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ORIGINAL ARTICLE

ABO-incompatible kidney transplantation in perspective of deceased donor transplantation and induction strategies: a propensity-matched analysis

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SUMMARY

Kidney transplant candidates are blood group incompatible with roughly one out of three potential living donors. We compared outcomes after ABO-incompatible (ABOi) kidney transplantation with matched ABO-compatible (ABOc) living and deceased donor transplantation and analyzed different induction regimens. We performed a retrospective study with propensity matching and compared patient and death-censored graft survival after ABOi versus ABOc living donor and deceased donor kidney transplantation in a nationwide registry from 2006 till 2019. 296 ABOi were compared with 1184 center and propensity-matched ABOc living donor and 1184 deceased donor recipients (matching: recipient age, sex, blood group, and PRA). Patient survival was better compared with deceased donor [hazard ratio (HR) for death of HR 0.69 (0.49–0.96)] and non-significantly different from ABOc living donor recipients [HR 1.28 (0.90–1.81)]. Rate of graft failure was higher compared with ABOc living donor transplantation [HR 2.63 (1.72–4.01)]. Rejection occurred in 47% of 140 rituximab versus 22% of 50 rituximab/basiliximab, and 4% of 92 alemtuzumab-treated recipients ($P < 0.001$). ABOi kidney transplantation is superior to deceased donor transplantation. Rejection rate and graft failure are higher compared with matched ABOc living donor transplantation, underscoring the need for further studies into risk stratification and induction therapy [NTR7587, www.trialregister.nl].

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Key words
ABO-incompatible kidney transplantation, alemtuzumab, deceased donor transplantation, living donor transplantation, patient and graft survival, rejection

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Trial registration
The Netherlands Trial Register [NTR7587, www.trialregister.nl]. This trial is approved by the institutional review board of the Erasmus Medical Center (MEC-2018-1325).

Introduction

The main barriers for kidney transplantation are shortage of donor organs and circulating antibodies in the recipient against potential donors. Historically, antibodies against blood group A and B antigens were clinically the most relevant antibodies against red blood cells and endothelial cells of the donor kidney. Given the blood group distribution in the general population, roughly one out of three transplant candidates will be blood group incompatible with an intended living donor. Especially blood group O recipients, approximately 40 percent of the population, are limited by the presence of circulating A/B antibodies. As a result, blood group O candidates have longer waiting list times on dialysis [1]. To enlarge the pool of potential donors, desensitization treatment was introduced to enable transplantation across incompatible ABO (ABOi) blood groups. ABOi desensitization programs have evolved at different sites, and protocols differ substantially in plasma exchange techniques and induction regimens. The game changer in ABOi kidney transplantation has been the introduction of rituximab [2]. This anti-CD20 monoclonal antibody made splenectomy obsolete [3]. Rituximab has been the cornerstone of ABOi kidney transplantation from the start of the 21st century onwards. Other induction therapies are thymoglobulin (ATG) and, to a lesser extent, alemtuzumab [4,5]. Rituximab is administered with or without basiliximab as induction treatment. Nowadays, ABOi kidney transplantation is considered safe and accounts for a substantial portion of the living donor program, ranging from roughly 5% in the UK to 28% in Japan [6]. However, two meta-analyses suggested inferior patient and graft survival in ABOi kidney transplantation compared with ABO-compatible (ABOc) living donor transplantation [7,8]. Infectious complications, rejection, and bleeding were observed more frequently in ABOi recipients, favoring ABOc transplantation.

When an ABOc donor is not available in clinical practice, patient and physician have to decide whether they should wait for a deceased donor sometime in the future or proceed with desensitization for transplantation with their ABOi living donor. Therefore, an additional comparison with outcomes after deceased donor transplantation is warranted. Recently, Massie *et al.* [9] performed such a comparison by analyzing ABOi recipients in the Scientific Registry of Transplant Recipients (SRTR) and compared their survival to waiting list registrants who received a (mostly deceased) donor transplant or remained on the waitlist. From 180 days post-transplant onwards, patient survival was better after ABOi kidney transplantation. It is unclear whether this survival benefit also relates to areas outside the United States. Furthermore, as both death with a functioning graft and antibody-mediated rejection occurred more frequently in ABOi recipients [7,8], it is not known whether recipients could benefit from a less or more intensive induction treatment. Nationwide, rituximab was used as the standard induction agent after the publication of Tyden *et al.* [10]. Since then, two protocol modifications have been made: addition of basiliximab to rituximab, or substitution of rituximab for alemtuzumab.

In order to compare outcomes after ABOi versus ABOc kidney transplantation, we selected a nationwide cohort of all consecutive kidney transplantations performed since the year of the first ABOi procedure onwards to answer the following research questions:

1. What is the difference in patient and graft survival after kidney transplantation with an ABOi donor compared with (i) an ABOc living donor and (ii) ABOc deceased donor?
2. What is the impact of different induction regimens for kidney transplantation with an ABOi donor on graft failure and patient survival with a functioning graft?

Methods

Patients

Data on all kidney transplantations performed since January 2006, the year of the first ABOi transplantation in the Netherlands, were obtained from the Dutch Organ Transplant Registry (NOTR Nederlandse Orgaan

Transplantatie Registratie). Written informed consent was obtained from all patients. We included procedures up to March 2019. Exclusion criteria were age below 16 at time of transplantation, combined liver–kidney or kidney–pancreas transplantations, and desensitization for HLA-incompatible kidney transplantation. Baseline anti-A/B titers were defined as low (≤1:8), intermediate (1:16–1:64), and high (≥1:128) IgG and IgM titers.

ABOi treatment protocol

ABOi candidates were advised to participate in the national kidney exchange program, for two rounds in general. If unsuccessful and baseline A/B titer was 1:256 or lower, candidates were deemed eligible for ABOi kidney transplantation. Desensitization consisted of immunoadsorption (IA) with Glycosorb® (Glycorex, Lund, Sweden), pre-operative initiation of tacrolimus, mycophenolate mofetil, and corticosteroids and Intravenous Immunoglobulin (IVIG) 0.5 g/kg one day preoperatively. Rituximab was the initial induction agent. After observing frequent rejection episodes, centers changed this protocol, resulting in the following three main regimens (details in Table S1):

1 Rituximab 375 mg/m^2 day minus 28.
2 Rituximab 375 mg/m^2 day minus 28 and basiliximab 20 mg intravenously day 0 intraoperatively and day plus 4.
3 Alemtuzumab 30 mg subcutaneously day minus 30 with or without additional 15 mg subcutaneously day minus 1.

