A Case of Desensitization, Transplantation, and Allograft Dysfunction

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Case Presentation: Dr. Michael Clarkson

A 41-yr-old Asian-American woman presented for evaluation for a second kidney transplant in 2005. She had a potential living kidney donor, a sister. The patient had developed end-stage renal disease from IgA glomerulonephritis. This was first diagnosed in 1989; in 1991, she underwent a preemptive living donor transplant in another hospital. The donor was another sister and the allograft was a 2-haplotype match. Despite this, her early post-transplant course was complicated by severe acute rejection (further information on this was not available). She also developed mitral valve endocarditis with septic embolization to the brain and, in addition to prolonged antimicrobial therapy, required craniotomy and insertion of a prosthetic mitral valve. Fortunately, she made an excellent recovery. She was later diagnosed as having chronic allograft nephropathy and resumed peritoneal dialysis in 2005 and stopped immunosuppression at that time. She had received blood transfusions in the early period after her first transplant but had had no pregnancies. The only other medical history was hypertension. Her functional status was excellent. Examination was unremarkable apart from the prosthetic mitral valve click.

Investigations showed the potential donor was a 1–2–2 human leukocyte antigen (HLA) mismatch with the potential recipient. The latter was blood group ABO-AB and had a panel reactive antibody (PRA) of 0% against class I HLA by the complement-dependent cytotoxicity (CDC) assay. However, by the more sensitive FlowPRA assay, the PRA against class I antigens was 35% and against class II antigens was 98%. The crossmatch against T cells of the potential donor was negative by the CDC (antihuman globulin) method but was strongly positive against B cells by the CDC (modified Amos) method: positive out to a 1:256 dilution. The patient was diagnosed as being very highly sensitized to class II HLA antigens of the donor and informed that the transplant would be very high risk, without some form of desensitization. Because the sister had no contraindication to donation, they were both enrolled in a live donor kidney exchange program (New England Program for Kidney Exchange). After 4 mo enrolled in this program, no suitable match was found, however, and the issue of desensitization against her living donor was again discussed. The risks of the procedure were explained in detail, and they made an informed decision to proceed with desensitization and transplantation.

Indeed, the B-cell crossmatch could not be rendered negative. After detailed discussion, all parties agreed to proceed with the transplant. Another dose of rituximab was given 12 h before the transplant, and plasmapheresis was performed on postoperative days (POD) 2 and 4 to prevent rebound in alloantibody. Basiliximab was used as the induction agent.

The renal allograft functioned immediately. Unfractionated heparin was started on POD1. On POD5, the creatinine was 1.0 mg/dl and the bladder catheter was removed. On POD6, the recipient complained of pain over the allograft and her plasma creatinine had increased from 1.0 to 1.7 mg/dl. Examination showed pulse 98, blood pressure 120/70 mmHg, temperature 38.0°C, and mild tenderness over the allograft. She was 2 kg over her dry weight but was otherwise unremarkable. Initial tests showed white blood cells (WBCs) 11.6 cells/mm³, hemoglobin 7.7 g/dl, platelets 260, tacrolimus trough 10.0 ng/ml, and lactic dehydrogenase (LDH) 161 (normal). The urine dipstick revealed 2+ protein, 2+ blood, 2+ leukocyte esterase. An urgent ultrasound showed intact blood flow in the transplant renal artery and vein; there was no hydronephrosis.

Clinical Discussion: Dr. Colm Magee

Here we have a 41-yr-old female with acute renal allograft dysfunction on POD6. Clearly, this is a high-risk transplant for many reasons. The patient had severe early acute rejection of her first allograft (despite it apparently being a 2-haplotype match) and was highly sensitized to class II HLA antigens. Indeed, the B-cell crossmatch could not be rendered negative...
by desensitization so she went to the operating room with a positive crossmatch. It’s now accepted that positive B-cell crossmatches at the time of transplant are associated with a real risk of antibody-mediated rejection (AMR), if they are due to antibodies against class II HLA (1). The rejection in her first transplant also raises the concern that she has antibodies to non-HLA, such as MICA antigens; there is increasing evidence that these are clinically important (2). So the risk of acute AMR in the first few post-transplant weeks in this patient is high. One should also not forget the potential for complications related to the prosthetic mitral valve. Ideally, anticoagulation would be prescribed within 24 to 36 h of the transplant surgery (as it was here), but obviously this increases the risk of bleeding in the allograft area and after any allograft biopsy. Conversely, subtherapeutic anticoagulation places her at risk of prosthetic mitral valve thrombosis and embolization. Finally, while she is at high risk of rejection, she is also at risk of infection as she has been very heavily immunosuppressed. The use of central venous catheters and bladder catheters further increases the risk of bacteremia and endocarditis.

