Transplantation of ABO A2 kidneys into O recipients: do IgM anti-A1 titers matter?

Tierney J, Shaffer D. Transplantation of ABO A2 kidneys into O recipients: do IgM anti-A1 titers matter?

Abstract: Background: The ABO blood subgroup A2 expresses lower levels of A antigen on the cell surface and is less immunogenic toward anti-A immunoglobulin present in blood type O or B recipients. Previous studies have shown successful kidney transplantation from A2 donors into O or B recipients with low pre-transplant anti-A titers. Previous studies suggest good results with recipient IgG titers <1:8. Few studies have specifically evaluated the importance of anti-A1 IgM titers on early outcomes following A2 to O or B kidney transplantation. Methods: We performed a single center, retrospective review of all A2 to O living donor kidney transplants. All recipients had pre-transplant anti-A IgG titers <1:8. IgM titers were measured in all recipients and were reported but not used to determine eligibility for transplant. Results: From 2001 to 2013, we performed seven consecutive A2 to O living donor kidney transplants. Early allograft dysfunction, acute rejection or thrombotic microangiopathy, occurred in four patients and were associated with high IgM titers despite low IgG titers. Conclusions: Our data show a high incidence of early acute rejection or thrombotic microangiopathy in A2 to O kidney transplants with high recipient anti-A IgM titers despite low IgG titers. Steps to lower anti-IgM pre-transplant may reduce the risk of early allograft dysfunction in A2 to O or B kidney transplants. Attention should be paid to IgM titers in establishing individual center selection criteria for A2 to B kidney transplants under the new UNOS kidney allocation system.

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The ABO blood subgroup A2 is known to express lower levels of blood group A antigen on the cell surface and is less immunogenic toward anti-A1 immunoglobulin present in serum of blood type O and B individuals (1, 2). Previous studies have shown successful kidney transplantation from ABO A2 or A2B donors into O or B recipients who have low pre-transplant anti-A titers (3–8). Some centers have reported higher early graft failure with IgG titers >1:8 (3, 4, 7), while others found no correlation between IgG titers and acute rejection or allograft loss (5). Published selection criteria for A2 to O or B kidney transplants similarly vary, from not using recipient anti-A titers to determine suitability for transplant (5) to selecting recipients with low anti-A IgG titers (<1:8) regardless of IgM titers (6–8). Another series included both IgG and IgM in selection criteria, performing plasmapheresis (PP) peri-operatively on patients with an IgM/IgG titer ≥1:8 and excluding patients with IgM/G > 1:64 (3). Few studies have specifically evaluated the importance of anti-A1 IgM titers on early outcomes including rejection and graft survival.

Patients with blood type B and O also have longer wait times to kidney transplant than blood group A patients once listed. Proponents of A2 to B or O kidney transplantation promote it as a strategy to increase organ availability, particularly to minorities who are disproportionately represented in the B blood group (6, 8). To that end, the new UNOS kidney allocation system (KAS) recently implemented in December, 2014 incorporated A2 to B allocation (non-A1 or non-A1B to B). The UNOS KAS does not provide anti-A titer criteria for A2 and A2B kidneys into B recipients. The UNOS KAS does not provide anti-A titer criteria for A2 to B transplants but states that each transplant program must establish a written policy regarding its program’s titer threshold for transplanting A2 and A2B kidneys into B recipients (9). We report a high incidence of early acute rejection (AR) or thrombotic microangiopathy (TMA) in A2 to O living donor transplant recipients with low IgG titers but high IgM titers and suggest that anti-A1 IgM titers may be important in predicting
the early success of A2 to O or B kidney transplants.

Methods

Study design

A single center, retrospective review of all A2 to O living donor kidney transplants.

Anti-A1 titers

We require anti-A1 IgG titers \(<1:8\) without pre-transplant conditioning to proceed with A2 to O or B transplants at our center. Recipients with anti-A IgG \(\geq 1:8\) are turned down for transplant from A2 donors. All recipients had anti-A1 titers measured using dithiothreitol (DTT) to precipitate IgM antibodies from the serum to isolate and measure levels of IgG antibodies. DTT pre-treatment consisted of one part of 50 mM DTT to nine parts of recipient serum incubated for 30 min at 37°C in waterbath. Untreated and DTT-treated sera are then serially diluted using twofold dilutions from 1:1 to 1:128. One drop of a 2% RBC cell suspension of A1 cells is added to a set of dilutions containing 80 µL of each dilution. The tubes are incubated for 30 min at room temperature, centrifuged, and then read for agglutination. Serum not treated with DTT contains both IgG and IgM components. Recipient IgM/IgG (i.e., untreated) titers were routinely measured and reported but not used in determining eligibility for transplant.