Induction therapy thus differed per center and over time (and not per patient characteristic). Maintenance immunosuppression consisted of prednisolone, tacrolimus 0.075–0.1 mg/kg daily, and mycophenolate mofetil 1000–2000 mg/daily during the first 6 months and was then continued according to local practice. Target tacrolimus trough levels were 10–15 μg/l initially. Targets levels have been changed to 8–12 μg/l in more recent years since the introduction of basiliximab and alemtuzumab.

ABOc treatment protocol

Since 2009, basiliximab was introduced as induction therapy in five out of six centers. Before, no induction was administered. The last center started basiliximab in 2014. T-cell depleting therapy thymoglobulin (rATG) was administered in a small subset of highly sensitized patients. One center administered rituximab in a clinical trial for several years (Table 1) [11]. Maintenance immunosuppression and tacrolimus trough levels were identical to ABOi recipients.

Outcome definitions

Graft failure was defined as the initiation of chronic renal replacement therapy, and death with a functioning graft was considered a competing event. Rejection was defined as treatment for a rejection episode, whether biopsy-proven or not. Administrative censoring was performed for follow-up after September 2019, ensuring that all patients had at least six months of follow-up.

Statistical methods

We presented means and standard deviations for normally distributed variables and medians and 25th and 75th percentiles for variables with a skewed distribution. We presented frequencies and proportions for categorical variables. Baseline differences were tested with one-way ANOVA, Kruskal–Wallis test, and χ^2 tests where appropriate. In order to deal with missing data for covariates, we performed multiple imputations using chained regression equations [12]. We created 20 imputed datasets that were used for further analysis. Imputation results were checked using diagnostic plots. We created a hypothesized causal model for the possible effect of having an ABOi donor on the risk of adverse outcome after kidney transplantation. The initial causal model was based on literature and expert opinion, visualized with a directed acyclic graph (DAG) using the dagitty.net software [13]. A DAG encodes assumptions about possible associations between variables in the data [14]. These assumptions include (conditional) independencies (i.e., the absence of an association) between variables and can be tested with regressions (see Appendix S1). This model identified four covariates for the adjustment set: recipient age, sex, blood group, and peak panel reactive antibodies (PRA). Next, we performed propensity score matching using this adjustment set within each center to ensure confounder balance across centers. We matched ABOi procedures to ABOc procedures in a 1:4 ratio. After matching, we estimated cumulative incidence of graft failure and survival probability with a function graft as mutually exclusive competing events. Next, a cause-specific Cox proportional hazards model was fitted to estimate the causal effect of having an ABOi donor compared with either an ABOc living or deceased donor. The proportional hazards assumption was checked by plotting Schoenfeld's residuals by time [15].

Table 1. Baseline characteristics of propensity score-matched recipients.

	ABO-incompatible	ABO-compatible living donor	ABO-compatible deceased donor	*P*-value vs. living donor	*P*-value vs. deceased donor
N	296	1184	1184		
Recipient age (years) (median [IQR])	54.0 [44.8, 64.0]	55.0 [46.0, 63.0]	58.0 [46.0, 66.0]	0.80	0.003
Recipient sex: male (%)	199 (67.2)	762 (64.4)	812 (68.8)	0.39	0.71
Recipient BMI (kg/m^2) (mean (SD))	25.6 (4.1)	26.0 (4.4)	26.5 (4.5)	0.15	0.002
Primary kidney disease (%)					
Diabetic nephropathy	19 (6.4)	103 (8.7)	151 (12.8)	<0.001	<0.001
Glomerulonephritis	68 (23.0)	193 (16.3)	162 (13.7)		
Urologic	6 (2.0)	12 (1.0)	19 (1.6)		
Polycystic kidney disease	58 (19.6)	139 (11.7)	90 (7.6)		
Vascular	69 (23.3)	237 (20.0)	340 (28.7)		
Benign/malignant tumor	3 (0.3)	3 (0.3)	7 (0.6)		
Other/not reported	57 (19.3)	474 (40.0)	394 (33.3)		
Hereditary nephropathies	17 (5.7)	23 (1.9)	21 (1.8)		
Preemptive	118 (39.9)	538 (45.4)	62 (5.2)	0.13	<0.001
Time on dialysis (days) (median [IQR])	216 [0, 548]	99 [0, 542]	1152 [644, 1707]	0.11	<0.001
Previous transplantation (*n*) (mean (SD))	1.16 (0.50)	1.05 (0.24)	1.10 (0.40)	<0.001	0.07
Recipient blood group (%)					
A	57 (19.3)	222 (8.8)	219 (18.5)	0.97	0.95
B	44 (14.9)	181 (15.3)	181 (15.3)		
O	195 (65.9)	781 (66.0)	784 (66.2)		
Donor age (years) (median [IQR])	55.00 [45.00, 63.00]	53.50 [45.00, 61.00]	56.00 [47.00, 64.00]	0.18	0.27
Donor sex: male (%)	126 (42.6)	489 (41.3)	660 (55.7)	0.74	<0.001
Total HLA mismatches (*n*) (mean (SD))	3.47 (1.39)	3.55 (1.52)	2.75 (1.43)	0.69	<0.001
Peak PRA (%)					
1–4%	259 (87.5)	1025 (87.5)	1019 (86.1)	0.48	0.09
5–84%	32 (10.8)	147 (12.4)	113 (9.5)		
85–100%	5 (1.7)	12 (1.0)	52 (4.4)		
ABO IgG titer (%)					
<1	23 (9.5)				
1:1	1 (0.4)				
1:2	27 (11.2)				
1:4	27 (11.2)				
1:8	34 (14.0)				
1:16	26 (10.7)				
1:32	27 (11.2)				
1:64	35 (14.5)				
1:128	25 (10.3)				
1:256	10 (4.1)				
1:512	7 (2.9)				
ABO IgM titer (%)					
<2	6 (2.8)				
1:1	13 (6.1)				
1:2	13 (6.1)				
1:4	34 (16.0)				
1:8	27 (12.7)				
1:16	47 (22.2)				
1:32	32 (15.1)				

Table 1. Continued.

