated Rejection 1604
   1.2 Cellular Rejection 1605
   1.3 Achieving Allograft Tolerance 1605
2. Pre-Transplant Evaluation of Immunological Risk 1605
   2.1 ABO Blood Group Determination 1606
   2.2 Detection of Donor-Specific Antibodies 1606
   2.3 Human Leucocyte Antigen Tissue Typing 1609
   2.4 Crossmatching 1609
3. Peri-Transplant Desensitisation 1610
   3.1 Intravenous Immune Globulin 1610
   3.2 Plasmapheresis 1612
   3.3 Rituximab 1612
   3.4 Kidney-Paired Donation 1612
4. Recipient Demographics 1612
   4.1 Recipient Race 1612
   4.2 Recipient Age 1614
5. Living versus Deceased Donor 1614
6. Procurement Variables 1615
   6.1 Ischaemia/Reperfusion Injury 1615
   6.2 Delayed Graft Function 1616
7. Post-Transplant Immunosuppression 1616
   7.1 Induction Immunosuppression 1617
   7.2 Maintenance Immunosuppression 1621
8. Conclusions 1623

Abstract

Renal transplantation in high-risk patients is a growing phenomenon. More patients are progressing to endstage renal failure, in the setting of an increased incidence of diabetes mellitus and cardiovascular disease. Current organ shortages and the use of more marginal donors have affected both patient and graft survival. Acute rejection has been minimised under modern immunosuppression; however, patient and long-term allograft outcomes have not improved concurrently. Specific understanding of donor, recipient and allograft variables associated with

stratification of patients as 'high risk for renal transplantation' is necessary to facilitate appropriate peri- and post-transplant pharmacotherapy. Induction and maintenance immunosuppression choices are different for high-risk patients and must be made to ensure optimal immunosuppression, while limiting patient and allograft toxicity.

The definition of 'high-risk' renal transplantation has evolved significantly over the past 2 decades. Initially, high risk was defined by the patient's probability of acute rejection, as a surrogate marker for early graft loss.[1] Traditionally, 'high risk' for acute rejection was evaluated based on race and the presence of an immunosensitising event prior to engraftment. Acute rejection was thought, and proven, to be a surrogate marker for long-term allograft survival in early clinical trials.[1-6] However, modern immunosuppression has both changed the risk of rejection and caused a shift in characterisation of the high-risk patient.

Improved clinician comfort with novel immunosuppressant agents and the study of more efficacious combinations, have substantially reduced the risk of acute rejection.[1,7,8] However, long-term patient and graft survival have not dramatically increased in the setting of lower acute rejection rates.[7] One-year adjusted, deceased donor allograft survival evaluated from 1994 to 2003 illustrates this modest improvement (83.7% to 89.1%).[9] Similarly, Meier-Kriesche et al.[3] re-emphasised the perceived overestimation of improved transplant outcomes in a recent analysis of the Scientific Registry of Transplant Recipients (SRTR) database. Their study indicated only a 2-year increase in graft survival half-life from 1988 to 1995.[3,9] Explanations for the limited improvement in long-term graft survival include the use of older donors, marginal or extended criteria grafts, and recipients with significant comorbidities.[8] Thus, the definition of high-risk renal transplantation is now extended to encompass factors such as donor and recipient age, donor cause of death, time to transplant, as well as several other pre- and post-procurement variables that influence graft survival.[8,10-16]

In order to characterise a patient as 'high risk' for renal transplantation, several variables must be considered. Risk stratification, at the time of transplant, will aid the multi-disciplinary team in the selection of immunosuppression and enable appropriate post-operative co-morbidity management. Therefore, we aim to describe the donor and recipient variables that place a patient at high risk for renal transplantation, and to provide a review of peri- and post-transplant strategies to maximise long-term graft and patient outcomes.

1. Terminology and Pathophysiology of Acute Rejection

Before describing the factors that contribute to the classification of a patient as 'high risk', it is necessary to briefly describe the pathophysiology of rejection and subsequent graft loss. Following renal transplantation, donor tissue is exposed to the host (recipient) immune system. This exposure can stimulate several host immune responses characterised by (i) antibody-mediated graft infiltration (antibody-mediated rejection), or (ii) T lymphocyte-mediated graft infiltration (cellular rejection). Host responses to allograft tissue can occur at any time post-transplant and are dependent on several factors.

1.1 Antibody-Mediated Rejection

Antibody-mediated or humoral rejection is characterised by the presence of preformed cytotoxic antibodies against donor antigens expressed on the surface of transplanted tissue epithelium. These antigens include AB blood group antigens, class I/class II human leucocyte antigens (HLA) and endothelial antigens.[17,18] These antibodies initiate a cascade of events, leading to generalised graft thrombosis, mainly concentrated in the arteries, arterioles and glomeruli. Biopsy findings are characterised by

severe vasculitis, glomerulitis, fibrin thrombi, fibrinoid necrosis, infarction and neutrophils in the peritubular capillaries.[19] Complement split products (C3, C4-C3d and C4d), which bind covalently to vessel walls during antibody-mediated rejection leading to thrombogenesis, are stained for on biopsy and are indicative of this type of rejection.[19] The presence of C4d deposition in the peritubular capillaries is 95% sensitive and 96% specific for antibody-mediated rejection.[19,20] Shortly after antibody infiltration, cessation of blood flow ensues leading to rapid cellular necrosis, with cortical and medullary infarcts and subsequent graft loss within the first 24 hours.[18]

An insidious form of antibody-mediated rejection has also been documented, but is not clearly described.[18] This chronic form of humoral rejection usually occurs >1 year post-transplant and leads to progressive allograft damage.[18]

Today, humoral rejection is a rare occurrence as a result of improved donor-recipient HLA matching and tissue typing; however, an incidence of 0–8% still exists in large transplant centres.[18,21] Risk factors for this type of humoral rejection include previous transplant, female sex, pregnancy, historically positive crossmatch and increased panel reactive antibody (PRA).[17] Often, these highly sensitised patients unexpectedly experience '*de novo*' antibody-mediated rejection, despite the absence of donor-specific antibodies and a negative crossmatch to their prospective donors prior to transplant, placing them at high risk for renal transplantation.[18,22]

1.2 Cellular Rejection

The most commonly encountered form of acute rejection is cellular rejection, which is mediated through an alloimmune response involving the development of naive and memory lymphocytes.[23] Donor antigen presenting cells (APCs) are the source of host immune system recognition and lead to the initiation of the signal transduction pathway of acute rejection. Donor APCs may activate the host immune system directly, through presentation of antigens to host lymphocytes, or indirectly, which occurs following host APC recognition of donor antigens.[24] The interaction between the APC and the host T-lymphocyte receptor will lead to an immune response; however, the magnitude of the immune response is dependent upon additional receptor interactions. To optimally stimulate the engagement of the immune cascade, 'co-stimulatory pathways' must also activated.[24] Specifically, (i) co-stimulator proteins, CD86 and CD80, on the surface of APCs stimulate CD28 on recipient T cells, which leads to (ii) initiation of the calcium calcineurin, renin-angiotensin aldosterone system (RAAS)-mitogen activated protein (MAP) kinase and nuclear factor κ pathways causing (iii) cytokine release and T-cell surface receptor expression, which induces (iv) cellular proliferation and clonal expansion of T-effector cells and alloantibody production.[23] T-effector cells, along with activated macrophages, B cells and various cytokines, infiltrate the graft-producing cellular rejection.[23] Biopsy sections of these kidneys will display mononuclear cell infiltration of the tubules (tubulitis) and intima of small arteries (arteritis).[23] The extent and severity of graft infiltrate and injury is graded using Banff 97 criteria, which also dictates treatment of the cellular rejection episode.[25]

1.3 Achieving Allograft Tolerance

The ultimate goal in transplantation is to achieve a state in which the host does not recognise the graft tissue as foreign and ceases immunoreactivity towards donor specific antigens. Events leading to 'host-graft adaptation' or 'tolerance' have been postulated; nevertheless, no definitive pathway has been proven. It is thought that both donor tissue and recipient immunomodulation must occur prior to true tolerance being achieved.

