Transplant outcomes of 100 cases of living-donor ABO-incompatible kidney transplantation

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Abstract

Background: Although ABO-incompatible (ABOi) kidney transplantation (KT) has been performed successfully, a standard preconditioning regimen has not been established. Based on the initial antidonor ABO antibody titers, an individualized preconditioning regimen is developed, and this study explored the efficacy and safety of the regimen.

Methods: From September 1, 2014, to September 1, 2020, we performed 1668 consecutive living-donor KTs, including 100 ABOi and 1568 ABO-compatible (ABOc) KTs. ABOi KT recipients (KTRs) with a lower antibody titer (≤1:8) were administered oral immunosuppressive drugs (OIs) before KT, while patients with a medium titer (1:16) received OIs plus antibody-removal therapy (plasma exchange/double-filtration plasmapheresis), patients with a higher titer (≥1:32) were in addition received rituximab (Rit). Competing risk analyses were conducted to estimate the cumulative incidence of infection, acute rejection (AR), graft loss, and patient death.

Results: After propensity score analyses, 100 ABOi KTRs and 200 matched ABOc KTRs were selected. There were no significant differences in graft and patient survival between the ABOi and ABOc groups ($P = 0.787$, $P = 0.386$, respectively). After using the individualized preconditioning regimen, ABOi KTRs showed a similar cumulative incidence of AR (10.0% *vs.* 10.5%, $P = 0.346$). Among the ABOi KTRs, the Rit-free group had a similar cumulative incidence of AR ($P = 0.714$) compared to that of the Rit-treated group. Multivariate competing risk analyses revealed that a Rit-free regimen reduced the risk of infection (HR: 0.31; 95% CI: 0.12–0.78, $P = 0.013$). Notably, antibody titer rebound was more common in ABOi KTRs receiving a Rit-free preconditioning regimen ($P = 0.013$) than those receiving Rit. ABOi KTRs with antibody titer rebound had a 2.72-fold risk of AR (HR: 2.72, 95% CI: 1.01–7.31, $P = 0.048$). ABOi KTRs had similar serum creatinine and estimated glomerular filtration rate compared to those of ABOc KTRs after the first year.

Conclusions: An individualized preconditioning regimen can achieve comparable graft and patient survival rates in ABOi KT with ABOc KT. Rit-free preconditioning effectively prevented AR without increasing the risk of infectious events in those with lower initial titers; however, antibody titer rebound should be monitored.

Keywords: ABO blood-group system; Kidney transplantation; Rituximab; Immunologic desensitization

Introduction

Living-donor kidney transplantation (KT) shows higher graft survival and patient survival than deceased-donor KT.[1] To overcome the shortage of organ donors, living-donor ABO-incompatible (ABOi) transplantation has been employed.[2,3] Recent developments and improvements in preconditioning therapy have succeeded in decreasing the titers of antibodies and achieving comparable transplant outcomes with those of ABO-compatible (ABOc) KT.[4,5]

The main principles of preconditioning are as follows: the depletion of pre-transplant ABO antibody using selective or semi-selective immunoadsorption, plasma exchange (PE), or double-filtration plasmapheresis (DFPP), and inhibiting its recurrence by eliminating B cells. However, no standardized preconditioning regimen has been established to date. There is a substantial variation in preconditioning regimens in clinical practice globally, including different combinations of available preconditioning forms, dosing, and frequency.[6-8] Despite reducing the risk of antibody-medicated rejection (AMR), intensive immunosuppression causes a higher incidence of infections.[9] A recently published meta-analysis reported an increased incidence of death among ABOi KT recipients (KTRs) due to severe bacterial and viral infections.[10] Whether all ABOi KT candidates should undergo antibody removal or B lymphocyte elimination and how to select individualized optimal preconditioning strategies scientifically remain unclear.

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Recently, based on the initial antidonor blood IgM and IgG titers, an individualized preconditioning regimen is developed.[11] This simplified regimen reduced the potential morbidity, minimized the immunosuppression burden, and reduced the costs. Here, we further examined the efficacy and safety of an individualized preconditioning regimen with a larger patient number and extended follow-up.

Methods

Study population

This study was approved by the Ethics Committee of the West China Hospital (No. 2019SHEN418). All recipients gave their written informed consent prior to KTs. From September 1, 2014, to September 1, 2020, we performed 1668 consecutive living-donor KTs, including 100 ABOi and 1568 ABOc KTs.

Individualized preconditioning regimen

The individualized preconditioning regimen has been explained in detail in our previous study.[11] Our individualized regimen has four stages: imitation (case 1, September 1, 2014), exploration (cases 2–19, December 2014–June 2016), improvement (cases 20–34, June 2016–April 2017), and stabilization (case 35 and later, May 2017–now).

