ORIGINAL ARTICLE

Outcomes and risk stratification for late antibody-mediated rejection in recipients of ABO-incompatible kidney transplants: a retrospective study

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SUMMARY

The required intensity of monitoring for antibody-mediated rejection (AMR) after of ABO-incompatible (ABOi) kidney transplantation is not clearly formulized. We retrospectively evaluated a single-center cohort of 115 ABO-incompatible (ABOi) kidney transplant recipients, of which 32% were also HLA incompatible (ABOi/HLAi) with their donors. We used an adjusted negative binomial model to evaluate risk factors for late AMR. Using this model, we risk-stratified patients into high- and low-risk groups for the development of late AMR; 26% of patients had at least one AMR episode; 49% of AMR episodes occurred within 30-days after transplant and were considered early AMR. Patients with an early AMR episode had a 5.5-fold greater incidence of developing late AMR [IRR = 5.5, (95% CI: 1.5–19.3), $P = 0.01$]. ABOi/HLAi recipients trended toward increased late AMR risk [IRR = 1.9, (95% CI: 0.5–6.6), $P = 0.3$]. High-risk recipients (those with an early AMR or those who were ABOi/HLAi) had a sixfold increased incidence of late AMR [IRR = 6.3, (95% CI: 1.6–24.6), $P = 0.008$] versus low-risk recipients. The overall incidence of late AMR was 20.8% vs. 1.5% in low-risk recipients. Changes in anti-A/B titer did not correlate with late AMR (IRR = 0.9 per log titer increase, $P = 0.7$). This risk-stratification scheme uses information available within 30 days of ABOi transplantation to determine risk for late AMR and can help direct longitudinal follow-up for individual patients.

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Introduction

ABOi transplantation was pioneered in Japan over three decades ago [1–4] and is now performed in many other parts of the world with exceptional results [5]. In fact, many groups have reported that short- and long-term outcomes of ABOi living donor kidney transplants are similar to those of ABO compatible transplants [6–13]. In the United States, only a small proportion (approximately 1%) of the kidney transplants performed each year are ABOi, yet since

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1995, 280 centers in the United States have successfully performed at least one ABOi kidney transplant [14].

In part due to its success, the landscape of ABOi transplantation has been evolving. At our center and others, ABOi transplantation is now being utilized as a means to increase rates of transplantation for patients sensitized to human leukocyte antigens (HLA). For sensitized patients, high levels of donor-specific HLA antibodies (DSA) increase risks for rejection and are associated with worse long-term outcomes [15,16]. Therefore for these patients, every effort must be made to identify a donor to whom the recipient has the least possible DSA. In many cases, this means intentionally selecting an ABOi donor if that donor affords the most favorable HLA match and therefore, a so-called double-barrier transplant, in which a donor and recipient are both ABOi and HLA incompatible (ABOi/HLAi), is often performed.

Because of this practice, the ABOi patient population has evolved into a heterogeneous one. Most centers that perform ABOi transplants have specific protocols pertaining to the postoperative management and monitoring of incompatible kidney recipients, but for the current ABOi population, a "one-size fits all" approach to postoperative monitoring may not be appropriate. The monitoring protocols that were developed in the early ABOi experience were centered on close surveillance for antibody-mediated rejection (AMR), with the thought that early detection and treatment of AMR would increase allograft longevity. These protocols were quite intensive compared to those used for recipients of compatible transplants and included more frequent routine laboratory testing, specialized tests such as antibody titer monitoring, and even protocol biopsies. These tests and procedures are costly over the lifetime of a transplant, and it is unclear whether monitoring with such intensity in the long term is always necessary. The purpose of this study was to retrospectively evaluate long-term outcomes of all ABOi transplants at our center, many of which were ABOi/HLAi, with the intent of risk stratifying for late AMR. By identifying phenotypes that place patients at higher or lower risk for late rejection events, long-term post-transplant monitoring might be individually tailored so as to minimize testing and interventions, but to do so in a way that does not compromise outcomes.