	ABO-incompatible	ABO-compatible living donor	ABO-compatible deceased donor	*P*-value vs. living donor	*P*-value vs. deceased donor
1:64	16 (7.5)				
1:128	17 (8.0)				
1:256	7 (3.3)				
Transplant center (%)					
A	11 (3.7)	44 (3.7)	44 (3.7)	1.00	1.00
B	19 (6.4)	76 (6.4)	76 (6.4)		
C	111 (37.5)	444 (37.5)	444 (37.5)		
D	50 (16.9)	200 (16.9)	200 (16.9)		
E	31 (10.5)	124 (10.5)	124 (10.5)		
F	74 (25.0)	296 (25.0)	296 (25.0)		
Year of transplantation (median [IQR])	2014 [2011, 2016]	2014 [2011, 2017]	2014 [2011, 2017]	0.048	0.055
Induction therapy (%)					
Alemtuzumab	92 (31.1)	1 (0.1)	2 (0.2)	<0.001	<0.001
Alemtuzumab + bortezomib	5 (1.7)	0 (0.0)	0 (0.0)		
Basiliximab	1 (0.3)	766 (64.7)	743 (62.8)		
Basiliximab + alemtuzumab	0 (0.0)	0 (0.0)	1 (0.1)		
rATG	1 (0.3)	17 (1.4)	46 (3.9)		
None	0 (0.0)	389 (32.9)	388 (32.8)		
Rituximab	146 (49.3)	11 (0.9)	9 (0.8)		
Rituximab + basiliximab	50 (16.9)	0 (0.0)	0 (0.0)		
Rituximab + basiliximab + eculizumab	1 (0.3)	0 (0.0)	0 (0.0)		

BMI, body mass index; HLA, human leukocyte antigen; IQR, interquartile range; PRA, panel reactive antibodies; rATG, rabbit anti-thymocyte globulin; SD, standard deviation.

In addition, we carried out two explorative analyses on induction therapy and recipient blood group, respectively. First, we limited the analysis to the three most commonly used induction regimens: rituximab, combination of rituximab/basiliximab, and alemtuzumab. To ensure comparability of results, administrative censoring at 5-year follow-up was performed in all three groups. As the induction therapies were determined by center and era, we did not expect that propensity score adjustment would alleviate confounding bias. We chose to estimate crude cumulative incidences and hazard ratios instead. Secondly, we performed a similar explorative analysis on ABOi recipient blood groups O, A, and B respectively.

Software and data

All analyses were performed on a digital research (DRE) platform, a secure cloud-based data analysis platform on Microsoft Azure architecture (www.andrea-consortium.org). We used dagitty.net (version 2.3), R version 3.5.1 with the RStudio shell (version 1.1.463).

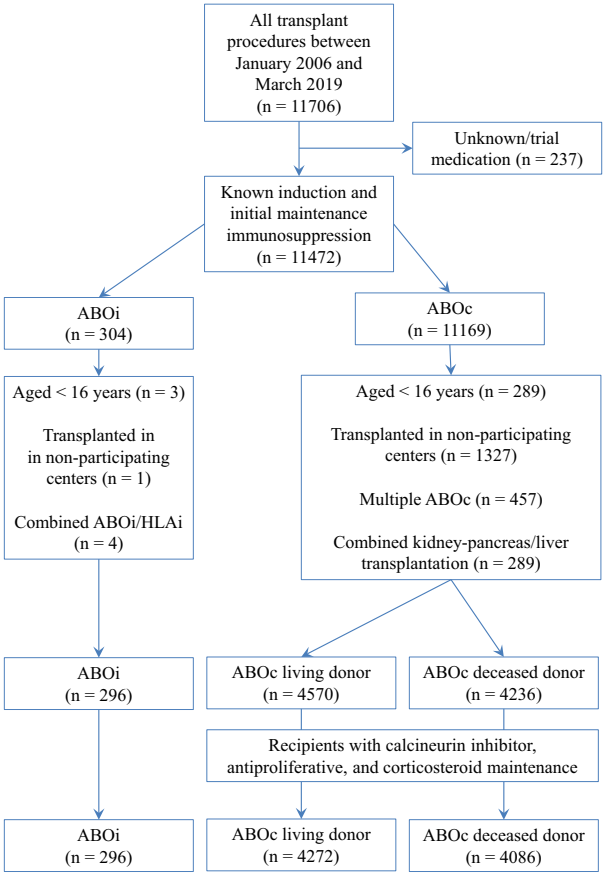
Results

Composition of the study cohort

Between January 2006 and March 2019, 11 706 kidney transplantations were performed. A total of 1 ABOi and 1327 ABOc procedures were excluded for reasons mentioned in Fig. 1. Two centers were excluded from the analysis as one performed only 1 ABOi procedure, and the other did not perform ABOi procedures at all. Of the remaining 8806 unique ABOc recipients, 448 were excluded because they did not receive standard maintenance immunosuppressive therapies with calcineurin inhibitors. In the end, 296 ABOi transplant recipients were included for matching as were 4272 ABOc living donor and 4086 ABOc deceased donor recipients. Table S2 describes baseline characteristics of this total, unmatched cohort.

Causal model and adjustment set

The final causal model is presented in Fig. S1. The implied conditional independencies and regressions for