It is useful to apply the same principles of diagnosis of acute kidney injury (or acute renal failure) in the native setting to the transplant kidney setting. Prerenal failure seems unlikely to fully explain the acute dysfunction as the patient is 2 kg over her dry weight and is not hypotensive. Postrenal failure has been excluded by ultrasound. So we are left with intrinsic causes; the urine dipstick findings are also consistent with this. The differential diagnosis is still broad and includes acute AMR, acute cellular rejection, acute pyelonephritis, acute tubular injury (due to sepsis), and renal complications related to thrombosis or endocarditis of the prosthetic mitral valve.

Acute tacrolimus nephrotoxicity seems unlikely as the trough is not high and this condition is not associated with fever. There are no laboratory features suggestive of an acute thrombotic microangiopathy. As she has fever and allograft tenderness, I am most concerned about rejection (especially AMR) and acute pyelonephritis. Arguably, the pyuria supports the latter, but I would not rely on this finding alone. I suggest urgently performing the following tests: urine culture, at least 2 sets of blood cultures, crossmatch against donor cells (by a technique that will not be invalidated by rituximab), and allograft biopsy. It is reasonable to give prophylactic antimicrobial therapy before the biopsy because of the concern that the procedure might cause bacteremia if pyelonephritis were present. I would not empirically give additional antirejection therapy as the differential diagnosis includes various forms of infection.

Pathology: Dr. Helmut Rennke

An allograft biopsy was performed (Figure 1). Light microscopy revealed severe changes in the tubulointerstitial compartment, mostly related to widespread interstitial inflammation by neutrophil polymorphs, with disruption of tubular basement membranes, formation of microabscesses, and casts composed of neutrophil polymorphs and cellular debris. No significant changes were seen in the glomeruli. There was no tubulitis or endothelialitis. There was diffusely positive staining of peritubular capillaries for C4d by immunofluorescence. The changes were most consistent with acute pyelonephritis. The clinical significance of C4d staining was difficult to interpret in the setting of such widespread and severe inflammation.

Case Presentation: Dr. Michael Clarkson

The flow cytometry crossmatch (using Pronase) was negative against donor T cells and weakly positive against donor B cells. After the biopsy result became available, the urine culture yielded >10^5 colonies/ml of Morganella spp. Blood cultures were negative. She was diagnosed as having 1) acute pyelonephritis and 2) C4d positivity of unclear significance. Intravenous fluids and high-dose ciprofloxacin were prescribed, and her symptoms and signs rapidly improved. The plasma creatinine fell to 1.0 mg/dl within 24 h of starting antibiotics. She received a total of 5 sessions of plasmapheresis with low-dose intravenous Ig after the transplant, to prevent rebound in antibody and was discharged on prednisone, tacrolimus, MMF, SMX-TMP, valganciclovir, and warfarin. The creatinine remained initially 0.9 to 1.0 mg/dl. Two months later, she returned for routine follow-up. Although she felt well, the creatinine had risen to 1.2 mg/dl. Examination was unremarkable. The prednisone dose was 10 mg/d and the MMF dose was 2 g/d. The tacrolimus trough was 11 ng/ml and the LDH 355 (107–231). The WBC was 9.4 cells/mm^3, hemoglobin 10.4 g/dl, and platelets 332,000; no schistocytes were seen on the blood smear. The urine dipstick showed no blood or protein. Ultrasound showed no hydronephrosis.