Materials and methods

Regulatory oversight and patient selection

The incompatible kidney transplant protocol reported here has been approved by the Institutional Review Board at Johns Hopkins. All patients provided specific written informed consent to proceed with incompatible kidney transplantation. A total of 125 living donor ABOi kidney transplants were performed at The Johns Hopkins Hospital between October 1, 1999 and December 18, 2012. A total of 115 patients were included in the analyses performed here. Ten patients were excluded for death within 30 days of transplant (one patient), graft loss within 30 days of transplant (two patients), or insufficient follow-up data, specifically, too few isohemagglutinin (anti-A/B) titer measurements to be included in our model (seven patients).

Desensitization and immunosuppression

The primary desensitization modality used for all patients in this cohort consisted of pre- and post-transplant plasmapheresis with the administration of low-dose intravenous cytomegalovirus immune globulin (CMVIg) after each plasmapheresis treatment, as previously described [17]. The number of planned plasmapheresis treatments was determined by the starting antibody titer (roughly one treatment lowers isohemagglutinins by one dilution) and was increased as necessary to obtain an antibody titer of 16 or less at the time of transplant. Routine splenectomy was initially performed, but this practice was discontinued. A subset of patients received anti-CD20 antibody (rituximab, 375 mg/m^2 intravenously) on the day prior to transplantation [18]. Induction immunosuppression was either with daclizumab (2 mg/kg intra-operative dose followed by 1 mg/kg every 2 weeks for five total doses) or antithymocyte globulin (1.5 mg/kg intra-operative dose followed by four additional daily doses of 1.5 mg/kg for a total dose of 7.5 mg/kg). A steroid pulse was given starting intra-operatively with a dose of either dexamethasone (100 mg) or methylprednisolone (500 mg) and was subsequently tapered to oral prednisone. Prednisone was reduced to 20 mg daily once therapeutic tacrolimus levels were achieved and tapered by 5 mg monthly to a final daily dose of 5 mg. Both tacrolimus (adjusted to a trough goal of 8–12 ng/dl) and mycophenolate mofetil (2 g daily) were started at the time of the first pretransplant plasmapheresis treatment. It is important to point out that the Hopkins' protocol evolved with regard to induction and B-cell ablative therapy as our understanding of the essential components of ABOi desensitization became more informed. For many years, our protocol has utilized ATG induction, plasmapheresis/IVIg (100 mg/kg), and maintenance immunosuppression without splenectomy or rituximab. However, patients with a double barrier (ABOi plus HLAi) did receive 375 mg/m^2 rituximab on the day prior to transplant.

Antibody titer monitoring and biopsies

Measurements of anti-A/B titers and HLA antibody titers were performed as previously described [17,19]. For ABOi kidney transplant recipients, our institution's protocol is to measure anti-A/B titers prior to the initiation of desensitization (the starting titer), before and after every plasmapheresis treatment, weekly for the first month after transplant, monthly for 3 months post-transplant, and every 3 months thereafter. For patients who were also HLAi with their donor, measurements of donor-specific anti-HLA antibody titers were performed on the same schedule. Additional measurement of anti-A/B and HLA antibody titers was performed on an as-needed basis in the setting of clinical change or suspicion for rejection. Protocol renal allograft biopsies were performed at 1, 3, 6, and 12 months post-transplant, and when clinically indicated.

Histopathologic analysis of biopsies and diagnosis of rejection

Biopsies were reviewed by a renal pathologist and graded according to the most recently published Banff criteria at the time the biopsy was performed, between the years 1999 and 2012 [20–25]. The diagnosis of AMR was made when there was pathological evidence of antibody-mediated injury in conjunction with detectable circulating antibody including a rise in either HLA or anti-A/B antibody or both. Because C4d staining is commonly observed in ABOi allografts, it was only considered a feature of AMR when microcirculatory inflammation was also present [26]. C3d staining was performed on biopsy samples as described. C3d positivity was interpreted as an indicator of AMR also in the context of other histopathologic features of inflammation [27]. Microvascular injury (MVI) scores were calculated retrospectively as the sum of the g- and ptc-scores in biopsies for patients diagnosed with AMR.