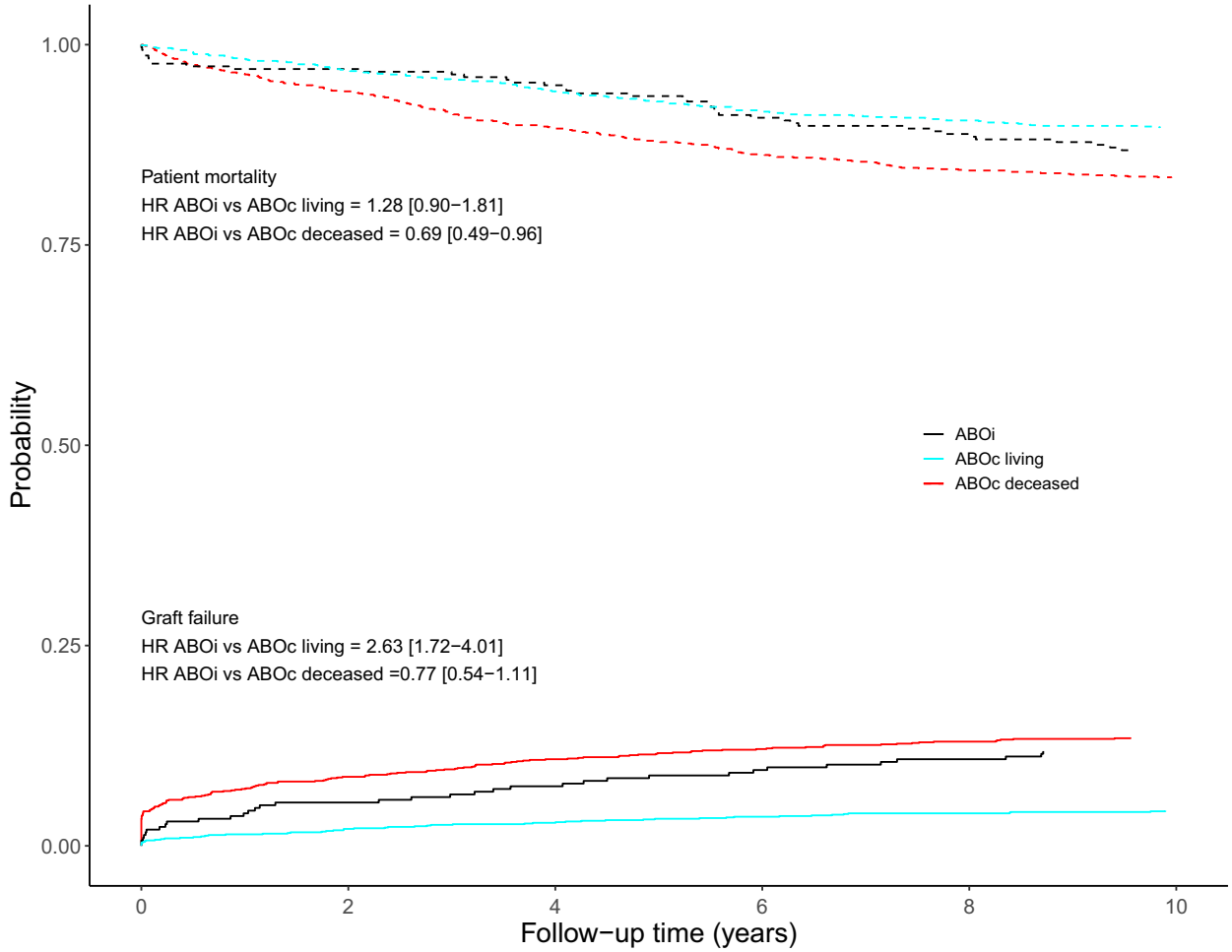
	years	0	2	4	6	8	10
ABOi		296	231	160	106	59	19
ABOc living donor		1184	877	574	380	224	108
ABOc deceased donor		1184	783	509	285	164	84

Figure 1 Composition of the study cohort.

this model are shown in Table S3. From the final causal model, the adjustment set included recipient age, sex, blood group, and peak panel reactive antibodies (PRA).

Comparison of ABOi versus matched ABOc living donor versus matched ABOc deceased donor recipients

In total, 296 ABOi transplant recipients were matched to 1184 ABOc living donor and 1184 ABOc deceased donor recipients. Recipient age, sex, donor age and sex, peak PRA, and number of HLA mismatches were comparable for ABOi and ABOc living donor (Table 1). Dialysis vintage was longest for ABOc deceased donor recipients (median 1152 vs. 216 days in ABOi vs. 99 days in ABOc living donor, $P < 0.001$). Retransplantation rate was highest for ABOi (16% vs. 10% in ABOc deceased donor and 5% in ABOc living donor, $P < 0.001$). Compared with ABOc deceased donor, ABOi recipients were younger (mean age 54 vs. 58 years, P: 0.003), with fewer male donors (43% vs. 56%, $P < 0.001$) and more HLA mismatches (total 3.5 vs. 2.8 mismatches, $P < 0.001$). Diabetic nephropathy as primary kidney disease was more common in ABOc deceased donor recipients (12.8% vs. 6.4% and 8.7% in ABOi and ABOc living donor recipients respectively, $P < 0.001$). Induction therapy in both ABOc living donor and ABOc deceased donor was basiliximab in two-thirds and none in approximately one-third of the procedures. Figure S2 shows these baseline characteristics with density plots. Baseline titers in ABOi recipients were median 1:16 for both IgG and IgM.



	years	0	1	2	3	4	5
Rituximab		146	136	131	126	114	97
Rituximab/ basiliximab		50	36	26	12	3	1
Alemtuzumab		92	85	71	46	38	21

Figure 2 Patient survival and cumulative incidence of graft failure in blood group incompatible (ABOi) kidney transplant recipients compared with matched blood group compatible (ABOc) living and deceased donor kidney transplant recipients. Outcomes for ABOi kidney transplant recipients ($n = 296$) were compared with propensity-matched ABOc recipients ($n = 1184$) from the same centers, with living and with deceased donors. The matching variables included the following: recipient age, peak panel reactive antibody levels, recipient blood group, and recipient sex. Kidney transplant recipients of an ABOi donor are marked in black, recipients with an ABOc living donor are marked in light blue, and recipients with an ABOc deceased donor are marked in red. The dashed lines represent patient survival with a functioning graft, and the solid lines represent kidney graft failure with patient death considered as a competing event.

Patient and graft survival after ABOi kidney transplantation

Patient survival in ABOi recipients was comparable with ABOc living donor recipients, with a hazard ratio (HR) for death of 1.28 [95% confidence interval (95% CI) 0.90–1.81, Fig. 2]. Patient survival was higher in ABOi compared with ABOc deceased donor recipients with a HR 0.69 [95% CI 0.49–0.96]. Cumulative incidence of mortality with a functioning graft was 3.0%, 6.4%, and 13.5% at 1, 5, and 10 years after ABOi transplantation. Cumulative incidence was similar for ABOc living donor recipients with 1.6%, 7.0%, and 10.4% at 1-, 5-, and 10-year follow-up. Mortality with a functioning graft was 3.7%, 6.4%, and 16.6% in ABOc deceased donor recipients.