Clinical Discussion: Dr. Colm Magee

Now we have mild acute renal allograft dysfunction, 10 wk after transplant. Again, an intrinsic renal cause seems most likely. The differential diagnosis differs somewhat from that described earlier but includes acute AMR, acute cellular rejection, acute tacrolimus nephrotoxicity, acute thrombotic microangiopathy, and BK-virus nephropathy. I am most concerned about acute
AMR; many cases we have seen in our desensitization program have had a somewhat indolent presentation, as here. Full-blown BK-virus nephropathy usually manifests later than this, but the patient has received heavy immunosuppression so it must be considered. Although the tacrolimus level is not very high, a component of acute tacrolimus nephrotoxicity is possible. However, in a high-risk patient such as this, I would not empirically reduce the dose and wait to see what happens to the plasma creatinine. The raised LDH raises the question of acute thrombotic microangiopathy, but there are no other laboratory features suggestive of this. I very much doubt there is clinically significant recurrence of IgA glomerulonephritis as the urine dipstick is bland. I suggest the following tests: crossmatch against donor cells, blood PCR for BK-virus and allograft biopsy. There is little evidence of acute pyelonephritis, but with her history, I would check a urine culture.

Pathology: Dr. Helmut Rennke
A biopsy was performed, and it showed focal neutrophil polymorphs and mononuclear cells in the peritubular capillaries (Figure 2). There was diffuse staining for C4d in these capillaries. No glomerulitis was noted. By electron microscopy, the architecture of the glomerular capillary walls was well preserved. Some peritubular capillaries revealed prominent swelling of the endothelium and layering of the basement membranes; inflammatory cells were present within the capillary lumina and in the interstitium surrounding the capillaries. There was no tubulitis or endothelialitis. These features were consistent with acute AMR. There were no features suggestive of polyoma virus infection, bacterial pyelonephritis, or recurrent IgA nephropathy.

Case Presentation: Dr. Michael Clarkson
The flow cytometry crossmatch was negative against donor T cells and positive against donor B cells. The urine culture was negative. She was diagnosed as having a relatively mild episode of acute AMR and was treated with pulse methylprednisolone and plasmapheresis. The creatinine returned to 0.9 to 1.0 mg/dl. Rituximab was administered after the course of plasmapheresis, and she was discharged on the same maintenance immunosuppression. Valganciclovir was stopped 6 mo after the transplant, but SMX-TMP was continued indefinitely. Eighteen months after transplant, she is feeling very well with a serum creatinine in the 0.8 to 1.0 mg/dl range.

Clinical Discussion: Dr. Colm Magee
This case illustrates well the high medical and immunologic complexity of certain patients receiving renal transplantation today. There are two parts to my discussion: the first focuses on desensitization to HLA and the second on acute AMR. The management of both conditions overlaps to a significant extent.

Desensitization to HLA
Sensitization to HLA is typically defined as the presence in the serum of antibody (usually IgG) to HLA. There are three main causes of this phenomenon: previous transplant, blood products, and pregnancy. Historically, the emphasis has been on sensitization to HLA-A, HLA-B (class I antigens), and HLA-DR (class II antigens), but there is growing recognition that antibodies directed against other HLA, or even non-HLA, are clinically important.

The definition of high sensitization varies; one is PRA >50%. By PRA, we are referring to the percentage of typical deceased donors against which the patient has in vitro evidence of toxic alloantibody. Importantly, the type of in vitro test used can have a major impact on the PRA result.

Highly sensitized patients form about 30% of those on the waiting list in the United States and Ireland. Complications of high sensitization include longer waiting time (some never receive a transplant from the list), limited access to living donor transplantation (because of positive crossmatches against interested donors) and, when transplant does occur, higher rates of rejection and allograft loss. Irreversible allograft loss from hyperacute and acute AMR has been of particular concern. Nevertheless, there has...
been major interest in recent years in improving access to transplantation for these patients (3). Options to improve such access include acceptable mismatch programs, paired living donor exchange, desensitization against a specific living donor, and desensitization to obtain a deceased donor transplant (3). I will concentrate here on desensitization, although I emphasize it is not the only option. Desensitization can be defined as follows:

1. In the setting of living donor transplantation: attenuating the humoral alloimmune response such that the patient becomes crossmatch negative against a specific donor.
2. In the setting of deceased donor transplantation (i.e., waiting on the list): attenuating the humoral alloimmune response, making it more likely the patient will receive a deceased donor transplant.

There are several reasons why desensitization is becoming more popular. First, there has been the general increase in living kidney donation and in second or even third transplants. So HLA-incompatible living donor pairs are asking what can be done for them! Second, we are able to diagnose acute AMR more quickly and more accurately than in the past (4). The current criteria for diagnosing acute AMR are shown in Table 1. Finally, we have more effective treatments for acute AMR (5).

