

## Case Report

# ABO Incompatible High-Titer Renal Transplantation without Splenectomy or Anti-CD20 Treatment

Dorry L. Segev<sup>a</sup>, Christopher E. Simpkins<sup>a</sup>, Daniel S. Warren<sup>a</sup>, Karen E. King<sup>b</sup>, R. Sue Shirey<sup>b</sup>, Warren R. Maley<sup>a</sup>, J. Keith Melancon<sup>a</sup>, Matthew Cooper<sup>a</sup>, Tomasz Kozlowski<sup>a</sup> and Robert A. Montgomery<sup>a,\*</sup>

Departments of <sup>a</sup>Surgery, <sup>b</sup>Pathology, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA  
\*Corresponding author: Robert A. Montgomery, rmonty@jhmi.edu

**Most successful protocols for renal transplantation across ABO incompatible (ABOi) barriers have utilized splenectomy as part of the pre-conditioning process. We recently described successful ABOi transplantation using anti-CD20 monoclonal antibody in lieu of splenectomy. In the current study, we hypothesized that plasmapheresis (PP) and low dose CMV hyperimmunoglobulin (CMVlg) alone would be sufficient to achieve successful engraftment of ABOi kidneys. We describe four blood type incompatible patients who received live donor renal transplants from A1 (two patients), A2 (one patient), and B (one patient) donors. All patients started with antihuman globulin (AHG) phase titers of 64 or higher and were pre-conditioned with PP/CMVlg but not splenectomy or anti-CD20. All 4 patients underwent successful transplantation and have a mean current serum creatinine of 1.1 (range: 0.9–1.2). There were no episodes of antibody mediated rejection. Rapid allograft accommodation may limit the need for long-term antibody suppression provided by splenectomy or anti-CD20, thereby eliminating the added infectious risk of these modalities and removing another disincentive to ABOi transplantation.**

**Key words:** B cells, flow cytometry cross-matching, hyperacute rejection, IVIG, kidney transplantation, plasmapheresis

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## Introduction

The early experience with ABO incompatible (ABOi) renal transplantation yielded poor results with most grafts being lost rapidly to antibody mediated rejection (1). Since 1985, several groups have reported improved outcomes

of ABOi renal allografts using protocols that included pre-transplant plasmapheresis (PP), splenectomy, and heavy immunosuppression to control isoagglutinin titers while the recipient was accommodating the incompatible graft (2–6). In two important papers by Alexandre et al., rapid graft loss was universal among the ABOi recipients who did not undergo splenectomy as a part of the pre-conditioning therapy (2,3). Following this report, splenectomy became a standard component of ABOi transplantation protocols.

Splenectomy, however, remains one of the most significant impediments to wider acceptance of ABOi transplantation by patients and their physicians (7). Avoidance of splenectomy and its accompanying life-long risk of blood born infections has been possible in select cases of patients with A2 donors. Blood group A has 2 subgroups. The A1 phenotype consists of both a qualitative and quantitative increase in A antigen expression on the renal endothelial surface. Blood group B antigen expression is intermediate between A1 and A2. In low-titer recipients of A2 organs, splenectomy and in some instances pre-conditioning may not be required (8–10). However, transplantation of A2 kidneys into recipients with anti-A titers  $\geq 32$  without pre-conditioning or splenectomy has been associated with excess graft loss (4). More recently, patients with non-A2 donors, or high-titer patients with A2 donors, have had pre-conditioning regimens that included anti-CD20 monoclonal antibody (rituximab) without splenectomy (11,12). The advantage of anti-CD20 over splenectomy is that it ablates the B-cell compartment during the period when the risk of graft loss from antibody-mediated rejection (AMR) is greatest, and then allows the humoral immune system to reconstitute with an intact spleen.