Immunosuppressive protocol

Immunosuppression consisted of antibody induction with either Thymoglobulin 6 mg/kg total dose over four d (first four patients) or a single dose of alemtuzumab 30 mg intravenously started intra-operatively (last three patients), a three-d methylprednisolone taper, and maintenance immunosuppression with cyclosporine (one patient) or tacrolimus (six patients), mycophenolate mofetil, and prednisone.

This study was approved by the Vanderbilt Institutional Review Board.

Results

In the period from 2001 to 2013, we performed seven A2 to O living donor kidney transplants at our institution. Patient demographics and pre-transplant anti-A1 antibody titers are summarized in Table 1. Pre-transplant IgG titers were \(<1:8\) in all recipients per protocol. Pre-transplant IgM/IgG titers ranged from 1:4 to 1:128.

Patient outcomes are summarized in Table 2. All patients transplanted had pre-transplant IgG anti-A1 titers \(<1:8\) per our protocol. Four patients had early allograft dysfunction with acute rejection (AR) or thrombotic microangiopathy (TMA) or both (mean seven d post-transplant; range 2–16 d). Of these 4 patients, three had IgM/IgG titers \(>1:8\) and one had IgM/IgG = 1:8.

Patient #1 had accelerated acute rejection with cortical necrosis unresponsive to IVIG, OKT3, and conversion from cyclosporine to tacrolimus and underwent transplant nephrectomy on POD 14. Anti-A titers were done at the time of acute rejection and had increased to 1:128. C4d staining and donor-specific antibody (DSA) measurements were not done.

Patient #3 had acute cellular rejection CCTT Type I and acute vascular rejection CCTT Type II, C4d positive, with creatine 3.0 mg/dL on POD#16. Neither anti-A titers nor DSA were measured at time of rejection episode. He was treated with intravenous methylprednisolone with good response but died in a motor vehicle accident with
a functioning graft and creatinine 1.7 mg/dL six months post-transplant.

The two most recent patients (#6 and #7) had low anti-A IgG but with high IgM/IgG pre-transplant and early allograft dysfunction on POD #3 and POD#2, respectively, with TMA consistent with antibody-mediated rejection, hyperacute rejection, or calcineurin inhibitor toxicity. Both had positive C4d staining on biopsy. Anti-A titers were low (IgG/IgM 1:8, IgG 1:2), and DSA was undetectable at time of biopsy in patient #7. Neither anti-A titers nor DSA were available prior to treatment in patient #6 although DSA was measured post-treatment on two occasions and was undetectable. Both patients were treated with five d of PP followed by a single dose each of IVIG 2 mg/kg and rituximab 740 mg IV with excellent responses. Patient #6 had an excellent response to PP, IVIG, and rituximab but died with a functioning graft with creatinine 1.4 mg/dL five months post-transplant. Patient #7 has excellent graft function 12 months post-transplant.

Of the three patients without rejection, only one had IgM/IgG titers > 1:8.

Discussion

Our data suggest that anti-A1 IgM titers are important in predicting the early success of A2 to O living donor kidney transplantation. There has been a relative paucity of data on the role or importance of IgM titers on A2 to O or B kidney transplantation. Nelson et al. (7, 8) have pioneered the use of A2 donors, both cadaveric and living, in non-A recipients. The cohort of recipients with IgG titers <1:8 did well with 94% graft survival at one and two yr. But in their earliest series before excluding patients with high anti-A1 IgG titers, they reported a 55% rate of early non-function in patients with high titers, that is, IgG > 1:8. Interestingly, all the recipients with early graft dysfunction were type O, whereas 19% of the type B recipients had titers >1:8 and all had early graft function. This suggests that type O recipients may have had an anti-A1 IgM/IgG component responsible for early graft dysfunction. The IgM/IgG titers were not reported in this series.

Norman et al. (3) is the first group to suggest the importance of the anti-A1 IgM/IgG titer as part of their protocol for A2 to O/B kidney transplantation. In addition to excluding patients with anti-A1 IgG titers greater than or equal to 1:8, they also excluded patients with anti-A1 IgM/IgG titers >1:64 and pre-conditioned patients with PP who had IgM/IgG titers between 1:8 and 1:64. In this series of 19 recipients, four (21%) had graft loss. The first patient in the series did not receive PP with IgM/IgG titers of 1:64 and had hyperacute rejection within 36 h.