2. Pre-Transplant Evaluation of Immunological Risk

It has been well characterised in the literature and through individual centre reporting that exposure to HLA antigens places the recipient at higher risk for rejection and early graft loss.[26-28] The risk associated with recipient exposure to HLA antigens prior to transplant is intuitive. Exposure leads to up-regu-

lation of the recipient's immune system, and clonal expansion of lymphocytes and antibodies directed at donor tissue. Graft survival in patients with peak or current PRA of >50% have 1-year graft survival rates on the order of around 80%; which is 10% lower than the overall average.[27,29] This effect is exaggerated in repeat transplant recipients, whose graft survival drops by an additional 10% when combined with an elevated PRA;[27] the higher rate of acute rejection observed in patients with an elevated peak or current PRA is also seen in those with a history of past pregnancies, or those with significant exposure to blood products.[30] In addition, immunological diseases associated with the development of endstage renal disease (ESRD) may contribute to early graft loss. For example, an analysis of patients with type 1 diabetes mellitus, whose disease aetiology is attributed to an autoimmune process, are at increased risk for primary graft loss and acute rejection. However, determination of an immunological source for graft loss has not been fully characterised, and early graft loss in such a patient population may be secondary to associated vascular disease or other factors. Other immunological causes of ESRD, such as lupus nephritis or focal segmental glomerulosclerosis, are associated with high recurrence rates leading to graft loss.[31-35]

Pre-transplant immunological risk evaluation comprises several components to stratify and determine early and late graft outcomes. High immunological risk patients are characterised as having an immunological barrier that predisposes them to suboptimal transplant outcomes, namely a higher rate of acute rejection or graft loss. Pre-transplant sensitisation to HLA alloantigens occurs through exposure to blood products, pregnancy or previous transplant. In recent years, the presence of an immunological barrier has been better detected and therapeutic strategies have been employed to circumvent these barriers responsible for poor outcomes. Figure 1 outlines the immunological screening strategies used to evaluate and stratify both donors and recipients, with a more detailed description in the subsequent paragraphs.

2.1 ABO Blood Group Determination

The ABO blood group is a group of antigens located on the surface of erythrocytes.[37] These carbohydrate or protein antigen structures initiate antibody production when exposed to a foreign host immune system.[37] To date, there are 250 known antigens in 29 blood group systems.[37] Incompatibility with the major blood group antigens (A, B, AB and A1) is the most clinically significant antigen group because of naturally occurring immunoglobulin (Ig)M and IgG circulating antibodies.[37] Transplantation across the major blood group antigens has resulted in hyperacute rejection and has been historically considered a contraindication to transplantation.[38] Recently, the shortage of organs has resulted in successful transplantation across this immunological barrier using pre-transplant splenectomy, plasmapheresis and immunosuppression.[39] In addition, the value of using A2 blood group patients in O and B blood group recipients has been successfully explored. The A2 group, a subgroup present in 20% of A blood group patients, is associated with lower immunological risk.[38] Lower immunological risk has been observed in A1 agglutination tests, where A2 positive sera do not agglutinate when testing for the A1 blood group.[38] Therefore, because A2 patients have lower levels of A group antigen, they immunologically behave more like O blood group (universal) donors.[38] However, before employing this practice, recipients should be screened to determine their level of anti-A1 antibody. Presence of high anti-A1 antibody would still preclude transplantation from an A2 positive donor unless the benefit of receiving a living donor kidney outweighs the risk of pre-transplant plasmapheresis.[38]

2.2 Detection of Donor-Specific Antibodies

Prior to implementation of modern methods for the detection of allogenic antibodies in recipients, kidney transplantation success and graft survival were based on chance. It was not until the late 1960s when the implications of a positive crossmatch and incidence of high panel reactive antibodies were fully realised. A report by Patel and Terasaki[40] evaluated 226 kidney transplant recipients whose

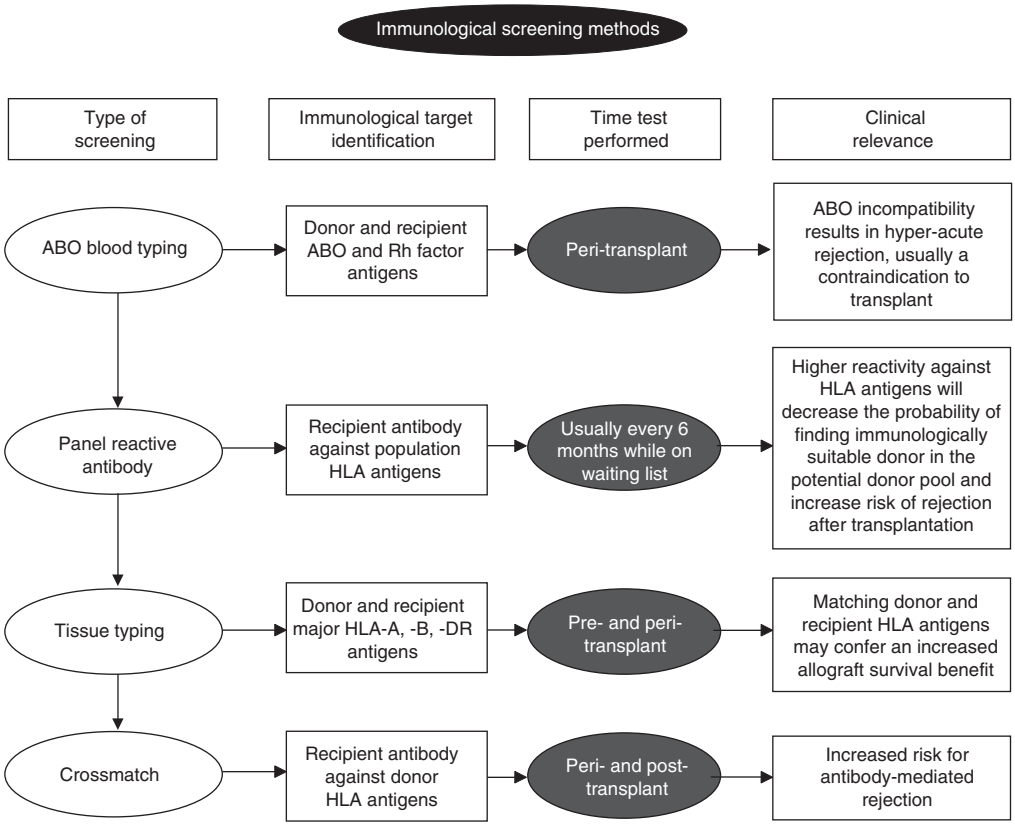

Fig. 1. Immunological screening methods are used to help determine organ allocation. Detection of immunological barriers prior to implanting the allograft may help predict the success of the transplant (graft and patient longevity) and allow for equity in organ allocation algorithms. *ABO blood typing* is performed during the initial potential recipient medical evaluation. ABO blood typing is used to determine which deceased donor list the patient will be placed on, or to allow continued evaluation of a potential living donor, who in most cases will be ABO compatible. Recipients who have preformed antibodies against ABO donor antigens will have immediate antibody graft infiltration and complement-dependent cytotoxicity. *Panel reactive antibody* (PRA) through complement-dependent cytotoxicity (CDC) or Flow technology is performed at baseline, following a sensitising event, and at institutional HLA laboratory-specified intervals (commonly every 6 months). Patients with a higher percentage PRA will move up on the deceased donor list, in that they will be given preferential allocation of an organ when one becomes available, contingent upon a negative final crossmatch. *Tissue typing* is used to determine which major histocompatability complex (MHC) A, B and DR HLAs the patient expresses. Additional information on the use of immunological screening methods in organ allocation can be found at the United Network for Organ Sharing (UNOS) website.[36] *Crossmatch* is done immediately before transplantation and may be performed following transplantation if an immunological event is observed in the allograft. Donor serum or lymph nodes are inoculated with recipient serum to determine the presence of preformed antibodies against donor tissue that could produce complement-dependent cytotoxicity. Antibodies against the A and B (MHC1) complex area confer the greatest risk for hyperacute rejection, and are a contraindication to transplantation. Following transplantation, an historical crossmatch may be performed if there is early, profound graft dysfunction. The recipient's current serum is taken and inoculated with pre-transplant donor serum to identify the presence of anti-donor antibodies. If present prior to transplant, the production of anti-donor antibodies will be up-regulated following transplantation and may be the cause of allograft dysfunction. However, findings must be clinically correlated with laboratory, pathological and patient-specific clinical conditions.

serum was tested against a panel of lymphocytes from random donors. This prospective analysis revealed that patients with a positive crossmatch, meaning there were circulating antibodies to donor lymphocytes present in recipient serum, produced an 80% graft failure rate.[40] In addition, a statistically significant difference was found with female patients in the incidence of immediate graft failure

when compared with males ($p < 0.01$). Similarly, history of pregnancy displayed a trend towards increased graft loss.[40] These observations served as a basis for our current assessment of immunological risk and stratification of patients as 'high immunological risk'. Currently, there are several techniques used to determine the presence of donor-specific antibodies and to predict post-transplant immunological risk of rejection.

As described in section 1.1, determining a patient's PRA reactivity prior to transplantation is commonplace in the pre-transplant evaluation. This test evaluates the presence of circulating antibodies to class I HLA antigens in the recipient's serum which, if present, may induce hyperacute rejection.[41] Moreover, the presence of even low levels of circulating antibodies has been correlated with an increased risk of acute rejection and graft loss.[40] Two methods are currently used to assess a patient's pre-transplant sensitisation: (i) complement-dependent cytotoxicity (CDC) PRA; and (ii) flow cytometry PRA or FlowPRA.