In contrast with the Japanese groups (13), the centers performing non-A2 ABOi transplantation in the US have continued PP after the transplant with a goal of maintaining anti-blood group antibody titers  $\leq 32$  during the first post-operative week (4,11,14). We hypothesized that long-term B-cell ablative techniques like splenectomy or anti-CD20 might not be necessary to achieve successful engraftment when using protocols that combine prolonged post-transplant PP with current maintenance immunosuppressive regimens containing anti-B-cell activity. By preventing early anti-blood group antibody rebound, the protocol described in this study may prevent AMR during the first 2 or 3 weeks post-transplant when it is most likely to lead to graft loss. In this report, we describe successful engraftment of

four ABOi kidneys from A1, A2, and B donors using PP and low dose CMV hyper-immunoglobulin (CMVlg) without splenectomy or anti-CD20, despite high initial anti-A or anti-B titers. We provide evidence that in some instances the ABOi barrier can be crossed with pre-conditioning regimens that are less intensive and immunosuppressive.

**Methods**

**Patient selection**

The 4 patients reported in this series were each referred for evaluation to the Incompatible Kidney Transplant Program of the Comprehensive Transplant Center at the Johns Hopkins Hospital. In each case the only available living donor patient was ABOi. All patients had a negative flow cytometric crossmatch with their donor. During the study period, a total of 8 patients underwent ABOi transplantation. Four patients were excluded from the study. Of these, one patient had a preexisting splenectomy due to trauma, and the other 3 patients had A1 donors and anti-A titers of 256. Additionally, one of the three was a blood group O and had an A1B donor.

**Desensitization protocol**

Following informed consent, all 4 patients were treated with a standardized protocol approved by the Johns Hopkins Institutional Review Board which included pre- and post-transplant PP/CMVlg (Figure 1). Quadruple, sequential immunosuppression was administered at the time PP/CMVlg was initiated. The number of pre-transplant treatments was dependent on the starting isoagglutinin titer and the goal of preconditioning was to reduce titers to ≤ 16. The protocol used in this study differs from the one previously reported as no anti-CD20 or splenectomy was utilized and post-transplant anti-A or anti-B antibody titers were maintained below 16 by extending the planned PP/CMVlg treatments beyond 2 weeks (11,14).

**PP/CMVlg**

PP treatment was performed using a COBE Spectra (COBE 2997, COBE BCT, Lakewood, CO) centrifuge-driven cell separator. One plasma volume was removed and 100% of the volume removed was replaced with either 50% crystalloid/50% albumin (5% solution) or 100% albumin (5% solution), depending on clinical assessment of optimal replacement fluid. FFP was used in some patients when an invasive procedure was planned or performed within a 24-h period of a PP treatment. Immediately following each PP treatment, patients received 100 mg/kg (dry weight) of CMVlg (CytoGam, Medimmune, Gaithersburg, MD).

**Immunosuppression**

All patients received induction with daclizumab (2 mg/kg initial dose and 1 mg/kg every 2 weeks for 5 total doses). Steroids were used perioperatively, including dexamethasone 100 mg intraoperatively and 25 mg every

6 h for 6 doses postoperatively, followed by prednisone 30 mg/day. Tacrolimus was administered initially at 0.1 mg/kg/day, and titrated to target levels of 10-12 ng/mL. Prednisone was reduced to 20 mg/day when therapeutic tacrolimus levels were achieved, and slowly tapered thereafter. Mycophenolate mofetil (MMF) was given at 2 gm/day in divided doses. Tacrolimus and MMF were started when preoperative PP/CMVlg was initiated (Figure 1).

**Biopsies and histology**

Surveillance biopsies were obtained at 1, 3 and 6 months after transplantation, and when clinically indicated by rising serum creatinine or decreasing urine output. In addition to routine light microscopy, all specimens were evaluated by immunofluorescence for C4d by a standard protocol, as previously described (15). Histologic criteria for AMR included the presence of diffuse linear C4d deposition in peritubular capillaries (PTC) in association with margination of polymorphonuclear leukocytes in PTCs.