Two main methods of desensitization have evolved: high-dose intravenous Ig or plasmapheresis (with low-dose intravenous Ig) (3). The high-dose intravenous Ig protocol involves administration of intravenous Ig (up to 2 g/kg) once monthly for several months (6–8). A variety of intravenous Ig preparations are available; all are pooled from multiple donors. The mechanisms of action of intravenous Ig in this setting are not fully understood. Proposed mechanisms include blockade of Fc receptors on mononuclear phagocytes, direct neutralization of alloantibodies (anti-idiotypic effects), inhibition of expression of CD19 on activated B cells, inhibition of complement, and inhibition of alloreactive T cells (9). Advantages of intravenous Ig include ease of administration, lack of immunsuppression and, with appropriate precautions, a low incidence of serious adverse effects. Disadvantages include high cost, perhaps batch-to-batch variability in efficacy, and, if high osmolality preparations are used, a risk of thrombosis and acute renal failure.

The plasmapheresis protocol typically involves plasmapheresis three times weekly, immediately followed by low doses of intravenous Ig (10–13). Daily tacrolimus and MMF are usually prescribed also. Advantages of this regimen include probable higher efficacy than intravenous Ig and a somewhat predictable time to transplant (based on the crossmatch titer). Disadvantages include high cost and the potential adverse effects of the plasmapheresis procedure itself: hypocalcemia, depletion of clotting factors, which increases the risk of bleeding especially after transplant, and reactions to fresh frozen plasma.

Desensitization to obtain a deceased donor transplant is difficult in that the availability of a compatible kidney cannot be controlled. The intravenous Ig protocol is probably more suitable in this setting as it can be continued over several months; it is difficult to continue plasmapheresis beyond several weeks.

There are no randomized controlled trials comparing the 2 protocols, but one nonrandomized study did suggest the plasmapheresis-based protocol was superior (14). Overall, short-term and medium-term results with both protocols have been encouraging. Rates of acute AMR have ranged from 7% to 80% (6,8,10,12–15). This wide range probably reflects differences in patient characteristics (such as the strength of the baseline crossmatch) and in thresholds for performing allograft biopsy. The reported incidence of adverse effects does not appear excessive. Long-term data are needed, of course, before the role of desensitization for sensitized patients can be better defined. For example, chronic rejection could be problematic, especially as there is evidence that donor specific antibody (DSA) and/or third party antibody often remain detectable, at least by sensitive techniques, after transplant in desensitized patients (16).

The Brigham and Women’s Hospital desensitization program has been in place since 2002 and has used a plasmapheresis-based protocol as described for this patient. These results, involving the transplantation of 28 patients, have recently been submitted for publication. Outcomes were promising, although the rate of acute AMR was 39% (17).

**Acute AMR**

The diagnostic criteria for acute AMR have been summarized in Table 1. Advances in C4d staining and in histocompatibility testing and the development of consensus definitions (4) have significantly improved our accuracy (and confidence) in diagnosing acute AMR.

The DSA are usually directed against HLA but, in the setting of ABO-incompatible transplant, are directed against either ABO-A, ABO-B, or both. Traditionally, class I HLA antibodies have been considered the main mediators of acute AMR, but there is increased recognition of the pathogenicity of class II HLA antibodies (1). Montgomery et al. reported that at least one third of their desensitized patients who developed acute AMR did so via class II HLA antibody alone (11). The case discussed here illustrates how troublesome class II HLA antibodies (or true-positive B-cell crossmatches) can be. The diagnosis and treatment of acute AMR due to class II HLA antibodies are similar to that for AMR due to class I HLA antibodies.