CDC PRA is characterised by inoculation of the serum of a patient with a reference group of lymphocytes that express a variety of HLA antigens.[40,42] The percentage of antigens that the patient has detectable antibodies to is then expressed as the PRA percentage (range 0–100%). This percentage can wax and wane over time, depending on the patient's exposure to foreign HLA antigens. Patients with very high PRA percentage are less likely to have a negative crossmatch to a prospective donor. Therefore, a negative crossmatch in these highly sensitised patients delivers a higher point allocation on the deceased donor list to ensure that these patients are given priority when they have a negative crossmatch. However, consideration of the following points is needed when evaluating the results of the CDC PRA: (i) test cell panels may not represent the entire spectrum of HLA antigens that a patient may react to; (ii) there is high inter-laboratory variability secondary to nonstandardised panels; and (iii) results may contain a high number of false positive results due to the presence of autoantibodies.[42]

In contrast, the FlowPRA is a more sensitive and specific technique to detect the presence of class I and class II HLA antibodies.[41,42] This method, which employs a flow cytometer, detects particles that pass through incident laser beams and correlates several light dispersion parameters with the presence of molecular interactions.[43] Initially, this technique was performed using spleen cells as the source of HLA antigens. However, the heterogeneity in panel pools, technical rigour of panel preparation, and the inability to determine whether antibodies were reacting to class I or class II antigens or autoantigens, made this method less than optimal.[41,42,44] An alternative method developed by Pei and colleagues[41] used microbeads coated with purified HLA class I or class II antigens. A total of 60 microbeads characterising 30 class I and 30 class II HLA antigens from a single cell line were selected to represent the most common HLA antigens as well as the most rare HLA antigens. Percentage PRA is then established by determining the percentage of microbeads that react positively with the patient's serum. There are advantages and concerns with the FlowPRA method. Specifically, this method decreases the incidence of false negative results because the antibodies react specifically with the HLA-coated beads. Also false positive results, or detection of autoantibodies, are also avoided because the beads are constructed after HLA antigen purification.[41] However, the extreme sensitivity and specificity of the FlowPRA method may not be able to decipher the harmful complement-fixing alloantibodies from the less harmful non-complement-fixing alloantibodies.[42] Although, these are not truly false positives, because the method is detecting the presence of an antibody, the clinical implication of non-complement-fixing alloantibodies is unknown.[42]

Many centres are now using the FlowPRA method, in combination with the standard CDC PRA to determine a patient's risk for rejection and graft loss.

2.3 Human Leucocyte Antigen Tissue Typing

During the initial transplant evaluation and at the time of transplantation, HLA tissue typing and determination of the presence of anti-donor HLA antibodies in the recipient's serum is performed. HLA tissue typing is used mainly to determine allocation of deceased donor organs. HLA class I molecules (HLA-A, -B or -C) are expressed on almost all nucleated cells in the body, and tissue typing can be performed on lymphocytes from the lymph nodes, blood or spleen.[45] HLA class II molecules (HLA-DR) are located on lymphocytes, specifically B lymphocytes.[45] HLA typing involves identification of the six major histocompatibility complex (MHC) or HLA molecules in both the donor and recipient. Matching of these antigens between the donor and recipient is correlated with graft outcomes.[45,46] Because of a substantial increase in graft longevity, a six-antigen match or zero-antigen mismatch between a deceased donor and prospective recipient constitutes preferential organ allocation.[47] Retrospective analysis of the US Renal Data System indicates that, under current immunosuppression, there was no difference in graft survival when outcomes were compared across 1–6 HLA mismatches.[47] However, cold ischaemia time, which is described in detail in section 6.1, did have an adverse affect graft outcomes, producing a decreased graft survival with each 12-hour increase in organ preservation time.[47] Despite longer cold ischaemia times associated with the transport of six-antigen-matched kidneys, subsequent analyses of these kidneys indicated that the benefit of the match outweighed the effect of cold ischaemia time.[48]

Although, six-antigen-matched kidneys afford the greatest allograft survival advantage, many clinicians will still consider all HLA matching in the assessment of a patient's risk for acute rejection and overall allograft function. The degree of HLA mismatch has been correlated with both early and late allograft outcomes in various studies. For example, in a recent review of unsensitised (PRA 0–9%) deceased donor recipient outcomes in the US, patients who had fewer HLA-B and -DR mismatches experienced significantly longer allograft survival, regardless of race.[49] However, HLA-A mismatches did not contribute to a change in allograft survival, compared with six-antigen-matched kidneys.[49] Roberts and colleagues[50] performed a similar evaluation and found that HLA-DR mismatch correlated with a statistically significant decrease in allograft survival compared with six-antigen-matched kidneys, but HLA-A and -B did not.[50] Single centre evaluations provide similar observations; however, immunosuppression is not standardised across either single centre or national transplant databases. Therefore, clinicians must evaluate the role of HLA matching on a patient-specific basis to determine its contribution to their risk assessment.

2.4 Crossmatching

Detection of preformed, recipient allogeneic antibodies to donor HLA antigens is performed immediately prior to transplant to predict the risk of hyperacute rejection (figure 1). This 'crossmatching', like PRA detection, involves several techniques that are used individually or in concert to determine the patients risk of rejection and graft loss prior to transplantation.[40] The CDC or 'standard' crossmatch, involves incubation of recipient serum with donor cells.[40,51] Following a specified period, direct cell cytotoxicity was observed to determine the presence of preformed antibodies to donor tissue.[51] This process has evolved to improve sensitivity and specificity by using anti-human globulin (AHG). AHG-complement-dependent T cell and B cell crossmatches detect low titre antibodies that do not fix complement.[51] The standard crossmatch has large interlaboratory variability and is a subjective determination of the presence of donor-specific antibodies, since observing complement-dependent cell lysis is the measure of reactivity.[51]

A more recently developed technique is the flow crossmatch, which can detect donor-specific antibodies without complement fixation.[51] Recipient serum is incubated with donor lypmphocytes that are stained with fluorochrome-conjugated anti-IgG antibody.[51] The major advantage of this technique is that antibody reactivity can be independently and simultaneously evaluated on donor T and B lympho-

cytes.[51] The flow crossmatch is also considered semiquantitative, which may remove the subjectivity seen in the standard method. However, like Flow-PRA, clinically irrelevant crossmatches may also be detected, producing false positive results. Nevertheless, the presence of a positive flow crossmatch in the setting of a negative CDC crossmatch has been associated with an increased risk of acute rejection.[52] ELISA and microparticle techniques, which are more sensitive and specific, may aid in deciphering unclear results. Time, cost and feasibility preclude all centres from employing new crossmatch technology, therefore the clinical implications of flow, ELISA and microparticle techniques remain unclear.

3. Peri-Transplant Desensitisation

The current shortage of allografts and the growing deceased donor waiting lists has stimulated more transplantation across previously incompatible immunological barriers. Transplantation across these barriers (such as a positive T cell CDC crossmatch, true positive B cell CDC crossmatch or across an ABO blood group) places the graft at high risk for hyperacute rejection.[53] Pre-transplant recipient desensitisation is aimed at (i) removing or neutralising circulating anti-HLA antibodies; and (ii) preventing the formation of new anti-HLA antibodies.[53] In the setting of newer, more potent induction and maintenance immunosuppression, highly sensitised patients and patients with a positive crossmatch living donor are able to undergo transplantation.

3.1 Intravenous Immune Globulin

Administration of intravenous immune globulin (IVIg) has been shown to reduce the presence or reactivity of circulating anti-HLA antibodies, as detected by a reduction in PRA or conversion of a previously positive crossmatch into a negative crossmatch.[54] IVIg is prepared from pooled healthy donor plasma.[55] Commercial pooled IVIg preparations contain intact IgG molecules with a distribution consistent with normal human serum.[55] Most preparations contain trace amounts of IgA, which can sensitise deficient patients after long-term administration.[55] Preparations also contain trace amounts of soluble CD4, CD8 and HLA molecules.[56] The half-life of exogenously administered IVIg, in an immunocompetent individual, is 3 weeks.[55] The specific mechanism of action of interest in desensitisation is not fully understood, but it is postulated that administration can attenuate host B- and T-cell activity, specifically the interaction and response to anti-HLA antibodies.[53] *In vivo* studies suggest that exogenously administered IVIg may also block IgM antibodies and allow deletion of the alloreactive B cells through binding to the Fcγ portion of the IIB receptor, thus preventing signal transduction.[57] IVIg interacts and down-regulates the synthesis of antibodies that are produced to attack the foreign graft tissue.[55] Antibodies targeted at the variable region of the T-cell receptor are also present in IVIg and may be responsible for the augmentation of T-cell function.[55] Antibodies against Fas (CD95), Fas ligand, are also present and can induce B- and T-cell apoptosis *in vitro*.[55] Additionally, the inhibition of cytokine stimulation, cytokine receptor antagonism and inhibition of complement activity may contribute to a decrease in PRA seen following administration.[58] There are no head-to-head trials comparing currently available commercial preparations and large intra-batch variability has also been observed.[53] It appears that a higher concentration of IgG is correlated *in vitro* with a reduction in PRA.[54] Therefore, some desensitisation protocols will use cytomegalovirus hyperimmune IgG, in an attempt to maximise IgG concentration; however, no data exist to indicate whether it is superior to standard IVIg preparations.[53]