Late antibody-mediated rejection and identification of risk factors

Early AMR episodes were defined as those that occurred on or before postoperative day (POD) 30. Late AMR episodes were defined those that occurred any time after POD30. We characterized the risk factors associated with late AMR by comparing the incidence rate ratio (IRR) estimated from a negative binomial model that was adjusted for the following variables: the incidence of early AMR, the presence of HLA incompatibility, the recipient's age, race, BMI, starting anti-A/B titer, and history of previous transplant. Each recipient contributed to the risk set from POD31 until the earliest of the following events: graft loss, death, or the last date of follow-up. The dispersion parameter alpha was 2.4 (95% CI: 0.7–0.9, $P = 0.004$), supporting the use of a negative binomial model over a Poisson model for this analysis.

Change in anti-A/B titer and late antibody-mediated rejection

Our clinical practice has been to continue longitudinal surveillance of anti-A/B titers for the lifetime of the transplant, and in this study, we sought to evaluate the clinical relevance of post-transplant changes in these titers. To this end, we defined the "baseline" anti-A/B titer as the titer measured at discharge from the admission on which the transplant occurred. In order to evaluate post-transplant changes in antibody titer and determine whether increased titers were associated with late AMR, we compared the baseline titer for each patient to the highest measured titer within 7 days prior to the first late AMR event. The association of an increase in anti-A/B titer with the incidence rate of late AMR was assessed using a negative binomial model, adjusting for the following variables: baseline titer, incidence of early AMR, presence of HLA incompatibility, the recipient's age, race, BMI, starting anti-A/B titer, and history of previous transplant. We also asked whether the value of the baseline titer modifies the effect of the increase in anti-A/B titer by estimating an interaction term between the two. For this analysis, each recipient contributed to the risk set from POD31 until the earliest of the following events: graft loss, death, the last day of follow-up, or 90 days after the last anti-A/B titer measurement.

Statistical analysis

Continuous variables were displayed as mean ± SD and categorical variables as *n* (%). Graft survival and AMR-free survival were described using Kaplan–Meier survival analyses. All analyses were performed using STATA 13.1/MP for Linux (College Station, TX, USA).

Results

Demographics

The average recipient age was 47 years old and ranged from 21 to 73 years old (Table 1). Over half (56.6%)

Table 1. Recipient characteristics.

N	115
Age—year ± SD	47 ± 13
Male—*n* (%)	65 (56.5)
Ethnicity—*n* (%)	
White	81 (70.4)
Black	27 (23.5)
Asian	4 (3.5)
Hispanic	3 (2.6)
ABO blood group	
O	96 (83.5)
A	11 (9.5)
B	8 (7.0)
Predesensitization anti-A or anti-B titer—median (IQR)	64 (32–256)
At transplant anti-A or anti-B titer—median (IQR)	8 (4–8)
Percent CPRA—median (IQR)	
Entire cohort	17 (0–92)
ABOi alone	0 (0–10)
ABOi/HLAi	75 (34–94)
HLA incompatible—*n* (%)	37 (32.2)
Transplanted via kidney-paired donation—*n* (%)	23 (20)
Number pretransplant plasmapheresis treatments—median (IQR)	4 (3–6)
Number post-transplant plasmapheresis treatments—median (IQR)	4 (3–6)
Previous kidney transplants—*n* (%)	
No previous transplants	72 (62.6)
One previous transplant	30 (26.1)
Two previous transplants	12 (10.4)
Three previous transplants	1 (0.9)
Body mass index—*n* (%)	
Underweight (<18.5)	2 (1.7)
Normal (18.5–24.9)	30 (26.1)
Overweight (25–29.9)	60 (52.2)
Obese (>30)	23 (20)
Diabetes—*n* (%)	
Type I	9 (7.8)
Type II	20 (17.4)

were male. A quarter of the patients had diabetes. The median starting anti-A/B titer was 64 (IQR 32–256), which was reduced to a median of 8 after desensitization and at the time of transplant (our cutoff for proceeding with transplantation is 16). There was no upper limit in terms of the starting anti-A/B titer that we considered acceptable for inclusion and several patients had titers of 512 or 1024. 37 recipients (32.2%) were ABOi/HLAi with their donor and 23 patients were desensitized to an ABOi KPD-matched donor. While the majority of the overall ABOi cohort was male, 56% of the ABOi/HLAi recipients were female. The majority of the patients (62.6%) were undergoing their first transplant, but 26.1% had had one previous kidney transplant, 10.4% had two, and 0.9% had three previous kidney transplants. The median CPRA of the ABOi alone subset was 0 (IQR 0–10), while the median cytotoxic CPRA of the ABOi/HLAi patients was 75 (IQR 34–94). Of the patients who were also HLAi with their donors, 33% had class I DSA only, 26% had class II DSA only, and 35% had both class I and class II DSA. The median number of pre- and post-transplant plasmapheresis treatments was 4 (IQR 3–6).