Acute AMR was a frequent complication of ABO-incompatible kidney transplantation but appears to be less common with the newer regimens being used. It is diagnosed by broadly similar criteria to those used outside the setting of ABO-incompatible transplantation, but there are some important differences. First, the noxious antibodies are naturally occurring and

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**Table 1. Criteria for diagnosis of acute AMR**

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<th>Criterion</th>
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<td>Allograft biopsy showing inflammation or thrombosis in the peritubular or glomerular capillaries or showing ATN-like minimal inflammation or showing arterial transmural inflammation/fibrinoid change</td>
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<tr>
<td>Diffuse staining of peritubular capillaries for C4d</td>
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<td>Donor specific antibody in the serum</td>
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Adapted from Solez et al. (4).
are directed against antigens of the ABO system. ABO-A and ABO-B antigens are expressed on the surface of multiple cells, including red blood cells, endothelial cells, and epithelial cells. In the laboratory, testing is not done against donor cells per se, rather, against a standard panel. Usually, acute AMR is associated with a rise in antibody (isoagglutinin) titer. Second, in the setting of ABO-incompatible transplantation, the presence of C4d alone does not imply rejection. Its presence has been well described in the setting of stable/good allograft function and in the absence of light microscopic changes of AMR (18). The reasons why C4d positivity is poorly correlated with “true” AMR in ABO-incompatible, as opposed to HLA-incompatible, renal transplantation are not clear. Interestingly, the phenomenon of accommodation appears to occur in some cases of ABO-incompatible transplantation. This is characterized by stable/good allograft function and the absence of capillaritis, in the setting of detectable isoagglutinin titers in the serum and continued expression of A/B endothelial antigen in the kidney. The mechanism of this accommodation is not well understood, but alterations in host gene expression may be important.

Occasional cases of AMR appear to be mediated by antibodies that are not directed against the “standard” HLA or ABO antigens. For example, antibodies against MICA antigens or monocyte antigens of the donor may be pathogenic (2). It’s possible that the patient discussed here had such antibodies as she had severe early rejection of her 2-haplotype matched allograft in 1991.

It is very important to liaise closely with the histocompatibility laboratory as to the best test to detect DSA in an individual patient. Different assays have very different sensitivities, and some can be affected by drugs administered to the recipient. For example, rituximab or high-dose intravenous Ig can interfere with the lymphocytoxicity and flow cytometry crossmatch against donor B cells. The rituximab effect can be circumvented by Pronase treatment of the B cells (19). Thymoglobulin can interfere with T-cell crossmatching (20). Enzyme-linked immunosorbent assay or FlowPRA tests are much less affected by administered drugs, but there is less experience with these tests in the clinical setting compared with assays using donor cells.

Acute AMR usually presents in the first few posttransplant months but can present late after transplant, especially if there is noncompliance. (By definition, severe AMR occurring within minutes to hours of the anastomosis of the allograft is termed hyperacute rejection. Hyperacute rejection requires the presence of preformed antibodies and is very rare today because of routine preoperative crossmatching.)

Acute AMR can occur in desensitized and nondesensitized recipients. Acute cellular rejection may coexist. Anecdotally, acute AMR after desensitization tends to be less severe (although more common) than that seen in nondesensitized cases (21). This could reflect effects of pretransplant immunosuppression (which is not the norm in standard transplant recipients) or the very close follow-up that desensitized patients receive (meaning earlier diagnosis of AMR). Symptoms and signs may be minimal but may include oliguria, low-grade fever, and tenderness over the allograft. The tempo of rise in plasma creatinine is variable; some cases are subclinical (identified only on protocol biopsy), whereas others present in a fulminant manner with oliguria and rapidly rising creatinine.

Treatment for acute AMR typically involves plasmapheresis (or immunoadsorption) + high-dose glucocorticoids + MMF + tacrolimus ± lymphocyte depleting antibody (5). Although only one small randomized trial has been published (22), this combination is generally considered to be the most effective in treating acute AMR and to offer much improved outcomes compared with historic controls. Intravenous Ig is usually given, either after each plasmapheresis session or as a large dose after completion of all plasmapheresis sessions. The rationale for administration of intravenous Ig is 1) to further suppress alloimmune responses and 2) to replenish IgG removed by plasmapheresis. Plasmapheresis is usually continued until the testing for DSA is negative, or, in the setting of ABO incompatibility, until the isoagglutinin titer is low positive only. Note, however, that the sensitivity of the tests for donor specific antibody and for isoagglutinin titer can vary significantly.

Overall, the regimen for treating acute AMR appears to be well tolerated, and serious complications of treatment seem uncommon. Of course, adverse effects of heavy immunosuppression remain a concern. Coagulopathy due to plasmapheresis can lead to serious bleeding in the early postoperative period or after renal biopsy, so it is important to adequately replace with fresh frozen plasma.