Rate of graft failure after ABOi was higher compared with ABOc living donor transplantation [HR 2.63 (1.72–4.01)] (Fig. 2). Rate of graft failure in ABOi was not significantly different from that after ABOc

Transplant International 2021; 34: 2706–2719

Table 2. Baseline characteristics of ABOi recipients according to induction therapy.

	Rituximab	Rituximab/basiliximab	Alemtuzumab	*P*-value
N	146	50	92	
Recipient age (years) (median [IQR])	54.0 [44.0, 62.8]	55.5 [46.0, 61.0]	57.5 [45.0, 67.0]	0.049
Recipient sex: male (%)	102 (69.9)	32 (64.0)	59 (64.1)	0.58
Recipient BMI (kg/m^2) (mean (SD))	25.20 (3.72)	25.37 (3.41)	26.62 (5.04)	0.04
Primary kidney disease (%)				
Diabetic nephropathy	9 (6.2)	3 (6.0)	7 (7.6)	0.26
Glomerulonephritis	35 (24.0)	13 (26.0)	19 (20.7)	
Urologic	2 (1.4)	1 (2.0)	3 (3.3)	
Polycystic kidney disease	27 (18.5)	11 (22.0)	17 (18.5)	
Vascular	39 (26.7)	15 (30.0)	13 (14.1)	
Other/not reported	27 (18.5)	5 (10.0)	23 (25.0)	
Hereditary nephropathies	7 (4.8)	2 (4.0)	8 (8.7)	
Preemptive	43 (29.5)	26 (52)	45 (48.9)	0.002
Time on dialysis (days) (median [IQR])	325 [0, 568]	184 [0, 572]	47 [0, 551]	0.03
Previous transplantation (*n*) (mean (SD))	1.17 (0.45)	1.14 (0.40)	1.14 (0.64)	0.88
Recipient blood group (%)				
A	22 (15.1)	9 (18.0)	26 (28.3)	0.16
B	24 (16.4)	8 (16.0)	11 (12.0)	
O	100 (68.5)	33 (66.0)	55 (59.8)	
Donor age (years) (median [IQR])	54.0 [43.0, 64.0]	57.0 [50.0, 63.8]	55.0 [45.0, 61.3]	0.37
Donor sex: male (%)	67 (45.9)	24 (48.0)	33 (35.9)	0.23
Total HLA mismatches (*n*) (mean (SD))	3.37 (1.39)	3.77 (1.27)	3.50 (0.71)	0.49
Peak PRA (%)				
1–4%	127 (87.0)	46 (92.0)	80 (87.0)	0.44
5–84%	18 (12.3)	3 (6.0)	9 (9.8)	
85–100%	1 (0.7)	1 (2.0)	3 (3.3)	
ABO IgG titer (%)				
<1	11 (8.9)	1 (3.0)	11 (14.3)	0.001
1:1	0 (0.0)	1 (3.0)	0 (0.0)	
1:2	16 (12.9)	0 (0.0)	11 (14.3)	
1:4	11 (8.9)	8 (24.2)	8 (10.4)	
1:8	16 (12.9)	11 (33.3)	5 (6.5)	
1:16	9 (7.3)	3 (9.1)	13 (16.9)	
1:32	18 (14.5)	3 (9.1)	6 (7.8)	
1:64	19 (15.3)	3 (9.1)	13 (16.9)	
1:128	15 (12.1)	3 (9.1)	5 (6.5)	
1:256	5 (4.0)	0 (0.0)	4 (5.2)	
1:512	4 (3.2)	0 (0.0)	1 (1.3)	
ABO IgM titer (%)				
<2	2 (1.8)	1 (5.9)	2 (2.6)	0.078
1:1	8 (7.1)	1 (5.9)	4 (5.2)	
1:2	3 (2.7)	2 (11.8)	8 (10.4)	
1:4	15 (13.4)	4 (23.5)	15 (19.5)	
1:8	12 (10.7)	4 (23.5)	11 (14.3)	
1:16	25 (22.3)	3 (17.6)	19 (24.7)	
1:32	21 (18.8)	1 (5.9)	7 (9.1)	
1:64	11 (9.8)	0 (0.0)	5 (6.5)	
1:128	14 (12.5)	0 (0.0)	2 (2.6)	
1:256	1 (0.9)	1 (5.9)	4 (5.2)	

deceased donor transplantation [HR 0.77 (0.54–1.11)]. Cumulative incidence of graft failure in ABOi was 4.1%, 8.8%, and 11.8% at 1-, 5-, and 10-year follow-up. By comparison, it was 7.1%, 11.6%, and 13.5% in ABOc deceased donor and 1.4%, 3.4%, and 4.4% in ABOc living donor recipients. Accordingly, eGFR at 1 year was 49.7 (SD 17.6) in ABOi compared with 55.1 (SD 17.1) in ABOc living donor ($P < 0.001$) and 48.9

Table 2. Continued.

	Rituximab	Rituximab/basiliximab	Alemtuzumab	*P*-value
Transplant center (%)				
A	8 (5.5)	0 (0.0)	2 (2.2)	<0.001
B	15 (10.3)	4 (8.0)	0 (0.0)	
C	86 (58.9)	0 (0.0)	25 (27.2)	
D	20 (13.7)	30 (60.0)	0 (0.0)	
E	14 (9.6)	16 (32.0)	0 (0.0)	
F	3 (2.1)	0 (0.0)	65 (70.7)	
Year of transplantation (median [IQR])	2011 [2009, 2013]	2017 [2016, 2018]	2015 [2013, 2017]	<0.001

BMI, body mass index; HLA, human leukocyte antigen; IQR, interquartile range; PRA, panel reactive antibodies; SD, standard deviation.

ml/min per 1.73 m^2 (SD 18.1) in ABOc deceased donor recipients (*P* 0.51). Proteinuria at year 1 was similar in all three groups [0.10 g/l median (IQR 0.0–0.20)]. Rejection occurred in 29%, 18%, and 19% of ABOi, ABOc living donor, and ABOc deceased donor recipients, respectively (*P*: 0.001). Baseline IgM titers correlated with rejection ($P = 0.0004$), whereas IgG titers had a non-significant impact on rejection ($P = 0.08$, Table S4). Graft survival was lowest in high IgG titers, with a HR for graft failure of 2.22 [0.81–6.10] in titers ≥1:128 vs. titers ≤1:8, Fig. S3a,b.