The average donor age was 46 years old with a range from 21 to 68 years old (Table 2). Over half of the donors were female (63.5%). The most frequent donor-recipient blood group combination was A into O (54.4%), followed by B into O (24.3%, Table 3).

Table 2. Donor characteristics.

N	115
Age—year ± SD	46 ± 12
Female–*n* (%)	73 (63.5)
Race–*n* (%)	
White	83 (72.2)
Black	25 (21.7)
Asian	4 (3.5)
Hispanic	3 (2.6)
ABO blood group—*n* (%)	
A1	58 (46.4)
A2	16 (12.8)
B	34 (29.6)
A1B*	14 (12.1)
A2B	1 (0.8)

*Two AB donors with A recipients were not subtyped therefore presumed A1B.

Table 3. Sum of blood group incompatibilities.

Donor blood type	Recipient blood type	*N*
A1	O	54
	B	5
A2	O	14
	B	2
B	O	28
	A	6
A1B	O	5
	A	5
	B	2
A2B	O	0
	A	0
	B	1

Renal allograft survival

Kaplan–Meier estimates of death-censored graft survival for the overall cohort were 99.1%, 92.9%, and 89.3% at 1, 3, and 5 years, respectively. For the ABOi alone subset, death-censored graft survival rates were 100%, 94.4%, and 92.3% at 1, 3, and 5 years, while for the ABOi/HLAi subset, death-censored graft survival rates were 97.2%, 89.2%, and 81.1% (Fig. 1).

Antibody-mediated rejection episodes

Over a median follow-up period of 45 months, 30 patients (26%) experienced at least one episode of AMR. Eight patients had more than one episode of AMR, for a total number of 42 independent episodes of AMR observed in the cohort over the follow-up period. Five patients had two independent AMR episodes, one patient had three independent episodes, and two patients had four independent AMR episodes. Of the 22 patients who had only a single episode of AMR, eight were ABOi/HLAi. Of the five patients who had two AMR episodes, four were ABOi/HLAi, and of the patients who had three or four independent AMR episodes, all were ABOi/HLAi; 38% of ABOi/HLAi recipients had at least one AMR episode compared to 21% of those who were ABOi alone [IRR = 2.5, (95% CI: 0.6–10.0), $P = 0.2$]. An episode of AMR was considered independent of a prior episode if between the biopsies there was either return of creatinine to or near baseline, and/or there was resolution of inflammation on an intervening biopsy. MVI scores were retrospectively calculated for all patients in whom AMR was diagnosed. The mean MVI score for biopsies at the time of a diagnosed AMR was 3.8 (SD 1.5); thus, there was a consistent picture of microvascular inflammation on these biopsies throughout the study period [28]. Among the ABOi/HLAi patients who had at least one AMR episode, a greater proportion had both class I and class II DSA compared to those who had no rejection episodes (50% vs. 29%, $P < 0.001$).

The time course of the development of AMR is illustrated in Fig. 2. AMR episodes occurred earlier and more frequently in patients who were ABOi/HLAi; 49% of the AMR episodes observed were early AMRs occurring within the first 30 days after transplant; 21% of the episodes occurred between POD31 and POD90, 11.6% occurred between POD91 and 6 months, 7% between 6 months and 1 year, and 11.6% beyond 1 year after transplant. AMR episodes were associated with an increase in DSA strength in 50% of those who were ABOi/HLAi. Among those who were ABOi alone at the time of transplant, 15% of AMR episodes were associated with either the development of *de novo* DSA or an increase in DSA strength of previously present DSA.