No single dosage strategy has been accepted as the gold standard for peri-transplant administration of intravenous immunoglobulin for purposes of desensitisation. Protocols available in the literature are summarised in table I. Infusion-related reactions, characterised by fever and chills, can be managed by slowing the rate of infusion and premedication with paracetamol (acetaminophen) and diphenhydramine. Rarely, anaphylactic reactions associated with preparations that contain high amounts of IgA in IgA-deficient patients have been

Table I. Published desensitisation protocols for renal transplant recipients

Study	Indication	Plasmapheresis	IVIg dosage	IVIg dosage interval	Other preoperative immunosuppression	Efficacy assessment	Outcome
Glotz et al.[61,62] (n=15)	Class I PRA ≥50% or positive T-cell CDC	No	2 g/kg	Every 4 weeks × 3 doses, given over 48 hours	No	PRA and CM repeated after every dose, success if antibody level fell ≥50% or negative CM	87% (13/15) transplanted, 11/13 transplanted with a deceased donor kidney with mean decrease in PRA of 80%, 15% (2/13) graft loss
Jordan et al.[58] (n = 27)	PRA ≥50% monthly for 3 months	No	2 g/kg vs placebo	Monthly × 4 months, then at 12 and 24 months	No	Serial PRA, treatment groups PRA returned to baseline 6 months after IVIg treatment	Time to transplantation decreased by 5.5 years, more acute rejection in treatment group
Sonnenday et al.[63] (n = 18)	Positive CM	Alternate-day	100 mg/kg[a]	Alternated with plasmapheresis until negative CM (mean three treatments needed)	MMF + tacrolimus initiated at start of desensitisation	Serial CM until negative	No hyperacute rejection, 5/18 had responsive AHR, 1 graft loss from noncompliance
Schweitzer et al.[64] (n = 15)	Positive CM to permit LD	3 × weekly for 6 treatments	500 mg/kg total dose, divided over 7 days	Initiated following first plasmapheresis	MMF + tacrolimus + corticosteroids initiated 4 weeks prior to scheduled transplant	CM at weeks 2 and 3 and preoperative once negative – transplant	n = 11 proceeded to transplant, acute rejection rate of 36%; n = 4 CM remained positive
Thielke et al.[65] (n = 16)	Positive flow cytometry CM defined >20 channel shift for T cell and >40 for B cell	Every other day starting 1 week prior to transplant	100 mg/kg	Following PP	Rituximab × 1 given in 6 patients	Negative CM by third treatment	n = 4 CM remained positive, 100% patient and graft survival, 25% (3/12) had humoral rejection
Stegall et al.[66] (n = 61)	Positive T-cell CM titres <1 : 16, three protocols compared	*Protocol 1*: (n = 32) daily following rituximab	100 mg/kg	Following PP	Rituximab 375 mg/m^2 × 1, days −4 or −7, splenectomy 18/32 patients performed	Negative T-cell CM at time of transplant, diagnosis of humoral rejection in first 6 weeks post-transplant	Negative CM 84% (27/32), humoral rejection 37% (11/30)
		Protocol 2: (n = 13) none	2.1–3 g/kg	1–3 days prior to transplantation	If negative CM not obtained protocol 1 used without splenectomy		Negative CM 36% (5/13), humoral rejection rate 80% (4/5)
		Protocol 3: (n = 16) daily following rituximab	100 mg/kg	Following PP	Rituximab 375 mg/m^2 × 1, days −4 or −7, thymoglobulin 1.5 mg/kg		Negative CM 88% (14/16), humoral rejection rate 29% (4/14)
Saito et al.[67] (n = 6)	ABO incompatible, no splenectomy	Double filtration × 2, day −5 and −2	None	None	MMF 20 mg/kg/day + corticosteroids 8 mg/kg started at day −28, rituximab 375 mg/m^2 days −14 and −2	Preoperative antibody titres	83% graft survival, n = 1 1A cellular rejection, 4/5 patients developed CMV infection

a CMV IgG used.

1A = 1A rejection (Banff 97 criteria); **AHR** = acute humoral rejection; **CDC** = complement-dependent crossmatch; **CM** = crossmatch; **CMV** = cytomegalovirus; **IVIg** = intravenous immunoglobulin; **LD** = living donor; **MMF** = mycophenolate mofetil; **PP** = plasmapheresis; **PRA** = panel reactive antibody.

reported. Pre-emptive and post-transplant administration of immunoglobulins should be done with a sucrose-free product. Sorbitol, glucose and sucrose are added to preparations as a stabiliser to prevent aggregation of components. Sucrose-containing intravenous IVIg preparations are responsible for the 90% of reported IVIg-associated adverse events, including acute renal failure.[59]Acute renal failure associated with IVIg administration is thought to be secondary to an osmotic nephrosis, characterised by swelling and vacuolisation of the proximal tubular cells. This osmotic nephrosis is thought to occur as a result of the high carbohydrate concentration in some preparations.[60] The risk of nephrotoxicity can be reduced by appropriate pre-administration hydration and slowing the infusion rate.[53]

3.2 Plasmapheresis

Plasmapheresis, used in the setting of desensitisation, is employed to directly remove all circulating anti-HLA alloantibodies.[22,53] Desensitisation protocols (table I) using plasmapheresis follow each plasma exchange with low-dose IVIg. Administration of IVIg is thought to prevent a rebound in alloantibody production by mechanisms previously mentioned.[53] Replacement fluids used to maintain intravascular oncotic pressure should be devoid of any immunological components. Therefore, albumin alone or combined with crystalloid based on the patient's serum albumin is preferred.

3.3 Rituximab

Rituximab is a chimeric human/murine monoclonal antibody directed against CD20 located on B cells but not mature plasma cells.[53,68] Administration of rituximab for desensitisation is usually reserved for patients at highest immunological risk.[53] Proposed mechanisms of action in the setting of allosensitisation include (i) apoptosis of precursor B cells effectively preventing new antibody formation; and (ii) inhibiting B-cell-mediated antigen presentation.[53] Currently, rituximab is used as a single administration of 375 mg/m^2 for the treatment of refractory humoral rejection in kidney transplant recipients.[68] The utility of rituximab is also being explored for transplantation across ABO-incompatible barriers. Sawada and colleagues[69] detailed four cases in which rituximab 375 mg/m^2 weekly for 3 weeks was given in combination with double filtration plasmapheresis plus splenectomy for plasmapheresis-refractory patients, with favourable graft outcomes. Rituximab is currently approved by the US FDA for the treatment of non-Hodgkin's lymphoma and in combination with methotrexate for refractory rheumatoid arthritis.[70]

3.4 Kidney-Paired Donation

Although desensitisation is an option for decreasing the risk of hyperacute and subsequent cellular rejection, it is not without risk of infection or malignancy. Moreover, desensitisation may not be appropriate for deceased donor waiting list candidates secondary to the unpredictability of organ availability.[53] An alternative option for candidates who have willing, but immunologically incompatible, donors is kidney-paired donation. This alternative matches donor-recipient pairs who are ABO-incompatible or have a positive crossmatch with other donor recipient pairs who have similar immunological barriers.[71]

4. Recipient Demographics

Acute rejection has been consistently identified as a substantial risk factor for chronic rejection and immunological graft loss; however, other donor/recipient factors also contribute to suboptimal graft outcomes.[2,4-6] Nevertheless, solid biological plausibility and consistency in a single predominant risk factor or group of distinct donor/recipient risk factors is lacking. Beyond pre-transplant recipient sensitisation to HLA, donor/recipient race and age, and ESRD aetiology are recurring risk factors that contribute to the risk of acute rejection and allograft loss, and place the recipient at high risk for renal transplantation.[8,72]

4.1 Recipient Race

Patients of African American descent are at an increased risk for suboptimal renal transplant outcomes and are accordingly classified as high risk for

transplantation. This classification stems from the evaluation of several prospective trials and analysis of large transplant cohorts indicating that African American recipients fare poorly compared with their Caucasian counterparts.[8] The rationale for the racial discrepancy in outcomes is multifactorial and is rooted in the access and allocation of organs, as well as increased immunological risk.[8,73]

Deceased donor waiting times have increased across all ethnicities, but the median waiting time for African American patients is more than 1.5 years longer than Caucasian patients (Organ Procurement and Transplantation Network [OPTN] data).[36] The deceased donor pool has historically been racially disproportionate, with 10.4% of donors being of African American descent versus 27.5% of waiting list candidates.[8] Several reasons have been suggested to describe these waiting time differences. Specifically, African American patients tend to rely more heavily on the deceased donor pool because of the inability to identify suitable living donors.[47,73,74] One single centre evaluation, reported that African Americans were 20% less likely to identify living donors compared with Caucasians, with the most frequent reason for exclusion being pre-existing disease (hypertension or glucose intolerance).[75] Consequently, the number of African American patients awaiting transplantation tends to grow beyond the supply of available organs. In addition, ABO blood types associated with longer waiting times are more predominant in the African American population (SRTR 2004[73]). Other immunological barriers are also presumed to influence the African American waiting times and have been postulated to contribute to the observed decreased allograft survival following transplantation. Specifically, African Americans tend to be more sensitised to HLAs pre-transplant, which translates into increased waiting times.[73,76] In addition, studies of minor blood groups, such as the absence of the Duffy antigen receptor, which confers a heightened immune response, have been implicated in the increased transplant immunological risk seen in African Americans.[77,78] However, studies of these minor antigen groups have been negative or equivocal.