**Results**

Four patients aged 42–72 underwent ABOi transplantation with isoagglutinin titers of 64 or greater by antihuman globulin (AHG) (Table 1). Three recipients were blood type O and one recipient was blood type A. Three donors were non-A2 blood type (2 A1 and 1 B). The kinetics of blood group antibody reduction, PP/CMVlg treatments administered, and serum creatinines for each patient are presented in Figure 2. After 4 or 5 preoperative PP/CMVlg treatments, anti-A or anti-B titers were 16 or lower at the time of transplant in each case. Between 5 and 8 PP/CMVlg treatments were administered by protocol postoperatively. Mean serum creatinine at 1 week was 1.1 (range: 0.8–1.6)

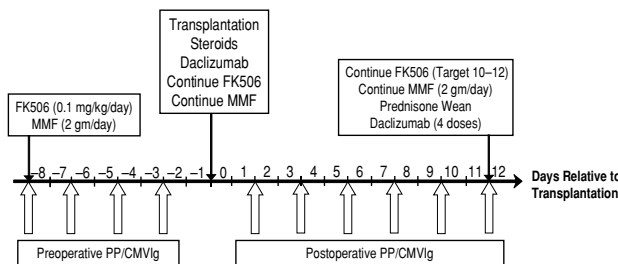
**Table 1:** Clinical characteristics and outcomes

Patient no	1	2	3	4
Recipient age	72	50	42	60
Cause of renal failure	FSGS	Thin BM	NS	HTN
Donor blood type	A2	A1	A1	B
Recipient blood type	O	O	O	A
No of previous Tx	0	0	2	0
Isoagglutinin titer, initial	64	64	128	64
Isoagglutinin titer, at Tx	2	8	16	2
Isoagglutinin titer, current	4	8	4	2
PP/CMVlg # preoperative Rx	4	5*	5	4
PP/CMVlg # postoperative Rx	5	8	7	6
Creatinine, 1 week	0.9	0.8	1.6	0.9
Creatinine, 1 month	1.1	0.9	1.1	0.9
<b>Creatinine, current</b>	<b>1.2</b>	<b>1.0</b>	<b>1.2</b>	<b>0.9</b>
Episodes of AMR	0	0	0	0
Episodes of cellular rejection	0	0	1**	0
Follow-up (weeks)	46	29	11	10

FSGS, focal segmental glomerulosclerosis; thin BM, thin basement membrane disease; NS, nephrosclerosis; HTN, hypertension; Tx, transplant; Rx, treatment. Titer, anti-A or anti-B antihuman globulin (AHG) phase titer; AMR, antibody mediated rejection.

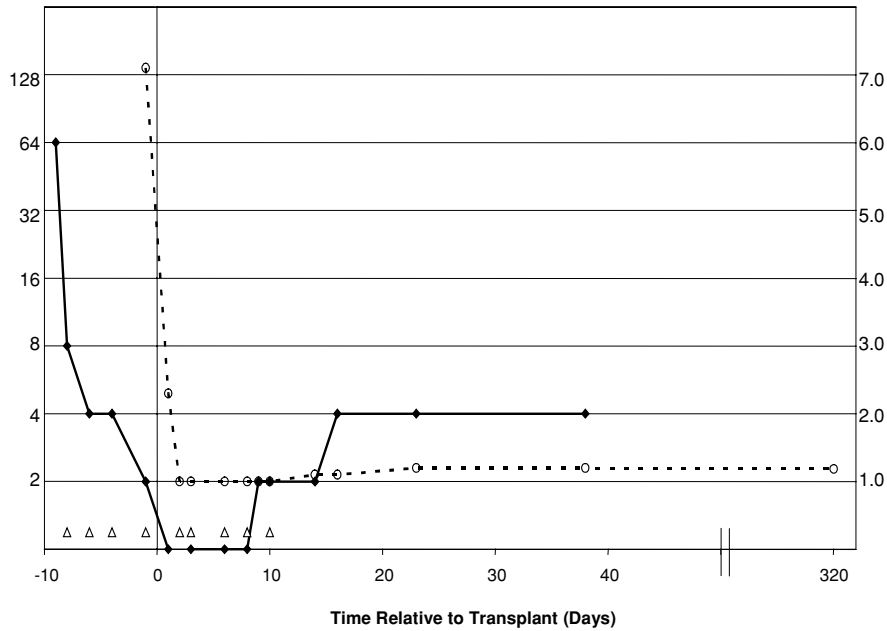
\*Patient underwent 4 PP treatments, developed line sepsis requiring cessation of desensitization, and rebounded to a titer of 64 before undergoing five preoperative treatments.