Late antibody-mediated rejection episodes

A total of 21 episodes of AMR were diagnosed at times beyond POD30 and considered late AMR. In 13 of these 21 instances, there was an elevation in the patient's serum creatinine that prompted the biopsy that diagnosed AMR. Two episodes were subclinical AMR diagnosed on

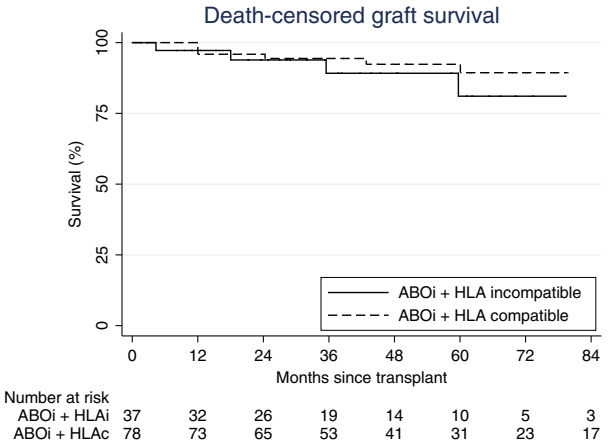

Figure 1 Renal allograft survival. Kaplan–Meier estimates of death-censored graft survival stratified by the presence of concomitant human leukocyte antigen incompatible (HLAi). Seventy-eight patients were ABO incompatible (ABOi) alone, and 37 patients were both ABOi/HLAi with their donors. Twenty-three patients were transplanted via kidney-paired donation.

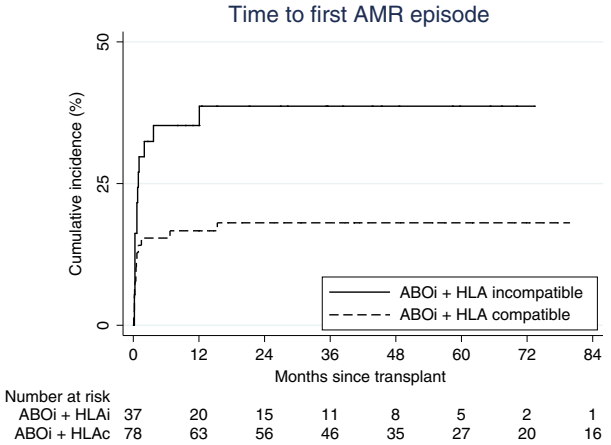

Figure 2 Timing of antibody-mediated rejection episodes. Time in months versus cumulative incidence of first antibody-mediated rejection (AMR) episode in ABO incompatible (ABOi) recipients stratified by the concomitant presence of human leukocyte antigen incompatible (HLAi). Seventy-eight patients were ABO incompatible only, and 37 patients were both ABOi/HLAi with their donors.

protocol biopsies (at 3 months in one case and at 12 months in the other). Neither anti-A/B titers nor anti-HLA titers in those cases had increased above baseline. In four cases of late AMR, there was an elevation in anti-A/B titer above baseline. In only one of these cases, which was diagnosed on POD71, was the elevated titer the only laboratory abnormality, and what prompted the biopsy. This patient was ABOi/HLAi. Of the three other instances, one was associated with medication nonadherence and non-detectable tacrolimus levels, one was in conjunction with a significant increase in donor-specific HLA antibody strength, and the other was associated with an elevation in creatinine that prompted the biopsy. Concomitant events such as cellular rejection, infection, and proteinuria were observed in many cases of late AMR (Table 4).

Risk stratification for late antibody-mediated rejection

In our multivariable model which included incidence of early AMR, HLA incompatibility, recipient's age, race, BMI, starting anti-A/B titer, and history of previous transplant, the incidence of early AMR was found to be significantly associated with the risk of late AMR after ABOi kidney transplantation [IRR = 5.5, (95% CI: 1.5–19.3), $P = 0.01$, Table 5]. A suggestive association between HLAi and late AMR was found [IRR = 1.9, (95% CI: 0.5–6.6), $P = 0.3$].

Table 5. Potential risk factors for AMR after ABO incompatible kidney transplantation.