Nevertheless, acute rejection and early graft loss occurs more frequently in African American patients than Caucasians.[79-81] African American recipients are continually over-represented in their cohort in the re-transplant population. A meta-analysis performed in 1998 evaluating 19 208 recipients with primary graft loss found that African Americans accounted for 31.2% of the population.[82] In contrast, African Americans during the same observation period accounted for 22% of primary deceased donor recipients.[82] Socioeconomic status, education and noncompliance are frequently cited as reasons for primary graft loss and suboptimal outcomes.[8,73,80,83,84] However, a significant contribution of immunological risk must be implicated in the increased incidence of allograft failure in the African American population.[8,73,80,83,84]

Hispanic ethnicity has also been reported as an independent predictor of premature graft loss. In a recent evaluation of 4471 adult transplant recipients in the US, the 10-year estimated probability of graft survival predicted an 11% difference in graft survival when Hispanics were compared with Caucasian recipients (64% vs 75%, $p < 0.001$).[85] Although Hispanic ethnicity is targeted as a high-risk patient population, there are no specific immunological causes associated with their premature graft loss. Recipient factors have been identified that may contribute to this patient population's high-risk stratification. Diabetes is the predominant cause of ESRD, accounting for 39% of the Hispanic patients on the deceased donor waiting list.[29] Therefore, pre-transplant microvascular disease and concurrent cardiovascular disease may contribute to poor allograft survival. In addition, Hispanic patients spend more time on haemodialysis, a known risk factor for inferior transplant outcomes.[86] Sequist and colleagues[87] retrospectively evaluated 388 Caucasians and compared them with 470 Hispanics to evaluate length of time till addition to a waiting list and subsequent transplantation. This evaluation revealed that Hispanic patients were 11% more likely to refuse transplant listing compared with Caucasians.

The most common reasons for refusal were their preference to remain on haemodialysis or significant concerns associated with financing their post-transplant treatment course. Once patients were referred to a centre for wait listing, Hispanic patients were 16% less likely than Caucasians to be listed. This observation is associated with the disproportionate number of Hispanic patients who fail to progress through their transplant work-up compared with Caucasians.[87]

4.2 Recipient Age

Recipient age is associated with an increased risk of suboptimal allograft outcomes. Paediatric recipients, in general, are known to have significant differences from adults in their metabolism, distribution and excretion of immunosuppressive medications, which contributes to their overall relative risk for acute rejection.[88,89] However, modern immunosuppression and routine use of induction therapy has diminished the risk of acute rejection and has contributed to improved long-term allograft survival.[90] Hwang and colleagues[90] recently evaluated the United Network for Organ Sharing (UNOS) database from January 1994 to December 2002 to evaluate risk factors associated with early allograft loss in paediatric deceased donor recipients. Recipients aged 2–5 years were at a 102% increased higher risk of graft loss within the first 3 months post-transplant versus recipients aged 6–12 years. Patients aged 13–20 years were 50% more likely to lose their graft compared with 6- to 12-year-olds. The predominant cause of early allograft loss in patients aged 2–5 years was primarily related to technical problems (thrombosis, primary non-function), while adolescent allografts succumbed to chronic changes related to non-compliance-induced acute rejection.[90,91]

Older aged adult patients are also considered high risk for kidney transplantation. The proportion of patients that will be diagnosed with ESRD is expected to grow significantly with more individuals living to 60 years or older with chronic diseases. Advancing age, similarly to African American race, has been subject to deceased donor allocation bias.[92] Traditionally, the distribution preference of standard criteria deceased donor organs has first been to patients with the longest life expectancy.[72] The risk of death increases with advancing age and time on dialysis; therefore, many older candidates will die while waiting for an organ.[92] Older patients with ESRD tend to have significantly more co-morbidities than younger patients, making them high risk for the transplant surgery itself and subject to more immunosuppression-induced complications, especially in the early post-transplant period.[93]

5. Living versus Deceased Donor

Living donors afford a substantial benefit to recipients by decreasing warm and cold ischaemia times and allowing for more accurate assessment of immunological match prior to transplantation.[92] Living donor graft recipients have decreased early transplant morbidity and mortality, and increased graft longevity compared with deceased donor recipients. Pre-donation co-morbidities, such as diabetes mellitus, hypertension or vascular disease, preclude living donors from donation because of their own increased risk for ESRD. However, use of such high-risk deceased donors is becoming more common, in an effort to increase the donor pool.[94]

Although deceased donors with a medical history positive for hypertension and diabetes are correlated with more post-transplant delayed graft function, the 1-, 2- and 5-year graft survival rates appear comparable to healthy donors.[95,96] However, the under-reporting of pre-donation renal function, use of pressors, and length in the intensive care unit do not allow full prediction of outcomes when using organs from patients with a history of diabetes and hypertension. Therefore, high-risk donors are characterised by (i) age (<5 or >55 years old); (ii) non-heart-beating donors; (iii) diabetes mellitus; (iv) hypertension; (v) race; or (vi) vascular disease.[8,10,14,15,97,98] Transplantation of older donor organs has been consistently associated with decreased graft survival.[8,94] The effect of donor age on patient survival was evaluated in a retrospective review of >50 000 primary deceased donor recipients, which found that 5- and 10-year survival begins to fall with donor age

36–40 years.[72] However, the current shortage of organs has been the stimulus for the 10% increase in the number of deceased donors over the age of 50 years seen in the past several years.[99,100]

Deceased donor allografts from African American donors have been correlated with an increased risk of graft loss in multivariate analyses of large transplant databases.[8,94] This finding may indicate that these grafts are subject to greater ischaemia/reperfusion injury or immunological factors that have not been fully elucidated.[8] Moreover, the susceptibility of African American patients to the sequelae of hypertension may be related to the failure rate of these grafts.[8]

Extended criteria donors are defined as donors aged >60 or 50–59 years with a history of hypertension, cerebral vascular accident as the cause of death, or terminal serum creatinine of >1.5 mg/dL.[95,100,101] Extended criteria donors are offered only to those patients who consent to accept them at the time of deceased donor listing, and may provide a considerable survival advantage for older recipients compared with remaining on haemodialysis.[92,102]

6. Procurement Variables

Deceased donors are defined as heart-beating or non-heart-beating donors. Historically, deceased donation was limited to organs procured following cardiac death. Standardisation of criteria to determine brain death, and improved patient and family education has enabled heart-beating donation, which has improved long-term graft outcomes.[103] The last two decades have revived the use of non-heart-beating donor organs as a result of the shortage of organs and need for an increased donor pool.

Outcomes associated with grafts obtained from both heart-beating and non-heart-beating donors are dependent on several variables. Age and pre-donation co-morbidities – such as a medical history of hypertension, diabetes or peripheral vascular disease – influence the graft's microvasculature and nephron density. This so-called pre-existing 'donor disease', which can be seen on biopsy, can influence the ability of the graft to sustain and survive ischaemia/reperfusion injury.