Sensitivity analysis

Sensitivity analysis where time on dialysis was added to the propensity score matching gave similar results to the model without dialysis vintage as a confounder (Fig. S4). HRs for patient death were 1.21 [0.85–1.71] for ABOi versus ABOc living donor, and 0.78 [0.55–1.09] for ABOi versus ABOc deceased donor recipients. Graft failure rate was 2.56 [1.68–3.90] for ABOi versus ABOc living donor and 0.81 [0.56–1.17] for ABOi versus ABOc deceased donor transplantation. Adjustment for diabetes mellitus as primary kidney disease gave similar results to the model without diabetes as a confounder (Fig. S5).

Induction therapy for ABOi kidney transplantation

Of the 296 ABOi recipients, 146 received rituximab, 50 the combination rituximab/basiliximab, and 92 alemtuzumab. Remaining recipients received either alemtuzumab/bortezomib ($n = 5$), basiliximab only ($n = 1$), rATG ($n = 1$), or rituximab/basiliximab/eculizumab ($n = 1$). As these numbers were very low, they were excluded in the induction analysis. Table 2 shows baseline characteristics of ABOi recipients by induction. Alemtuzumab-treated recipients were older, 57.5 vs. 54.0 and 55.5 in rituximab and rituximab/basiliximab, respectively (*P*: 0.049). Rituximab-treated recipients were less often transplanted pre-emptively, 29.5% vs. 52% of rituximab/basiliximab and 48.9% of alemtuzumab-treated recipients (*P*: 0.02). Of note is the earlier median year of transplantation for rituximab, 2011, vs. 2017 in rituximab/basiliximab and 2015 for alemtuzumab. This resulted in longer follow-up of 6.6 [IQR 4.2, 9.0] years for rituximab vs. 1.97 and 3.00 years for rituximab/basiliximab and alemtuzumab-treated recipients. Follow-up was therefore truncated at 5 years.

When compared to rituximab, patient survival was not statistically different for the other induction regimens. Respective HRs were 2.01 [0.53–7.55] and [1.12 [0.52–2.41] for rituximab/basiliximab and alemtuzumab compared with rituximab (Fig. 3). However, graft survival trended to be superior for rituximab/basiliximab and alemtuzumab compared with rituximab with HR for graft failure of 0.31 [0.04–2.41] and 0.84 [0.35–2.05], respectively. Rejection occurred in 47% of rituximab vs. 22% of rituximab/basiliximab vs. 4% of alemtuzumab-treated recipients ($P < 0.001$). Renal function at 1 year was better for rituximab/basiliximab and alemtuzumab with eGFR of 51.0 (SD 19.6) and 52.8 (SD 17.4) respectively compared with 47.3 ml/min per 1.73 m^2 (SD 17.0) in rituximab (*P*: 0.06). Table 3 shows causes of death by induction (follow-up truncated at 5 years). An infectious cause of death was observed in 2 out of 10 in rituximab, versus none in both rituximab/basiliximab and alemtuzumab. Malignancy was cause of death in 1 out of 6 in rituximab, vs. 1 out of 2 in rituximab/basiliximab and 2 out of 6 in alemtuzumab-treated recipients. BK nephropathy