\*\*Banff 2A rejection diagnosed on a 1-month surveillance biopsy.

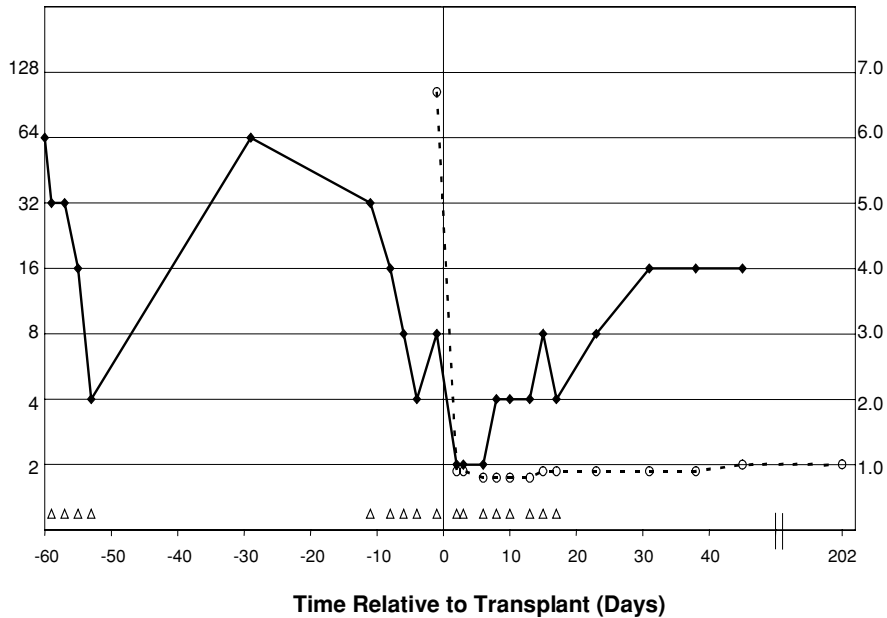


**Figure 1:** Schematic illustrating treatment protocols for ABO incompatible transplantation without splenectomy or anti-CD20 treatment.

Patient 1.



Patient 2.



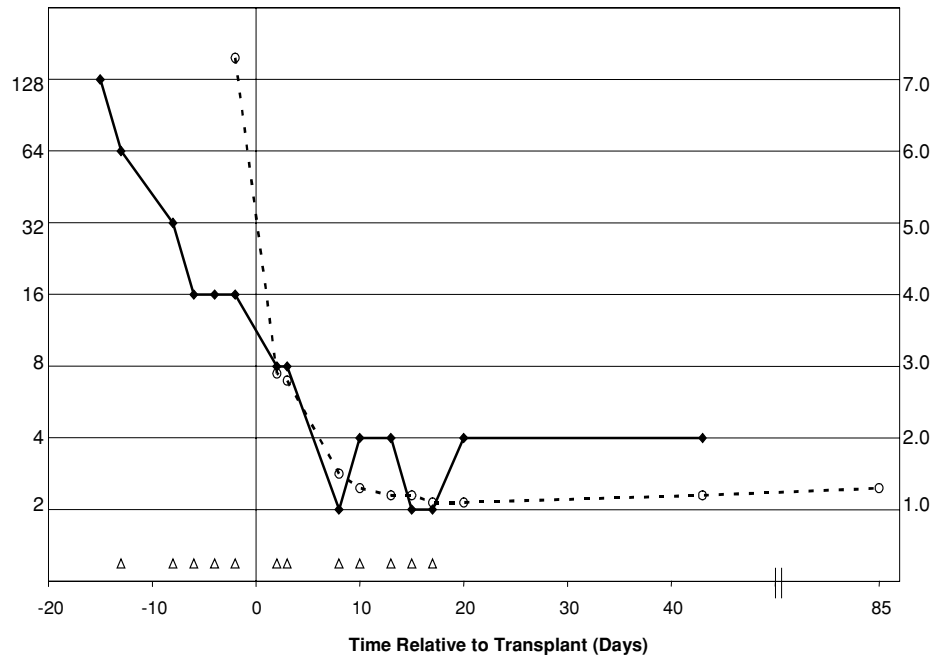
—◆— = Left axis, AHG Titer (log2), Δ = PP/CMVlg, .....○..... = Right axis, Serum Creatinine