6.1 Ischaemia/Reperfusion Injury

Ischaemia/reperfusion injury is one of the most frequent and elusive complications following renal transplantation, and is related to warm and cold ischaemia time. Following prolonged periods of ischaemia, tubular cells will experience decreased adenosine triphosphate generation in the setting of anoxia and enzyme activity is altered. Upon reperfusion, toxic oxygen free radicals are produced which induce local inflammation and up-regulation of the complement and coagulation cascade.[104] Although preservation solutions and cold temperature tissue storage can maintain electrolyte balance and slow the tubular cell metabolic rate, prolonged ischaemia time increases the production of anaerobic respiration byproducts. These byproducts and alterations in normal homeostasis manifest marked graft inflammation. Ischaemia/reperfusion injury is manifested through slowed or delayed graft function, characterised by acute tubular necrosis on biopsy. Several theories postulate the implications of prolonged cold and warm ischaemia and its effect on increasing graft alloreactivity and subsequent acute rejection.[105] Bryan and colleagues[28] theorised that longer cold ischaemia time was associated with increased humoral antibody response. Therefore, they evaluated 90 patients who experienced immunological graft loss and had at least 3 HLA class I AHG-PRA values performed subsequent to graft loss. Patients were unsensitised (PRA <10%) primary transplant recipients. Patient baseline demographics were well matched, and patients were stratified to compare the effect of cold ischaemia time <15 hours versus ≥15 hours. Factors implicated in increased graft loss were: three or four HLA-A,B mismatch and cold ischaemia time ≥15 hours. The development of class I directed antibody was significantly greater in the group with a cold ischaemia time ≥15 hours. Therefore, as historically assumed, longer cold ischaemia time ≥15 hours independently increases the risk for developing class I AHG in patients who subsequently rejected their grafts.

Table II. Risk factors for delayed graft function

Donor	Recipient	Graft	Immunological
Older age (≥50 years)	African American race	Non-heart-beating donor	Poor HLA matching
Female	High peak or current PRA	Prolonged cold ischaemia time	No T-cell-depleting induction
Serum creatinine at time of donation	Previous transplant		
	Time on dialysis		
Pre-donation hypotension	Weight mismatch		
CVA cause of death			
Medical history of hypertension			

CVA = cerebrovascular accident; **HLA** = human leucocyte antigen; **PRA** = panel reactive antibody.

Since immunogenicity increases with increased cold ischaemia time, through up-regulation of MHC, cytokines and adhesion molecules, the importance of adequate immunosuppression in the early post-transplant period cannot be overemphasised.[28]

Ischaemia/reperfusion injury has now become a target for novel immunomodulating agents. Several compounds, administered to donors prior to procurement or during the transplant surgery, are being explored in early phase clinical trials. The hope is that these agents can attenuate host-allograft responses to ischaemia/reperfusion injury and prevent its sequelae, such as delayed graft function, post-transplant.

6.2 Delayed Graft Function

Delayed graft function may be defined through several different methods, such as rising serum creatinine or unchanged during 3 post-transplant days, or a rise in serum creatinine above 3 mg/dL on the fifth post-transplant day. The most common, objective definition used is the need for dialysis within the first week post-transplant, with subsequent recovery of function.[106] The clinical effect of delayed graft function is not well characterised, but has been implicated in an increased risk of acute rejection, prolonged hospitalisation and increased hospital costs.[105] Risk factors for delayed graft function are listed in table II. Appropriate identification of patients who are likely to experience delayed graft function will facilitate early postoperative haemodynamic and immunosuppressive management. Use of T-cell-depleting induction can delay administration of nephrotoxic maintenance immunosuppression, such as calcineurin inhibitors, to ensure graft perfusion and recovery.

7. Post-Transplant Immunosuppression

Induction and maintenance immunosuppression can influence both the short- and long-term allograft outcomes. Agent choice must be evaluated based on both donor and recipient factors, and immunological risk, as well as risk of early graft dysfunction. Figure 2 outlines the complex matrix of variables that must be evaluated to determine the optimal immunosuppression regimen. In the last decade, routine induction therapy and use of tacrolimus and mycophenolate mofetil (MMF) in place of ciclosporin (cyclosporine) and azathioprine has significantly reduced the risk of acute rejection.[107] However, even in the setting of more potent immunosuppression and shorter ischaemia times, the use of marginal grafts from older, hypertensive donors has not improved graft or patient survival.[107] In addition, over immunosuppression places the patient at high risk for infection and malignancy. Stratification of patients into high-risk groups (table III) will aid in determining what type of induction and maintenance immunosuppression should be employed.

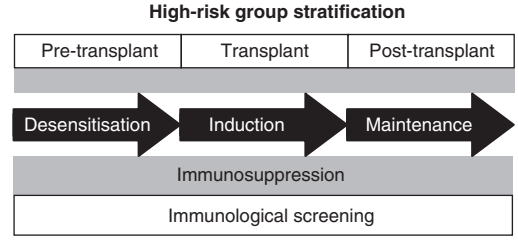

Fig. 2. The tactical approach to the high-risk kidney transplant patient involves an integration of patient, donor and allograft variables along with immunological screening at all time points surrounding kidney transplantation. These variables provide the basis for use of immunosuppression pre- and peri-transplant.

7.1 Induction Immunosuppression

Induction therapy is used in high-risk patients to achieve immediate, profound immunosuppression. Use of these agents in the setting of high immunological risk, expected acute tubular necrosis or delayed graft function may enable perioperative calcineurin inhibitor sparing, while maintaining adequate immunosuppression. Induction therapy, given at the time of engraftment, halts the immune system's immunoreactivity and prevents lymphocyte replication and clonal expansion. A recent multivariate analysis of antibody induction evaluated 24 901 deceased donor transplants performed from 1999 to 2001, using the UNOS database.[108] This analysis indicated that high-risk patients (paediatric, re-transplant and patients who received a graft from a donor >55 years old, with more HLA-DR mismatches, or prolonged cold ischaemia time defined as >26 hours) were more likely to receive induction therapy. Graft and patient survival was significantly higher in patients who received induction versus those who did not (90.1% and 92.1% vs 88% and 90.9%, respectively). Choice of induction therapy did not contribute to overall risk of graft loss or death, but patients who received rabbit antithymocyte globulin (ATG) were 24% less likely to have an episode of acute rejection compared with those who received interleukin (IL)-2 receptor antagonists (odds ratio 0.76; CI 0.59, 0.77).[108] Although this evaluation was retrospective, it illustrates that administration of induction therapy is nearly universal in high-risk patients. Selection of the induction agent is based on presumed efficacy associated with centre-specific maintenance immunosuppression. The three most commonly used induction agents are

Table III. High-risk group stratification in renal transplantation

Factor	Implication	Recipient peri-transplant management	Risk of acute rejection
Donor			
History of cardiovascular disease	Graft microvascular disease	Calcineurin inhibitor minimisation	Unknown
Older age	Decreased nephron density	Calcineurin inhibitor minimisation	Unknown
African American race	Increased ischaemia/reperfusion injury	Increased immunosuppression	Increased
CVA cause of death	Loss of sympathetic outflow and peri-procurement hypotension, increased risk of delayed graft function	Aggressive blood pressure management	Increased
Recipient			
Older age	Longer time on HD, increased peri-transplant co-morbidities, increased risk of infection	Modified immunosuppression, aggressive co-morbidity management	Decreased
Cardiovascular disease	Perioperative risk of cardiovascular event	Aggressive cardiovascular management	Unknown
African American race	Increased risk for acute rejection	Increased immunosuppression	Increased
Immunological aetiology of ESRD	Increased risk for acute rejection, risk of recurrence	Increased immunosuppression	Increased
Immunological status blood product exposure pregnancy previous transplant	Elevated PRA, positive crossmatch, longer deceased donor waiting time	Desensitisation, increased immunosuppression	Increased
Graft			
Prolonged warm/cold ischaemia times	Increased HLA expression, delayed graft function	Increased immunosuppression	Increased
Donation after cardiac death	Decreased graft perfusion peri-procurement	Increased immunosuppression	Increased

CVA = cerebrovascular accident; **ESRD** = endstage renal disease; **HD** = haemodialysis; **HLA** = human leucocyte antigen; **PRA** = panel reactive antibody.

ATG, alemtuzumab and anti-CD25 monoclonal antibodies.

ATG is a polyclonal antibody produced from immunisation of horses (Atgam®)[1] or rabbits (Thymoglobulin®) with human lymphoid tissue.[23] These polyclonal antibodies target several cell surface lymphocyte antigens, resulting in a profound reduction in CD3+ lymphocytes. Early trials comparing the potency of horse- versus rabbit-derived anti-lymphocyte globulin revealed that the rabbit preparation may be superior. Brennan and colleagues[109] performed a single-centre, randomised trial comparing rabbit versus horse ATG. Patients received induction therapy for 7 days in combination with ciclosporin, azathioprine and corticosteroids. Patients at high immunological risk received MMF in place of azathioprine. Following statistical adjustment for maintenance immunosuppression differences, 1- and 5-year acute rejection and graft outcomes favoured patients who underwent induction with rabbit ATG. Patients who received rabbit ATG experienced a lower rate of acute rejection at 1 and 5 years compared with those who received horse ATG (4 vs 25%, p = 0.014).[109,110] Profound and extensive lymphopenia was also observed in the rabbit antithymocyte group compared with the horse antithymocyte group.[110] This characteristic stimulated hypotheses aimed at observing the effect of ATG in patients who are at high risk for delayed graft function and/or acute rejection.