There is no uniform definition of what constitutes “complete reversal” of acute AMR, but presumably this would incorporate: 1) return of GFR to baseline, 2) negative serologic testing for DSA or other antibody, and 3) resolution of histopathologic changes. In practice, repeat biopsy is not essential if points 1 and 2 are achieved. Resistant acute AMR can be defined as ongoing allograft dysfunction associated with persistent histopathologic changes of AMR and continued demonstration of DSA in the serum. Note that the presence of DSA alone (without allograft dysfunction and histopathologic changes), while of concern, does not constitute resistant acute rejection. Continued presence of DSA in the setting of stable allograft function has been reported. Therapies for resistant AMR have not been well studied, but options include continued plasmapheresis, high-dose intravenous Ig, and rituximab. Small series have reported success with splenectomy (23), the rationale being that this rapidly removes a large number of plasma cells. It is important to weigh carefully the risks and benefits of further aggressive therapies in patients with resistant rejection, particularly when the repeat biopsy shows significant irreversible damage.

In conclusion, there have been major improvements recently in desensitization and in the management of AMR. Patients such as the one described herein can now be transplanted in specialist centers, but it is important to remember that the protocols being used are still in evolution and that the transplants still remain relatively high risk.
Questions

Dr. Ajay K. Singh, Brigham and Women’s Hospital, Harvard Medical School: Thank you, Colm, for an excellent discussion. You talked briefly about your experience in the Brigham with desensitization of patients. Have you, or anyone else, looked at the predictors of lack of response to the desensitization protocol or of antibody-mediated rejection among those patients that have undergone desensitization?

Dr. Colm Magee: Yes that has been an important question. Colleagues at John’s Hopkins have the largest desensitization experience in the world and have reported that the most difficult patients to desensitize (with the plasmapheresis protocol) are those with high baseline titers or reactivity to certain HLA-DR antigens or where the intravenous Ig protocol has failed (11,24). The experience from both Hopkins and Brigham is that the risk of acute AMR is highest in those with multiple previous sensitizing events, very high PRA, and very high baseline titers (especially to class II HLA) (11,17).

Dr. Ajay K. Singh: My second question. Why have there not been any randomized clinical trials looking at the efficacy of different desensitization protocols? We have reasonable surrogate endpoints such as the antibody titer, we have hard endpoints in terms of survival of the allograft, and we have a fairly robust patient population.

Dr. Colm Magee: Desensitization protocols have developed in a number of centers in the United States and elsewhere, but these have not been based on the results of randomized controlled trials. I think that there is certainly room for more collaboration, especially since the numbers at any individual center are fairly small. One important question, for example, is the efficacy and safety of the plasmapheresis versus the high-dose intravenous Ig protocol. There was one trial from the Mayo Clinic that compared the two protocols, but it did not use a randomized design (14). However, it did report superior outcomes with plasmapheresis. We also don’t know the value of rituximab in this setting or which induction agent to use. So, I agree, a lot of work needs to be done.

Dr. Peter Conlon, Consultant Nephrologist, Beaumont Hospital: I wanted to comment on the use of a desensitization protocol as it relates to deceased donor transplantation. Here in Ireland, we have experience with 13 patients over 3 to 4 yr. One of the biggest difficulties with the process is the issue of balancing utility and equity. With 30% of our waitlisted patients being highly sensitized (over 100 patients in Ireland), we can’t offer desensitization to all patients. Desensitization is expensive and is not medically appropriate for everyone. Another issue is the high attrition rate. Looking at our outcomes with about 2.5 yr of follow-up, we have observed about a 65% allograft survival rate. To my mind, this represents poor use of a deceased donor kidney. So I think it would be very important to see what the long-term survival would be with desensitized living donor transplant recipients. Finally, while we used a hybrid of the Hopkins and Cedars Sinai desensitization protocols for our deceased donor program, the logistical issues with regards to the timing of plasmapheresis were challenging; the surgeons understandably did not like plasmapheresis to be performed immediately before the transplant.

Dr. Colm Magee: Peter, you raise some important issues. I agree that balancing utility and equity is challenging in any deceased donor program and more so if it involves desensitization. One advantage of a living donor desensitization program is that you are effectively adding a kidney to the donor pool and not depriving anyone on the waitlist of a kidney. I believe this avoids the concern you have about appropriate use of the limited supply of deceased donor kidneys. Of course, it is essential to inform potential living donors of the relatively high risk that their donated kidney could be severely rejected or even lost in the first few post-transplant weeks.