Variable	IRR (95% CI)	*P*-value
Incidence of early AMR	5.5 (1.5–19.3)	0.01
HLA incompatibility	1.9 (0.5–6.6)	0.3
Age (per 10 years)	1.3 (0.8–2.2)	0.3
Non-White race (versus White)	0.7 (0.1–3.5)	0.6
Starting anti-A/B titer (per log increase)	0.9 (0.7–1.3)	0.6
History of previous kidney transplant	2.1 (0.5–9.2)	0.3
Recipient body mass index (kg/m^2)	1.1 (0.9–1.2)	0.3

Based on this risk factor analysis, we divided the cohort into two risk groups. The "high-risk" group ($n = 48$) for late AMR included those who had an early AMR episode, those who were ABOi/HLAi, or both. The "low-risk" group ($n = 67$) for late AMR included those who neither had an early AMR nor were HLAi with their donors. The cumulative incidence of late AMR episodes among these two risk groups is illustrated in Fig. 3. The high-risk group had a sixfold greater incidence of late AMR compared to the low-risk group [IRR = 6.3, (95% CI: 1.6–24.6), $P = 0.008$]. The overall incidence of late AMR in the high-risk group was 20% vs. 1.5% in the low-risk group. Death-censored graft survival at 1, 3, and 5 years

Table 4. Details of biopsy-proven late antibody-mediated rejection episodes.

	Days after transplant	Cr elevated	ABO titer elevated	DSA titer elevated	Concomitant event
31–90 days	32	Y			None
	45	Y	Y		Line infection
	61				Proteinuria
	69	Y			Proteinuria
	71		Y		None
	78				Cellular rejection
	80				Protocol biopsy
	85	Y			Skin wound infection
91 days–6 months	95		Y	Y	None
	99	Y			Thrombosed hemorrhoid
	112	Y			Diarrheal illness
	148	Y			Subtherapeutic CNI level
	176				Protocol biopsy
6 months–1 year	197	Y			CMV viremia
	204		Y		Medication noncompliance
	295	Y			None
>1 year	367				Protocol biopsy
	467	Y			Cellular rejection
	1587	Y		Y	None
	2966	Y			Cellular rejection
	3104	Y			Sinusitis
Sum	21 total AMRs	13/21	4/21	2/21	

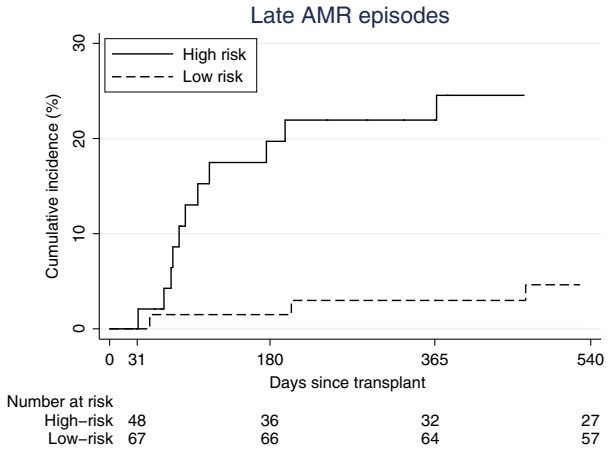

Figure 3 Timing of late antibody-mediated rejection episodes. Time in months versus cumulative incidence of late antibody-mediated rejection (AMR) episodes in ABO incompatible (ABOi) recipients stratified by high-risk [presence of early antibody-mediated rejection (AMR) and human leukocyte antigen incompatible (HLAi)] and low-risk groups (no early AMR and ABOi alone).

was 100%, 96.7%, and 94.4% in the low-risk group compared to 97.8%, 86.8%, and 81.0% in the high-risk group (Fig. 4). We repeated our negative binomial regression to determine whether additional risk factors for late AMR could be identified within these subgroups and found none (Table 6).

Increase in anti-A/B titer as a predictor of late AMR

Thirty-one patients were observed to have an increase in their anti-A/B titer compared to the baseline titer, and two patients had a decrease in anti-A/B titers time compared to their baseline titer. Eight recipients who developed at least one late AMR had increases in their titer. Two patients who developed AMR had decreases in their titer. Twenty-five patients who had increases in their titers had no AMR (Table 7). We observed no association between increase in anti-A/B titers above baseline and the development of late AMR [IRR = 0.9, (95% CI: 0.4–1.8), $P = 0.7$].