It is well known that early calcineurin inhibitor administration can prolong delayed graft function. In addition, potent antiproliferatives, specifically sirolimus (rapamycin), have been postulated to prolonged acute tubular damage and necrosis.[111] Animal models of ischaemia/reperfusion injury have demonstrated less infiltrate and endothelial damage when these lymphocyte-depleting polyclonal antibodies are administered prior to engraftment.[112] Charpentier and colleagues[113] performed a multicentre, randomised, open labelled, prospective, parallel group evaluation to determine whether rabbit ATG administration could allow for the delay of calcineurin inhibitor initiation. A total of 555 patients were enrolled and randomly assigned to receive tacrolimus alone, tacrolimus plus rabbit ATG, or ciclosporin plus rabbit ATG in combination with azathioprine and corticosteroids. Although this study was limited by only 6 months of patient follow-up, the incidence of acute rejection was significantly higher in the group who did not receive rabbit ATG induction (tacrolimus 30% vs rabbit ATG/tacrolimus 21%, p = 0.003). Tacrolimus in combination with rabbit ATG was also superior to ciclosporin with rabbit ATG (21 vs 35%, p = 0.004).[113] Moreover, polyclonal antibody induction has been used to decrease overall maintenance immunosuppression in high-risk patients. Woodle and colleagues[114] reported a single-centre analysis of 308 patients that evaluated the outcomes of several high-risk patient groups (African American, repeat transplant, peak PRA >50%, delayed graft function, and deceased donor) utilising an early corticosteroid withdrawal protocol, defined as corticosteroid withdrawal by postoperative day 7. These results revealed that those patients at high risk for acute rejection sustained lower acute rejection rates in the setting of rabbit ATG induction.[114]

Rabbit ATG is historically considered the induction of choice for high immunological risk or those patients at high risk for delayed graft function. However, the exact dose and duration of therapy needed in these high-risk patients is unknown. Infusion-related reactions, secondary to cytokine release, are common. Pre-administration of corticosteroids, paracetamol and diphenhydramine are often advocated to prevent associated hypotension, fever and shortness of breath.[115] Initial rabbit ATG infusions should be administered over a minimum of 6 hours; subsequent doses may be administered over 4 hours if tolerated.[115] This interval may be extended up to 24 hours for patients who are intolerant to the cytokine release or fluid volume.[115]

Anti-CD25 monoclonal antibodies were traditionally used for low to moderate immunological risk patients. Cost, toxicity, administration constraints and immunosuppressant potency of rabbit antithymocyte induction led to the investigation of

1 The use of trade names is for product identification purposes only and does not imply endorsement.

these agents in high-risk patients. Anti-CD25 monoclonal antibodies bind to the IL-2α receptor chain on activated T cells to prevent propagation of the T-cell response.[116] The biological half-life of these agents correlates with their ability to attach to the receptor. These agents, in contrast to polyclonal antibodies, do not provide T-cell depletion. Moreover, anti-CD25 agents may also be utilised in combination with aggressive maintenance immunosuppression for high immunological risk patients who are at significant risk for haematological or infectious complications of T cell-depleting induction.

Recently a meta-analysis of randomised trials utilising anti-CD25 antibodies for induction was performed by Webster and colleagues.[116] The analysis revealed that anti-CD25 induction did reduce the risk of overall acute rejection by 33% (0.67; CI 0.6, 0.75) compared with placebo, but did not affect overall graft loss. Compared with monoclonal or polyclonal antibody induction, anti-CD25 antibody provided a similar reduction in the risk of acute rejection and graft loss. Haematological and other adverse effects were more frequent with non-anti-CD25 antibody induction. However, of the 38 trials included in the analysis, only 11 trials included patients with PRA >50%, and 8 trials included repeat transplant recipients.[116]

Limited evaluation of these agents in high-risk patients has precluded their use as a universal induction agent. The one notable exception is the use of these agents in African Americans. For example, Haririan and colleagues[117] recently reported a single-centre evaluation of 88 African American recipients comparing basiliximab with rabbit ATG. Results revealed that graft and patient outcomes were similar between groups; however, a significantly larger proportion of patients in the rabbit ATG group were at high immunological risk (re-transplant, high PRA). Graft survival was 86% in the rabbit antithymocyte group versus 81% in the basiliximab group ($p = 0.84$). However, the rate and severity of acute rejection was clinically significant between groups: 29% of basiliximab recipients had an acute rejection, with 50% of those rejections characterised as moderate to severe (Banff grade ≥IB) versus 14% in the rabbit antithymocyte group. Additional analyses revealed that decreased graft survival was associated with increasing number of HLA mismatches and development of delayed graft function.[117] This study provides evidence to support the use of anti-CD25 monoclonal antibodies in African American recipients who have low immunological risk and probability of delayed graft function.

Anti-CD25 induction is more effective than no induction in high-risk patients; however, current evidence indicates that these agents may not be able to afford the protection of polyclonal antibody induction in delayed graft function or in the setting of high PRA.

Alemtuzumab has recently been utilised in high-risk patients to obtain the short dosage scheme associated with anti-CD25 monoclonal antibodies, but providing the lymphocyte deleting effects similar to the polyclonal antibodies. Alemtuzumab is a humanised monoclonal antibody against CD52, which causes apoptosis of both B and T lymphocytes. Compared with polyclonal antibodies, alemtuzumab induces profound lymphopenia lasting several months post-transplant. Bloom and colleagues[118] recently evaluated the immunodepleting effects of alemtuzumab and found that T lymphocytes remained at 50% of baseline at 36 months following administration of 40mg. Alemtuzumab is well tolerated, although infusion reactions similar to those observed with T lymphocyte-depleting agents have been reported.[119]

Like anti-CD25 monoclonal antibodies, alemtuzumab has been used successfully in low-risk patient populations to minimise maintenance immunosuppression. To date, a limited number of high-risk patients receiving alemtuzumab induction have been evaluated in clinical trials. Review of published trial demographics is limited by exact reporting of high-risk variables. However, from the available literature, a total of 465 patients have received alemtuzumab induction (table IV). Baseline transplant and recipient demographics revealed that 6.6% (31/465) had a PRA >20%, 16% (74/465) were of African American descent, 9% (43/465) experienced

Table IV. Summary of high-risk demographics for alemtuzumab induction in published trials

Study	Induction	Maintenance	PRA >20%	African descent [n (%)]	DGF [n (%)]	Deceased donor [n (%)]	Repeat transplant [n (%)]
Kirk et al.[120] (n = 7)	Alemtuzumab 0.3 mg/kg × 3 doses (n = 6) or × 4 (n = 1)	None	[a]	2 (29)	0 (0)	0 (0)	0 (0)
Flechner et al.[121] (n = 22)	Alemtuzumab 30mg × 1	Sirolimus + MMF	2 (9%)	0 (0)	2 (9)	8 (36)	0 (0)
Vathsala et al.[122] (n = 30)	Alemtuzumab 20mg × 2 (n = 20) vs no induction (n = 10)[b]	CsA + AZA + corticosteroids	2 (7%)	0 (0)	5 (17)	14 (47)	0 (0)
Ciancio et al.[123] (n = 44)	Alemtuzumab 0.3 mg/kg day 0 and day 4	FK + MMF	[a]	17 (39)	4 (9)	29 (66)	0 (0)
Watson et al.[124] (n = 99)	Alemtuzumab 20mg × 2 (n = 33) vs ATG (n = 7) or no induction (n = 59)	CsA monotherapy vs CsA + AZA + corticosteroids or CsA + sirolimus	15 (15%)[c]	[a]	38 (38)	99 (100)	11 (11)
Kaufman et al.[125] (n = 278)	Alemtuzumab 30mg × 1 (n = 123) vs basiliximab (n = 155)	FK + MMF	[a]	48 (17)	[a]	89 (32)	[a]
Shapiro et al.[126] (n = 343)	No induction (n = 152) vs ATG (n = 101) vs alemtuzumab (n = 90)	Tacrolimus + sirolimus or MMF + corticosteroids vs spaced weaning of tacrolimus monotherapy in lymphoid-depleted patients	63 (18%)	46 (13)	[a]	230 (67)	60 (17)
Knechtle et al.[127] (n = 1397)	Alemtuzumab (n = 126) vs anti-CD52 (n = 799) vs ATG (n = 160) vs other[d] (n = 312)	Tacrolimus or CsA + MMF + low-dose corticosteroids vs tacrolimus or CsA + MMF + corticosteroids	[a]	98 (7)	218 (16)	744 (53)	278 (20)
Total number			**82**	**211**	**267**	**1213**	**349**
Total alemtuzumab			**31**	**74**	**43**	**238**	**61**

a Not reported or unable to interpret.

b Randomised.

c Sensitisation defined as presence of class I or II specific antibodies, actual titres not presented in analysis, 'highly sensitised' patients in control group received rabbit ATG.

d Group includes muromonab-CD3 (OKT3), ATG, unspecified or no induction.