Another advantage of a living donor desensitization program is that the logistics are easier. The plasmapheresis and the surgery can, to a significant extent, be planned and coordinated together. Perioperative ischemia time can still be minimal. And the patient only needs a limited number of days to weeks of immunosuppression before the transplant, unlike in the deceased donor setting, where the timing of the transplant is unpredictable.

Your point about attrition is well taken. Although the short-term and medium-term outcomes with desensitization and transplantation across a positive crossmatch have been encouraging, longer-term outcomes are not yet available. I am concerned that chronic antibody-mediated rejection will be problematic. There is accumulating evidence that the presence of alloantibody after transplant is associated with poorer outcomes in the general transplant population (25), and we know that alloantibody remains detectable in some patients after desensitization and transplantation (16). Furthermore, Gloo et al. reported results of protocol biopsies performed at 12 mo in HLA-incompatible, ABO-incompatible, and conventional allografts (16). All three groups had relatively low rates of chronic histologic changes, a somewhat reassuring finding. However, transplant glomerulopathy, a form of injury that has been associated with chronic antibody mediated damage, was more common in HLA-incompatible recipients. On a more positive note, some patients have clearly benefited greatly from this procedure. For example, the first patient desensitized and transplanted in the Brigham is more than 5 yr out with a much improved quality of life and a plasma creatinine of 0.8 mg/dl.

Dr. Yvonne O’Meara, Consultant Nephrologist, Mater Hospital: How do you handle the issue of a persistent low titer positive crossmatch pretransplant in a recipient who has undergone your desensitization protocol? I had a patient who was transplanted and had detectable donor specific antibodies by the Lumixen assay but not by the standard crossmatch assay. Could you discuss the predictive value of the different antibody assays? Are some tests too sensitive?

Dr. Colm Magee: Our protocol specified that a negative lymphocytotoxicity crossmatch (by antihuman globulin method) against donor T cells and a negative or near-negative lymphocytotoxicity crossmatch against donor B cells (by modified Amos method) were required before proceeding with the transplant. We did not aim for a negative flow cytometry crossmatch or negative antigen specific tests as we felt these tests were very sensitive and achieving negative results would have been very difficult. That is not to say that we thought the
presence of low levels of donor specific antibody was not clinically significant. It’s reasonable to assume that low levels at the time of transplant adversely affect outcome, but the question is to what extent? Ultimately, you’ve got to decide what’s best for the individual patient and obtain informed consent.

Dr. Ajay K. Singh: As you know there is a so-called “sponge theory” with regards to the failed allograft kidney and alloantibody levels. In a patient with a failed allograft, who has resumed dialysis, do you remove the kidney with the risk that the panel reactivity may go high and make it even more challenging to desensitize? Do you leave the kidney in? What do you make of the theory that the failed allograft functions as “a sponge” adsorbing antibodies?

Dr. Colm Magee: This is an interesting topic. We have seen several cases at both the Brigham and Women’s and Beaumont Hospitals, where removal of the allograft was associated with an increase in PRA. In one case, the patient developed a positive crossmatch against their intended living donor and this crossmatch had previously been negative.

The theory is that the allograft is acting as a sponge, adsorbing the low levels of alloantibody being produced. Removal of the allograft means that the alloantibody is now not adsorbed and plasma levels rise. And perhaps the nephrectomy surgery itself (with release of antigens into the circulation and sometimes the need for transfusion of blood products) also stimulates an immune response. The counterargument to this is that the need for allograft nephrectomy is merely a marker of severe rejection and an already activated immune system. And then after nephrectomy, the immunosuppression is often abruptly stopped. My guess is that it’s a combination of all of the above. Having said that, I would not let these theories greatly influence my decision as to whether or not an individual patient needed an allograft nephrectomy. As it’s not a benign procedure, I reserve it for patients who are requiring significant amounts of immunosuppression to control the rejection while on dialysis or who are having complications from the rejection (such as fever or gross hematuria).