**Figure 2: Antihuman globulin (AHG) phase anti-A or anti-B (as applicable) titers, serum creatinine and plasmapheresis events with relation to date of operation.**

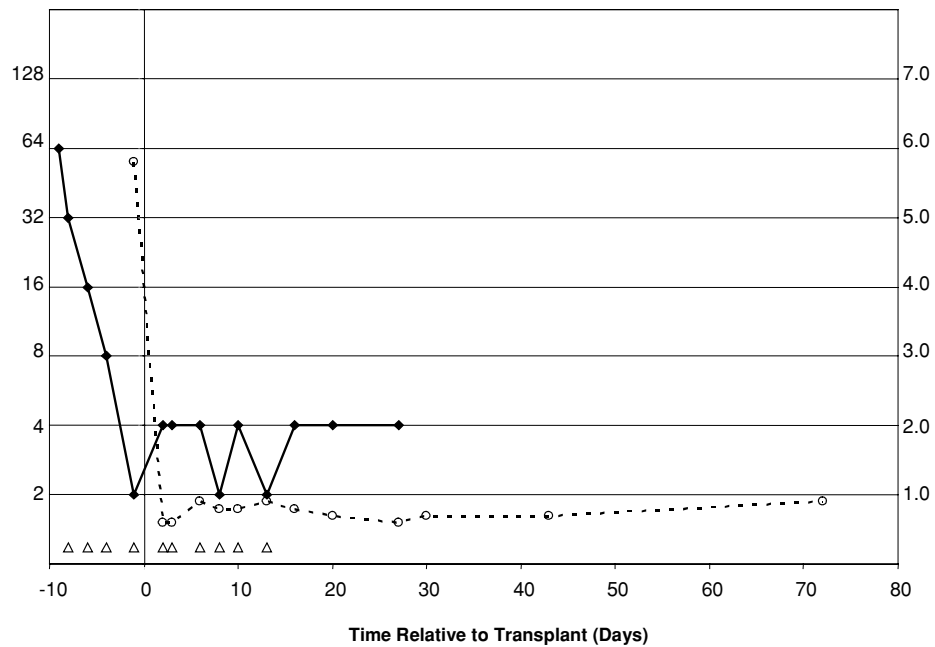
and at 1 month was 1.0 (range: 0.9–1.1). Follow-up ranged from 10 to 46 weeks, with mean serum creatinine of 1.1 (range: 0.9–1.2) at the time of most recent follow-up. There were no cases of AMR. All patients had C4d deposition in the peritubular capillaries without evidence of margination of neutrophils or rejection on surveillance biopsies, as has

been previously observed among accommodated ABOi allografts (11,14). One case of Banff 2A cellular rejection was diagnosed on surveillance biopsy and was treated successfully with pulse steroids, with a complete resolution of rejection upon repeat biopsy. Current ABO antibody titers remain 16 or below in all 4 cases.

Patient 3.



Patient 4.