ATG = antithymocyte globulin; **AZA** = azathioprine; **CsA** = ciclosporin; **DGF** = delayed graft function; **FK** = tacrolimus; **MMF** = mycophenolate mofetil; **PRA** = panel reactive antibody.

delayed graft function, 50% received a deceased donor kidney, and 13% were repeat transplants.[120-127] Direct comparison of trial outcomes is difficult because of different primary outcomes and maintenance immunosuppression. Moreover, the small number of high-risk patients in each individual trial limits interpretation of use for high-risk patients. Therefore, only two trials,[125,127] representing well accepted, modern maintenance immunosuppression strategies, are described here.

Kaufman and colleagues[125] performed a single-centre, retrospective review of two prednisone-free immunosuppression protocols. Induction and maintenance immunosuppression are listed in table IV. In addition, patients received 3 days of methylprednisolone, 500mg on day 0, 250mg on day 1 and 125mg on day 2. Three-year graft and patient survival were similar between those receiving alemtuzumab induction and those receiving basiliximab: 92% versus 96% ($p = 0.16$) and 96% versus 98% ($p = 0.13$), respectively. No differences were seen with regard to race or donor source. One-year acute rejection rates were also similar: 14.9% in the alemtuzumab group versus 13.5% in the basiliximab group. However, acute rejections occurred a mean 97 (median 143) days sooner in patients who received basiliximab induction versus alemtuzumab induction. Induction with either agent appeared to be well tolerated, with low rates of cytomegalovirus infection (4% in the alemtuzumab group vs 5% in the basiliximab group). Three-year graft function was also similar between groups.[125] Although only a small number of high-risk patients are represented in this trial, it is important to observe the relatively low risk of acute rejection and the ability to avoid long-term corticosteroid exposure with induction. The authors did not stratify their results to evaluate outcomes in patients who experienced delayed graft function, and did not report whether high immunological risk patients were included (PRA >20% or repeat transplants).

Knechtle and colleagues[127] performed a similar analysis of their single-centre experience. Patients were excluded if they received a living related or HLA-identical allograft. Induction and maintenance therapy are listed in table IV. The authors indicated that rabbit ATG was preferred for repeat transplants. Choice of calcineurin inhibitor was at the discretion of the treating physician and was introduced when serum creatinine was <3 mg/dL. Patients in the alemtuzumab group received low-dose corticosteroids (prednisone 10mg) versus corticosteroids tapered to 10mg over 3 months in the other treatment groups. Length of follow-up was not specifically delineated in the study; however, from the depicted survival analyses it appears to be around 1 year post-transplant. Compared with other treatment groups, patients receiving alemtuzumab had lower rates of acute rejection ($p = 0.037$). Similarly to the previous experience described, administration of alemtuzumab was not associated with an increased incidence of infection.[127]

Overall, from the current published experiences, longer follow-up of alemtuzumab-treated patients will be needed to characterise the benefit of associated corticosteroid minimisation. In addition, a larger number and stratification of high-risk patients is needed to determine long-term risk of rejection and graft loss.

7.2 Maintenance Immunosuppression

The goals of maintenance immunosuppression in all solid organ transplant recipients, including patients at high risk for kidney transplantation, are to maintain efficacy while limiting toxicity. Specifically, to determine the appropriate combination of immunosuppressive medications, these regimens must consider the risk of (i) delayed graft function; (ii) endocrine and metabolic adverse effects; (iii) infection; (iv) malignancy; and (v) acute rejection.

Corticosteroids have been the cornerstone of solid organ transplant immunosuppression for >40 years.[128] Despite their longevity in solid organ transplantation, a complete description of corticosteroid-induced immunosuppression has not been fully elucidated.[128] Administration of corticosteroids produces the following *in vivo* activities: sequester of CD4+ lymphocytes into the reticuloendothelial system, blockade of cytokine expression and subsequent clonal expansion of cell-mediated

immunity.[128,129] Often, corticosteroids are administered at very high doses for the first several days post-transplant and discontinued or aggressively tapered to afford early immunosuppressive effects but limit long-term exposure. Exposure to long-term corticosteroids produces several complications (i.e. infectious, endocrine and cardiovascular), which has driven the development of corticosteroid-sparing and corticosteroid-free regimens. Traditionally, these regimens were avoided in high immunological risk populations, but the advent of more potent induction agents and use of tacrolimus and mycophenolic acid have reduced the risk of acute rejection in the setting of early corticosteroid withdrawal.[130]

A recent analysis by Woodle and colleagues[114] details experience with early corticosteroid withdrawal in high-risk patient populations. This evaluation examined risk factors for acute rejection in 308 kidney transplant recipients who underwent early corticosteroid withdrawal (<7 days of corticosteroids). Maintenance immunosuppression contained a combination of MMF, in addition to a calcineurin inhibitor with or without sirolimus. Analysis revealed that patients at highest risk for acute rejection were similar to previous trials where corticosteroids were maintained. Risk factors for acute rejection included elevated current PRA (>25%), repeat transplants, African American race, female gender and delayed graft function. Administration of T-cell-depleting induction appears to reduce the risk of acute rejection in those high-risk populations, presumably by allowing therapeutic levels of maintenance immunosuppression to be achieved before the effects of induction are diminished.[114]

The ability to achieve adequate levels of maintenance immunosuppression in the early post-operative period is often limited by graft function and patient tolerance. Calcineurin inhibitors, which inhibit secretion of IL-2 and other cytokines, interrupt both humoral and cell-mediated immunity to produce their immunosuppressive effects. Following the widespread use of ciclosporin in combined regimens in the early 1980s, overall acute rejection rates fell from >70% to <40 % on average, with dramatic improvements in graft and patient survival.[131-133] Following the introduction of tacrolimus, rejection rates fell even further, allowing for more effective maintenance immunosuppression minimisation despite offering limited improvement in graft survival. However, early post-transplant administration of calcineurin inhibitors results in constriction of the afferent glomerular arterioles. Glomerular hypoperfusion contributes to tubular ischaemia and fibrosis. This effect is pronounced in the early post-transplant period, manifested by prolonged recovery from acute tubular necrosis and delayed graft function. Therefore, calcineurin sparing is usually employed to aid in graft recovery during periods of slow or delayed graft function.[134] Calcineurin sparing is variably defined but usually involves delaying introduction until serum creatinine levels have dropped significantly from pre-transplant values, along with consideration with clinical assessment of the patient's volume status.

Adjunct maintenance immunosuppressive medications include: MMF (the ester prodrug of mycophenolic acid), enteric-coated mycophenolic acid or sirolimus. Mycophenolic acid, an antiproliferative agent which selectively inhibits T-lymphocyte proliferation, is used in most modern immunosuppression regimens. However, the appropriate dose in African American and high immunological risk patients is still unknown. It appears that African American patients may require more MMF to maintain similar efficacy compared with their Caucasian counterparts; however, this is not based on their pharmacokinetic profile but rather the correlation appears to be with their risk of rejection.[135,136] In the initial trials utilising MMF, high doses produced lower acute rejection rates in African American recipients.[135] This observation may be due to the long onset of action observed in mycophenolic acid administration where full biological activity is delayed until 3–4 weeks post-administration in corticosteroid-containing regimens. Also, the lack of adequate induction therapy in these patients may have contributed to increased risk of acute rejection. Importantly, mycophenolic acid dose escalation is

often limited by gastrointestinal and haematological adverse effects.

Sirolimus, also a potent antiproliferative agent, exhibits similar but less nephrotoxic activity to ciclosporin and tacrolimus. This agent has been promoted as a long-term nephron-sparing agent compared with long-term calcineurin inhibitor exposure; however, its potent antiproliferative activity in the setting of delayed graft function and acute tubular necrosis may be detrimental. A study comparing 132 patients who experienced delayed graft function and had either received early sirolimus versus no sirolimus revealed prolongation of delayed graft function associated with sirolimus administration.[134] Moreover, induction therapy was compared among groups and revealed that T-lymphocyte-depleting induction produced significantly lower early acute rejection rates overall, but especially in African American recipients. The authors attributed prolonged delayed graft function in the sirolimus group to its antiproliferative effects which delayed nephron recovery, because long-term outcomes, such as graft loss and patient survival, were similar among racial groups.[134]

8. Conclusions

The definition of 'high-risk' renal transplantation has dramatically changed in the setting of newer, more potent immunosuppression. High risk now must be defined in terms of associated long-term graft and patient outcomes. Appropriate early characterisation of the patient's risk for acute rejection and adverse outcomes will aid in determining the appropriate induction and maintenance immunosuppression.

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