A subsequent report by Ishida et al. (10) highlighted the role of IgM in causing rejection in ABO-incompatible grafts. They reported high titers of both IgG and IgM in 12 patients who lost grafts due to humoral rejection, whereas eight stable patients had elevated IgG but low IgM titers. Moreover, they found that high-dose IVIG blocked anti-blood type antibodies, especially IgM.

We have subsequently performed two transplants from A2 donors into B recipients with low pre-transplant IgG titers but high IgM/IgG titers of 1:64 treated with peri-operative PP and single doses each of high-dose IVIG and rituximab. These had an excellent early post-transplant course without rejection or other allograft dysfunction.

There are several weaknesses to our study. First, this is a retrospective review of a small number of patients. As such, neither anti-A titers (IgM/IgG and IgG) nor DSA was available at the time of rejection or biopsy in all patients. A previous case report showed humoraly mediated vascular rejection POD #9 in an A2 to O living donor pediatric transplant in the setting of rising anti-A titers and recommended early post-transplant monitoring of titers (11). We plan on performing prospective post-transplant anti-A monitoring of A2 to B

**Table 2. Patient outcomes**

<table>
<thead>
<tr>
<th>Pt</th>
<th>IgG titer</th>
<th>IgM/IgG titer</th>
<th>Biopsy?</th>
<th>Path</th>
<th>Intervention</th>
<th>Transplant outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1:4</td>
<td>1:32</td>
<td>POD#8</td>
<td>ACR/AVR</td>
<td>OKT3, IVIG</td>
<td>Transplant nephrectomy POD #14</td>
</tr>
<tr>
<td>2</td>
<td>1:4</td>
<td>1:128</td>
<td>4 1/2 yr</td>
<td>Focal ATN</td>
<td>None</td>
<td>Functioning</td>
</tr>
<tr>
<td>3</td>
<td>1:4</td>
<td>1:8</td>
<td>POD#16</td>
<td>ACR/AVR/C4d+</td>
<td>Methylprednisolone</td>
<td>Death with functioning graft</td>
</tr>
<tr>
<td>4</td>
<td>1:4</td>
<td>1:8</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>Functioning</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>1:4</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>Functioning</td>
</tr>
<tr>
<td>6</td>
<td>1:2</td>
<td>1:64</td>
<td>POD#3</td>
<td>TMA/C4d+</td>
<td>PPx5, IVIG, rituximab</td>
<td>Death with functioning graft</td>
</tr>
<tr>
<td>7</td>
<td>1:2</td>
<td>1:32</td>
<td>POD#2</td>
<td>TMA/C4d+</td>
<td>PPx5, IVIG, rituximab</td>
<td>Functioning</td>
</tr>
</tbody>
</table>

ACR, acute cellular rejection; AVR, acute vascular rejection; TMA, thrombotic microangiopathy; PP, plasmapheresis; IVIG, intravenous immune globulin.

Do IgM anti-A1 titers matter?
deceased donor kidney transplants under the new UNOS KAS. Second, although all recipients in our series were unsensitized with 0% PRA and thus felt to be at low risk for early or accelerated anti-HLA antibody-mediated rejection, post-transplant DSA prior to rejection treatment was available in only one patient.

Nevertheless, our experience with seven consecutive A2 to O living donor kidney transplants suggests the importance of anti-A1 IgM/IgG titers in predicting early graft dysfunction and graft survival in A2 to O/B recipients. This has immediate relevance as the new KAS has begun to allocate A2 deceased donor kidneys to type B recipients (9). There currently are no antibody titer guidelines published in the new KAS, but each center will have the responsibility of listing B recipients based on their own selection criteria and their interpretation of the importance of recipient pre-transplant anti-A titers. Our data support that of Norman et al. (3) in suggesting a protocol that serially monitors anti-A1 antibodies in potential recipients with reasonable cutoffs for both IgG and IgM/IgG titers and PP or pharmacologic means of lowering antibody levels in those with IgM/G ≥ 1:8. While the available data including our series support an anti-A1 IgG titer threshold of <1:8, the safe IgM/IgG titer has yet to be defined. Our data suggest an increased risk of early allograft dysfunction in A2 to O kidney transplants in recipient with high (≥1:8) pre-transplant IgM/IgG titers.

In conclusion, although good long-term outcomes were achieved, our data show a high incidence of early AR and/or TMA and suggests caution in transplanting A2 kidneys into O recipients with high anti-A IgM titers despite low IgG titers. Steps to lower anti-IgM pre-transplant may reduce the risk of early AR or TMA. Attention should be paid to IgM titers in establishing individual center selection criteria for A2 to B kidney transplantation under the new UNOS KAS.

Authors’ contributions
Joshua Tierney and David Shaffer: Participated in research design, performance of the research, data analysis, and writing of the paper.

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