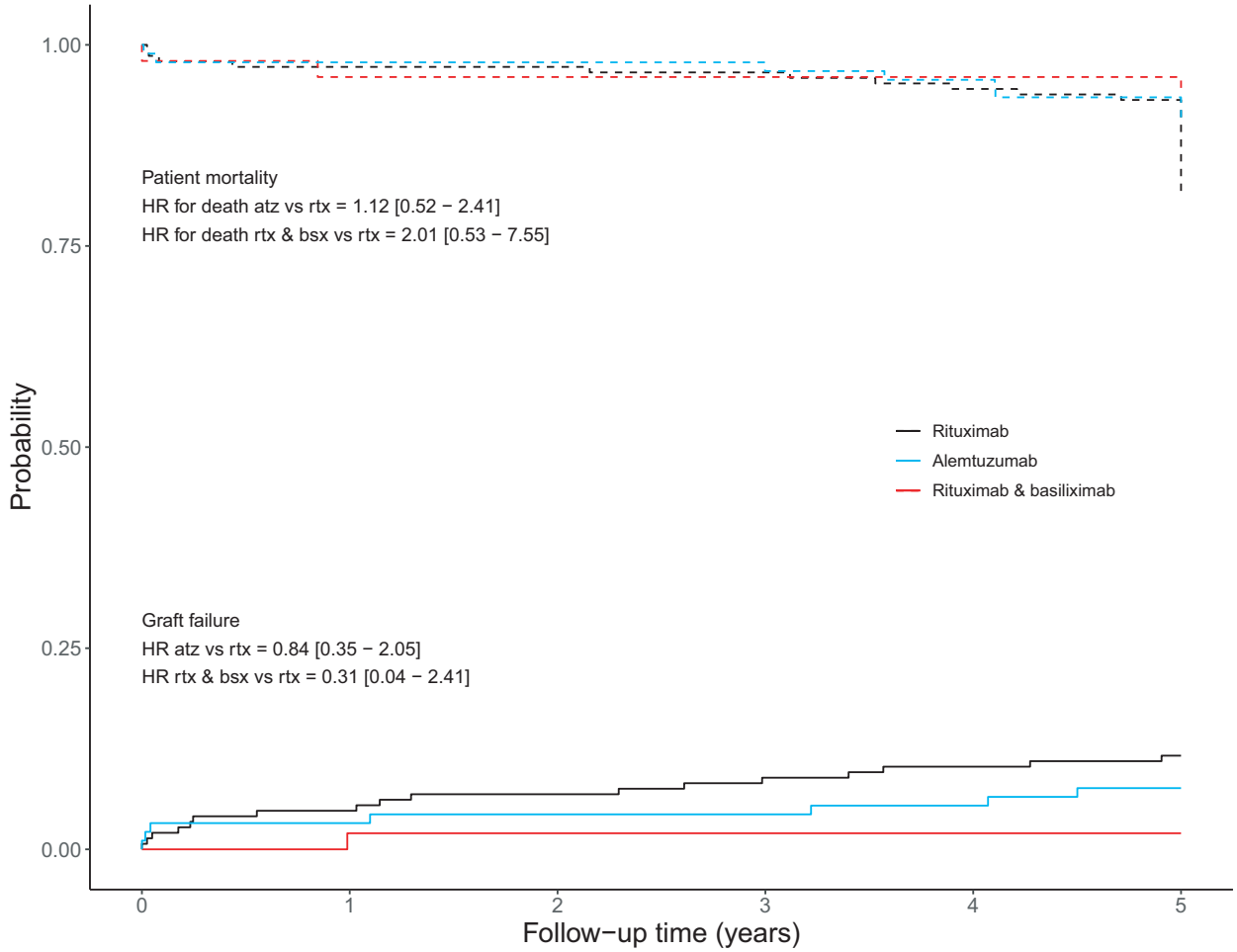


Figure 3 Patient survival and cumulative incidence of graft failure in blood group incompatible (ABOi) kidney transplant recipients by induction therapy. Outcomes for ABOi kidney transplant recipients were compared according to the different induction regimens: rituximab ($n = 146$, black), rituximab/basiliximab ($n = 50$, red), and alemtuzumab ($n = 92$, light blue). The dashed lines represent patient survival with a functioning graft, and the solid lines represent kidney graft failure with patient death considered as a competing event.

Table 3. Cause of death in ABO-incompatible kidney transplant recipients stratified for induction therapy.

	Rituximab ($n = 146$)	Rituximab/basiliximab ($n = 50$)	Alemtuzumab ($n = 92$)
Follow-up duration (years) (median, [IQR])*	5.0 [4.2, 5.0]	2.0 [1.0, 3.0]	3.0 [2.0, 5.0]
Total deaths	10	2	6
Cardiovascular	2 (20.0)	1 (50.0)	0 (0.0)
Infection	2 (20.0)	0 (0.0)	0 (0.0)
Malignancy	1 (10.0)	1 (50.0)	2 (33.3)
Other	3 (30.0)	0 (0.0)	2 (33.3)
Unknown	2 (20.0)	0 (0.0)	2 (33.3)

*Note that follow-up was truncated at 5 years.

was histologically diagnosed in seven patients, respectively, in 4% of rituximab, 4% of rituximab/basiliximab, and 0% of alemtuzumab-treated recipients. A sensitivity analysis excluding rituximab induction is depicted in Fig. S6.

Recipient blood group in ABOi kidney transplantation

Compared with blood group A, O recipients had, although not significant, the highest crude cumulative

incidence of graft failure with HR of graft failure of 2.07 [0.61–6.96] (Fig. S7 and Table S5). HR of graft failure for blood group B recipients compared with A was 1.35 [0.27–6.67].

Discussion

We performed a propensity score-matched analysis of patient and graft survival in a large cohort of ABOi kidney transplant recipients and compared this with ABOc living and ABOc deceased donor recipients. A total of 296 patients who underwent ABOi kidney transplantation were compared with respectively 1184 ABOc living donor and1184 ABOc deceased donor procedures. We showed that patient survival with a functioning graft after ABOi was superior to ABOc deceased donor transplantation. Death-censored graft survival was similar in these groups. Conversely, graft survival in ABOi kidney transplant recipients was inferior to ABOc living donor recipients, while patient survival was similar.

Living donor kidney transplantation programs result in inequity for blood group O recipients by allowing "universal donor" O donation to all blood group combinations. Deceased donor programs try to overcome this unbalance by ABO-identical allocation. Nevertheless, UK transplant registry O candidates wait twice as long as A candidates and four times longer than AB candidates [16]. The longer waiting list for O recipients warrants desensitization programs. However, recent meta-analyses have shown that outcomes after desensitization for ABOi kidney transplantation are inferior to ABOc living donor transplantation [7,8]. Yet, the comparison with ABOc living donation does not paint a complete picture. Our study demonstrates that ABOi recipients have better patient survival than recipients of a deceased donor transplant. This is in line with the recent publication from the United States by Massie *et al.* [9], demonstrating superior patient survival compared with matched waiting list candidates who did or did not proceed to kidney transplantation. This means that both in an American and in an European cohort, a survival benefit of ABO-incompatible kidney transplantation over deceased donor transplantation has been demonstrated, a concordance that has not been found in HLA-incompatible kidney transplantation [17,18].

These outcomes affirm current policies to prefer ABOi living donor over ABOc deceased donor transplantation. It justifies proceeding with and development of desensitization programs when an ABOi living donor is available.

Although in line with meta-analyses, the inferior graft survival compared with ABOc living donor is in contrast by some registry and single-center studies [19–21]. One of the explanations for reported differences in graft survival after ABOi kidney transplantation could be blood group distribution. Blood group O recipients tended to have inferior outcomes in our explorative analysis, which is in line with other reports on adverse outcomes in O recipients, possibly mediated by higher anti-A/B titers [22,23]. These findings underscore the importance of reporting recipient blood group in ABOi literature. In our study, two-thirds of the ABOi recipients had blood group O. This high percentage is due to a relatively high distribution of blood group O in the Netherlands of approximately 47% [24] and because non-O recipients are more successful in the national kidney exchange program [1].

Another explanation for the inferior graft survival after ABOi as opposed to ABOc living donor transplantation might be rituximab induction without interleukin-2 (IL-2) receptor blockade (IL-2RAb). Rituximab/basiliximab or alemtuzumab induction trended toward better graft survival compared with rituximab alone. The survival analysis excluding rituximab induction, however, still revealed inferior graft survival as compared to *matched* ABOc living donor transplantation. When ABOi kidney transplantation was launched in Europe in the first decade of the 21th century, no standard induction therapy was administered to low- and moderate-risk ABOc recipients. Basiliximab became standard after an extensive Cochrane review on the use of IL-2RAb. This review showed that although there was no difference in mortality, risk of graft loss at year one was reduced compared with no induction [25]. An ABOi kidney transplantation registry by Opelz *et al.* [26] did not show a benefit of adding basiliximab to rituximab. Nevertheless, in our study evidently less rejection and a trend toward better graft survival and renal function with combined T- and B-cell directed induction can be distinguished. The beneficial effect of the addition of basiliximab to rituximab in ABOi as compared to the modest improvement in graft survival with basiliximab in the ABOc Cochrane review suggests an additional immunological risk in ABOi. This is in contrast to the original Swedish protocol that propagated ABOi kidney transplantation reporting very low rejection rates [27], but the higher rejection rate in our ABOi compared with ABOc recipients is in line with later reports describing cellular rejection rates in up to one-third of all ABOi kidney transplantation [28,29]. Higher rejection rates were also observed for rituximab/basiliximab versus rituximab/ATG in a single-center ABOi

induction comparison, however, with similar graft survival [30].