Dr. Michael Clarkson, Consultant Nephrologist, Cork University Hospital: I agree that there is substantial anecdotal evidence to support a sponge phenomenon; however, I doubt that this is plausible from an immunologic standpoint. In many of these patients, this may simply reflect discontinuation of immunosuppression.

Dr. Peter Conlon: Can I ask you about you about the New England Organ Bank Sharing program? We would be interested in exploring this in Ireland. What type of legislative framework do you need to introduce this? Were specific laws brought in? Could you provide us more details on this program? As well, are both transplantations done simultaneously—i.e., there is no issue with one party acting out?

Dr. Colm Magee: My understanding is that no new laws were introduced or needed to start the program within New England. Rather, the New England Program for Kidney Exchange was formed through a consensus of New England Organ Bank centers. However, I believe legislation has been organized at the federal level to allow national paired donation. You are correct that the donor and recipient surgery occurs at the same time for both pairings; that’s very important. I believe it’s coordinated by phone calls between operating rooms. The website of New England Program for Kidney Exchange provides a lot of useful information on kidney exchange.

Dr. Paul Phelan, Nephrology Registrar, Beaumont Hospital: If the pretransplant crossmatch had been negative in this patient, would you still have done plasmapheresis in the postoperative period?

Dr. Colm Magee: Yes, absolutely, because of the problem of rebound in alloantibody. This could be due to return of IgG from the extravascular space into the vascular space (i.e., equilibration) and, more importantly, continued production of donor specific antibody. Indeed, production of donor specific antibody likely increases due to the intense antigenic stimulus of the allograft. The higher the titer of alloantibody before desensitization, presumably the higher is the likelihood of a rebound. Thus, we routinely do plasmapheresis after transplantation of a desensitized patient, at least 2 sessions. But if the baseline titers are high, as here, we would do more than 2 sessions. I want to emphasize, however, that plasmapheresis after transplant is not a benign procedure. Its complications include bleeding and hypotension. Also, induction antibodies, such as basiliximab or thymoglobulin, will be removed by plasmapheresis.

Dr. Peter Conlon: Do you routinely measure donor specific antibody after transplant to guide plasmapheresis afterwards? Did you see positive antibodies but no clinical syndrome of rejection? What about the role of protocol kidney biopsies?

Dr. Colm Magee: We did not routinely measure donor specific antibody after transplant, but some centers have reported success with that strategy (14). Yes, donor specific antibody can be detectable in the absence of overt rejection (16). We simply prescribed plasmapheresis according to the baseline crossmatch titer. We did not do protocol kidney biopsies, but in practice we had a very low threshold for performing them, for example, an increase in plasma creatinine of 0.2 mg/dl or more.

Dr. Denise Sadlier, Consultant Nephrologist, Mater Hospital: Was the pyelonephritis in the case you presented just bad luck? And second, did it occur because of over-immunosuppression?

Dr. Colm Magee: This was the only case of pyelonephritis in our series. This patient had no further episodes of pyelonephritis. I suggest that she developed this infection from both the effects of several weeks of immunosuppression and plasmapheresis and the presence of the bladder catheter and ureteric stent.

Dr. Ajay K. Singh: Monitoring of CD19+ cells in the peripheral blood is being used to evaluate responsiveness to rituximab. Do you use CD19 monitoring? Also, could the 39% rate of AMR reflect inadequate dosing of rituximab? Could you discuss the rationale for rituximab, the protocol that you use, and whether you use higher doses of rituximab in patients at higher risk?

Dr. Peter Conlon: We used 4 doses of rituximab in patients undergoing desensitization for a deceased donor transplant.

Dr. Colm Magee: We monitored CD19 positivity in selected patients, to “ensure” that dosing was adequate. There are no randomized controlled trials to support the use of rituximab in
this setting, but the rationale is that it eliminates the B cells that ultimately transform into plasma cells producing donor specific antibodies. Rituximab might also affect the alloimmune response in other ways. Typically, we reserved rituximab (at 1 to 2 doses of 375 mg/m²) for patients being desensitized who had high baseline titers against their donors. We also used it post-transplant at the same dose in those with severe or resistant AMR. Rituximab has the reputation of being a relatively safe drug, but I think it’s prudent to be careful with its use in patients who are already receiving plasmapheresis, interleukin-2 receptor blockade, steroids, MMF, and tacrolimus.

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Disclosures
None.

References
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