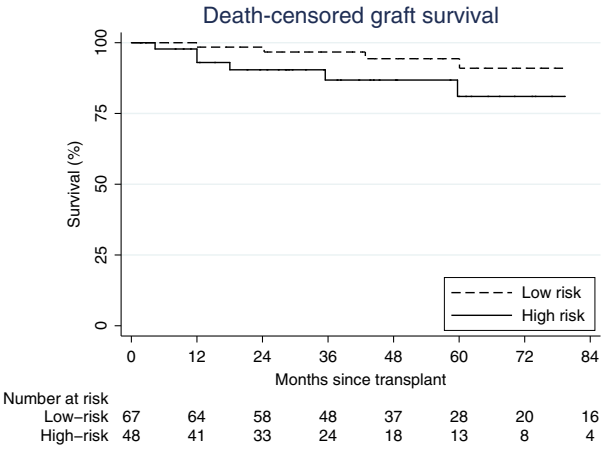

Figure 4 Renal allograft survival. Kaplan–Meier estimates of death-censored graft survival stratified by high-risk [presence of early antibody-mediated rejection (AMR) and human leukocyte antigen incompatible (HLAi)] and low-risk groups [no early antibody-mediated rejection (AMR) and ABO incompatible (ABOi) alone].

Table 6. Potential risk factors for late AMR stratified by subgroup.

	High-risk (n = 48)				Low-risk (n = 67)			
	IRR			P	IRR			P
Age (per 10 years)	0.8	1.4	2.6	0.3	0.5	1.1	2.4	0.9
Nonwhite					0.7	4.7	33.3	0.1
Starting titer	0.7	1.2	1.8	0.5	0.5	0.9	1.5	0.7
Previous tx	0.5	3.3	21.9	0.2	0.1	1.5	14.9	0.7
BMI	0.9	1.0	1.2	0.8	0.9	1.0	1.2	0.6

Discussion

In this single-center series, we describe the incidence, timing, and patterns of AMR in 115 recipients of ABOi kidney transplants. In our cohort, in whom the median follow-up time was 45 months, half of all the AMR episodes that were observed occurred within the first 30 days after transplant. Recipients who were ABOi/HLAi were at increased risk for having any AMR episode (early or late), compared to those who were ABOi alone. To gain a further understanding of the risk posed by attempting to cross both ABOi and HLAi barriers simultaneously versus ABOi alone, and to help guide our long-term management of these patients, we asked whether we

Table 7. Late AMR events in high-risk group by change in anti-A/B titer.

Increase in anti-A/B titer (log increase)	Late AMR event		Total
	No	Yes	
−1	0	2	2
0	13	2	15
1	15	3	18
2	9	1	10
3	1	1	2
4	0	0	0
5	0	1	1
	38	10	48

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could identify factors that were present early that portended greater risk for late AMR. The most significant risk factor for the development of late AMR was the occurrence of an early AMR. The incidence rate of late AMR was 5.5-times greater among those patients who had an AMR episode within 30 days of transplant [IRR = 5.5, (95% CI: 1.5–19.3), $P = 0.01$]. Recipients who were ABOi/HLAi trended toward increased risk for late AMR [IRR = 1.9, (95% CI: 0.5–6.6), $P = 0.3$]. Using this information, which is available for all ABOi recipients at 30 days post-transplant, we stratified patients into high-risk and low-risk groups. We observed that the high-risk group had a sixfold greater risk for the development of late AMR [IRR = 6.3, (95% CI 1.6–24.6), $P = 0.008$]. Interestingly, neither starting A/B titers prior to transplantation nor increases in titer post-transplant were predictive of AMR [29,30].

The primary limitation of this study is the sample size. While for a single center in the United States, this represents a large cohort of ABOi kidney transplant recipients, this study would certainly be strengthened by a larger sample size, such as that which would be generated by way of a national incompatible registry. Further, the single-center nature of this study potentially limits its generalizability to other centers where different desensitization protocols may be used, and different post-transplant monitoring policies may be in place.