The anti-CD52 molecule alemtuzumab also targeting monocytes and NK cells was administered in roughly one-third of ABOi recipients in our study. Although alemtuzumab has gained interest as induction therapy by halving acute rejection episodes in ABOc kidney transplantation [31], its use in ABOi kidney transplantation is rare [5]. During the long follow-up of up to 5 years in alemtuzumab-treated patients in our study, we observed a low rejection rate and similar graft survival as in ABOc living donor transplantation without an increase of infection as cause of death. The lower rejection rate in our ABOi cohort of recipients treated with alemtuzumab versus rituximab/basiliximab induction raises the question whether innate immune cells contribute to higher rejection rates after ABOi kidney transplantation [32–34]. As blood group A/B epitopes are so-called T-cell-independent epitopes, rapid IgM production can occur without T-cell help. Innate immune cells are necessary for IgG class switch and for the production of long-lived plasma cells [35]. In future studies, the benefit of targeting the innate immune system of ABO-incompatible kidney transplant recipients with for example alemtuzumab should be further explored [33].

A strength of our study is the propensity matching of both ABOc deceased donor and ABOc living donor controls in a large group of kidney transplant recipients from multiple centers. The national registry and verification in all participating centers led to complete ABOi data that could be compared with control recipients from the same center to alleviate residual bias. In the Netherlands, a national kidney exchange program is operative and ABOi couples are advised to participate in this program for two rounds, so chances to find an ABOc living donor had been exhausted in this study. An important consideration is that the vast majority of the ABOc cohort received basiliximab induction, which is in line with other European centers administering IL-2RAb to low- and standard-risk recipients. This practice differs from the United States where the percentage of recipients receiving lymphocyte depleting therapy is almost 75% [36].

A limitation to this study is that it is a retrospective analysis. Induction depended on center and era and follow-up differed. The survival analysis excluding rituximab only was center-biased, and the graft survival in controls had also improved over time. Rituximab was administered in the earlier period of ABOi, and rituximab/basiliximab and alemtuzumab in later years. Treatment for rejection without biopsy confirmation was also included in the outcome definition of rejection. When comparing patient survival of ABOi to deceased donor transplantation, we did not account for immortal time bias: candidates dying while waiting for a deceased donor transplant were not included in the comparison. We did perform a sensitivity analysis on dialysis; however, dialysis vintage remained different between ABOi and ABOc living and ABOc deceased donor recipients. Next, blood group analysis was not propensity matched and selection by indication is a limitation in these two analyses. Information on donor A2 blood group was not standard available.

To conclude, this study demonstrates improved patient survival with ABOi compared with matched ABOc deceased donor kidney transplantation. However, graft survival is inferior to matched living donor ABOc, supporting the continued effort to find an ABOc living donor. The addition of T-cell-targeted induction next to B-cell depletion reduces rejection, underscoring the need for further studies into risk stratification and induction therapy. It is high time for a randomized controlled trial in ABOi kidney transplantation.

Authorship

AW, MJ and MB: conceived the idea to perform this study. AW, JB, HB, AV, PD, JS, MC, FR, AZ, FB, AN, MA, MGB, MJ and MB: took part in acquisition of the data. AW, JB, MJ, and MB: had full access to the data (digital research environment) and take responsibility for the integrity of the data and the data analysis. JB: performed the statistical analysis. AW, JB, MJ, and MB: drafted the manuscript. AW, JB, HB, AV, PD, JS, MC, FR, AZ, FB, AN, MA, MGB, MJ and MB: critically revised the manuscript and approved the final version.

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Conflicts of interest

Jan AJG van den Brand is part-time employed by and has stock options for Binnovate Digital Health B.V. Binnovate Digital Health was not involved in the present research. The other authors of this manuscript have no conflicts of interest to disclose as described by *Transplant International*.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendix S1. Supplemental materials.

Table S1. Treatment protocols in the six centers performing ABO-incompatible kidney transplantation.

Table S2. Baseline characteristics of the unmatched cohort.

Table S3. Conditional independencies.

Table S4. Correlation of baseline anti-A/B titers and the occurrence of rejection in ABO-incompatible kidney transplant recipients.

Table S5. Baseline characteristics of the ABOi group by recipient blood group.

Figure S1. Directed acyclic graph of the causal model.

Figure S2. Baseline characteristics of the matched cohort represented with histograms.

Figure S3. (a) Patient survival and cumulative incidence of graft failure in blood group incompatible (ABOi) kidney transplant recipients according to baseline anti-A/B IgG titers. (b) Patient survival and cumulative incidence of graft failure in blood group incompatible (ABOi) kidney transplant recipients according to baseline anti-A/B IgM titers.

Figure S4. Sensitivity analysis of patient survival and cumulative incidence of graft failure in blood group incompatible (ABOi) kidney transplant recipients compared to matched blood group compatible (ABOc) living and deceased donor kidney transplant recipients; *dialysis duration prior to transplantation added to the propensity-score matching*.

Figure S5. Sensitivity analysis of patient survival and cumulative incidence of graft failure in blood group incompatible (ABOi) kidney transplant recipients compared to matched blood group compatible (ABOc) living and deceased donor kidney transplant recipients; *diabetic nephropathy as primary kidney disease added to the propensity-score matching*.

Figure S6. Sensitivity analysis of patient survival and cumulative incidence of graft failure in blood group incompatible (ABOi) kidney transplant recipients compared to matched blood group compatible (ABOc) living and deceased donor kidney transplant recipients; *rituximab induction was excluded from the analysis*.

Figure S7. Patient survival and cumulative incidence of graft failure in blood group incompatible (ABOi) kidney transplant recipients by recipient blood group.

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