—◆— = Left axis, AHG Titer (log2), Δ = PP/CMVlg, -○- - = Right axis, Serum Creatinine

Figure 2: Continued.

**Discussion**

In this report we describe our initial experience with the avoidance of splenectomy and anti-CD20 in a PP/low dose IVIG based protocol for ABOi renal transplantation.

Splenectomy continues to represent a major barrier to wider adoption of ABOi methodologies as one of the solutions to the organ shortage. In small series, the monoclonal antibody anti-CD20 has been shown to be an effective substitute for splenectomy but the effect of this

B-cell ablative drug on infectious morbidity and mortality is unknown. We propose that with the advent of newer, more potent maintenance immunosuppressive regimens including drugs with anti-B-cell activity like mycophenolate mofetil, the requirement for splenectomy and/or anti-CD20 should be re-explored. Furthermore, by including protocol post-transplant PP treatments to control anti-A or anti-B antibody titers in the early postoperative period when the rate of AMR is highest, successful engraftment may be less dependent on long-lasting B-cell ablation or suppression.

We describe 4 patients with initial ABO antibody titers  $\geq 64$ , 3 of which had non-A2 donors, in whom acceptable short term outcomes were achieved using PP/low dose CMVlg without splenectomy or anti-CD20. All 4 patients have functioning grafts with a serum creatinine of  $\leq 1.2$ . While the follow-up is relatively short in this cohort, the absence of any early episodes of AMR after discontinuation of PP/CMVlg is very encouraging because the overwhelming majority of graft losses after ABOi transplantation are due to AMR and occur in the first three post-transplant months (16). The protocol utilizes sequential quadruple immunosuppression and every other day PP/CMVlg treatments extending beyond 2 weeks post-transplant in order to maintain ABO antibody titers  $\leq 16$  during the period in which we hypothesize accommodation is occurring (17).

Starzl et al. first proposed that by reducing lymphoid mass and enhancing the efficacy of immunosuppressive medications, splenectomy would improve the outcomes of renal allo-transplantation (18). However, among immunosuppressed patients, splenectomy was shown to increase long-term morbidity and mortality due to infectious complications, principally sepsis from encapsulated bacteria (19,20). The practice was largely abandoned with the exception of ABOi transplantation where it was thought to be crucial to the successful engraftment of the kidney (2,3). This conclusion was based on a study by Alexandre et al. in which graft loss due to AMR was 100% among patients who received an ABOi allograft but did not undergo splenectomy (3). After this report, splenectomy became part of the standard immunosuppressive protocol utilized by the Japanese groups and in a recent report by Takahashi et al., of the outcomes of 441 ABOi kidney transplants performed at 55 centers in Japan, 98% of the patients received splenectomy (13). Ishida and colleagues called into question the utility of splenectomy after finding that the suppression of ABO antibodies after splenectomy was not significantly different from ABO antibody levels reported from centers that did not utilize splenectomy (21).

We and others recently proposed the use of anti-CD20 as an alternative to splenectomy in pre-conditioning regimens utilizing either immunoadsorption or PP/CMVlg to deplete anti-blood group antibodies (11, 12, 22). In an attempt to further limit the long-term suppression of the humoral arm of the immune system and increase access to ABOi trans-

plantation, we tested the hypothesis that successful engraftment could be achieved without excessive levels of immunosuppression and the concomitant risk of infectious complications. During the study period patients with A1 donors and isoagglutinin titers  $>128$  were excluded and were transplanted after splenectomy. Only patients with isoagglutinin titers of  $\leq 128$  are currently considered candidates for this protocol at our institution. There are many examples in the literature of graft loss from severe AMR following ABOi transplantation. Currently pre-conditioning regimens reported by centers performing non-A2 ABOi include either splenectomy or anti-CD20. We caution that while the initial results are encouraging, wider adoption of splenectomy and anti-CD20 avoidance should be predicated on the demonstration of long-term efficacy in larger, controlled studies.

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