In our center's experience, as in others, graft survival outcomes after ABOi kidney transplants are excellent [1,4,17,31]. Because of these favorable results, the role of ABOi transplantation has evolved from a means simply to enable transplantation between an ABOi living donor and recipient, and more into a tool we utilize to facilitate transplantation of our most highly sensitized patients. Kidney-paired donation (KPD) has greatly enhanced our ability to find the best donors for these patients. For our ultra-sensitized patients (PRA >97%), we frequently enter an AB blood type into the KPD database regardless of the true ABO type in order to improve their exposure to the rare genotypes that provide the best opportunity for successful HLA directed desensitization [26,31–34]. As a result of this practice, 32% of the patients in our ABOi cohort underwent a combined ABOi/HLAi transplant; 20% of the cohort benefited from the paradigm of combining desensitization and kidney-paired donation for the purpose of identifying a donor for whom donor-specific antibody strength is the lowest.

When the likelihood of finding a compatible match is low, choosing a donor for whom the sensitized recipient has low strength HLA antibodies, regardless of ABO incompatibility [35], minimizes the intensity of desensitization and may improve outcomes [9,36]. Thus, the contemporary ABOi recipient population has evolved into a heterogeneous one and we have a need to understand better how the outcomes and risks of this heterogeneous population compare to the reported results of standard ABOi only patients. This analysis will give us better insight into the timing and nature of AMR in this group and provide guidance related to the utility of close monitoring of isohemagglutinins and tissue. As a whole, the group is likely at greater immunologic risk compared to compatible recipients, yet at lower risk compared to the most highly sensitized HLAi recipients. By studying the ABOi recipient population as a whole we could ask whether there were factors in the peri-transplant period that would enable us to risk stratify patients for purposes of longitudinal monitoring. We have defined a high-risk group, which consists of those who were ABOi/HLAi with their donors, those who experienced an early AMR episode (within the first 30 days after transplant), or both. This high-risk group comprised 42% of our cohort and the incidence of late AMR in the high-risk group was sixfold higher than in the low-risk group.

Identifying patients who are at high-risk for rejection before they actually present with rejection will help us to tailor our long-term surveillance practices so that they are efficient, cost-effective, and appropriate given the patient's risk phenotype. For those who manifest the high-risk phenotype, a determination can be made within the first month post-transplant that they will need long-term careful follow-up and should not have their maintenance immunosuppression tapered over time. However, the benefits of overly vigilant longitudinal monitoring including more frequent routine laboratory tests, specialized testing such as antibody titer monitoring, and even invasive procedures such as protocol biopsies are not likely to outweigh the additional costs or the additional risks of these procedures in the low-risk group. Their maintenance immunosuppression can be managed more like ABOc patients.

In this study, we have undertaken a retrospective review of our ABOi outcomes and AMR (early and late) rates. This is one of the largest single-center ABOi cohorts reported outside of Japan. Once again we have affirmed previously reported outstanding results of ABOi transplantation. These results do not differ from ABO compatible live donor transplants and argue for a wider adoption of this technique to expand live donation. Our protocol now involves only plasmapheresis and low-dose IVIg, without rituximab, along with

conventional induction and maintenance immunosuppression [18,37–39]. The level of additional immunosuppression over and above a compatible transplant is mild and short-lived.

The simultaneous expansion of KPD and effective desensitization protocols have made it possible to combine the two modalities and select the best HLA-matched donor from the KPD pool for a recipient. This has created another ABOi population that little is known about presently. When crossing both the ABO and HLA barriers simultaneously in highly sensitized patients (with or without KPD), the rates of AMR are greater, graft survival outcomes are not quite as good but the patient survival is far better than waiting on dialysis for a compatible kidney [15]. However, our ability to offer this option to patients in need will depend upon a better understanding of the diverse population of patients who are now receiving ABOi kidneys. As an emerging field, incompatible transplantation has developed to a point where sufficient data have been generated to identify best practices and manage costs more effectively. Moving forward, cooperation and data sharing between centers performing these transplants will be essential to this ongoing process.

Authorship

BEL: involved in research design, data acquisition, data analysis, and writing of the manuscript. SB: involved in research design, data analysis, and writing of the manuscript. ESK: involved in data acquisition, data analysis, and writing the manuscript. MJH: involved in research design and data acquisition. KEK: involved in data acquisition and writing of the manuscript. FFN: wrote the manuscript. NA: wrote the manuscript. NND: wrote the manuscript. AS: wrote the manuscript. NMD: wrote the manuscript. DLS: involved in research design, data analysis, and writing of the manuscript. RAM: involved in research design and writing of the manuscript.

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Conflict of interest

The authors declare no conflict of interests.

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