As reported previously, the frequency of subtype A2 in East Asian populations was <1%. Hence, we did not subtype blood group A into A1 or A2.[11] In the study period, a gel card technology is only used to measure the antidonor IgG titers. Initially IgM titers were measured using a tube test for cases 1 to 19, then a gel card test was used. Based on the initial IgM and IgG titers, an individual preconditioning protocol was administered. When the initial antibody titers were ≤ 1:8 (lower titer), ABOi KT candidates received only oral immunosuppressants (OIs) for 2 to 4 weeks before transplantation. When the antibody titer was 1:16, in addition to OIs they received antibody-removal treatment, either PE or DFPP. When the antibody titer reached ≥1:32 (higher titer), rituximab (Rit) (MabThera, Roche, Shanghai, China) was additionally required. A single dose of 200 mg Rit was administered 2 to 4 weeks before KT with an additional 100 mg if $CD19^+CD5^+$ B cell count reached >10/μL 1 week after the first dose. After transplantation, antibody titers were monitored on days 1,3,5, and 7 within the first week, every week within the first month, and every month for 1 to 12 months within the first year. Rebound titer was defined as an antibody titer of ≥16 after 2 weeks of transplant for patients with an antibody titer of < 16 at transplantation. For patients with antibody titers ≥16 (such as 16 and 32) at transplantation, the rebound titer was defined as when the antibody titer increased compared with that at transplantation. Post-transplant PE/DFPP was performed only when the antibody titer was at least 1:32 or AMR was diagnosed.

Immunosuppressive therapy

As described in our previous study,[12] while antithymocyte globulin (ATG), (Sanofi Genzyme, Cambridge, MA, USA, 25 mg administered for 3–7 days) was used as induction therapy in patients receiving repeated KT or with positive pre-transplant panel reactive antibody (PRA), basiliximab (Simulect, Novartis, Switzerland, 20 mg on days 0 and 4) was used as induction therapy in other patients. No induction therapy was administered to patients with fully compatible human leukocyte antigen (HLA) and negative PRA. The triple immunosuppression regimen for KTRs consisted of tacrolimus (Prograf; Astellas Fujisawa, Osaka, Japan), mycophenolate mofetil (MMF) (Cellcept, Roche, Nutley, NJ, USA), enteric-coated mycophenolate sodium (EC-MPS), (Myfortic, Novartis, Switzerland), and prednisone. ABOi KTRs underwent oral immunosuppression 2 to 4 weeks before transplantation. On the day of the transplantation, tacrolimus and prednisone were discontinued, and the dose of mycophenolic acid was increased to 2000 mg/day (MMF) or 1440 mg/day (EC-MPS). ABOc KTRs received 1000 mg MMF/EC-MPS 1 day before transplantation. Intravenous methylprednisolone was administered intraoperatively at a dose of 500 mg, and 200 mg/day on days one to three, followed by oral prednisone (60 mg/day, tapered to 5 mg/day within 2 weeks) for both ABOi and ABOc KTRs. Tacrolimus was reinitiated on post-transplant day two, and the target trough level was 5 to 10 ng/mL for the first 3 months, 4 to 8 ng/mL for months 4 to 12, and 4 to 6 ng/mL thereafter.

Clinical outcomes

The primary outcomes were acute rejection (AR), infection, graft loss, and patient death. Graft loss was defined as the reestablishment of long-term dialysis or estimated glomerular filtration rate (eGFR) of <15 mL/min. AR was diagnosed clinically based on a significant increase in serum creatinine levels of 50% or more within 3 days, which was not explained by other reasons, including BK polyomavirus (BKV) infection, cytomegalovirus (CMV) infection, bacterial urinary tract infection, ureteral stricture, and urinary stones. If necessary, AR was diagnosed by biopsy based on Banff 2009 criteria. Tissue sections were stained with hematoxylin and eosin, methylamine silver periodate, and Masson and visualized under light microscopy. For immunofluorescence experiments, we examined the complement (C) 3, C1q, immunoglobulin G (IgG), IgA, IgM, fibrinogen, and C4d. AR was treated primarily with bolus doses of methylprednisolone and ATG, if refractory. Infection refers to symptoms requiring medical intervention, including wound, pulmonary, urinary tract, skin, BKV infection, and CMV infection. Blood samples were used for CMV quantitative nucleic acid testing using qualitative polymerase chain reaction (PCR). CMV infection was defined as detecting positive CMV pp65 antigenemia and DNA with or without clinical symptoms. Oral immunosuppressive agents were adjusted, and venous ganciclovir was prescribed after the diagnosis. Screening for BKV DNA in serum via PCR was performed routinely in our hospital. Patients were considered positive if the serum BKV PCR was >100 copies/mL. Immunosuppressive therapy was adjusted according to the amount of BKV DNA (>100 copies/mL).

Propensity score analyses

ABOc KTRs were selected as the control group. To limit the potential confounding due to indication and increase

comparability between the ABOi and ABOc groups, we conducted propensity score analyses.[13] Covariate factors included donor and recipient sex, age, body mass index, type of dialysis, induction therapy, and warm ischemic time. The process was performed using the "nearest" method at a ratio of 1:2 using the "Matchit" package of R software (version 4.0.0, https://www.r-project.org/).

Statistical analyses

Continuous data satisfying normal distribution were reported as mean and standard deviation and compared using Student's *t*-test; or was reported as median and range and compared using Wilcoxon rank-sum test. Categorical data are presented as percentages and were compared using the Chi-squared test or Fisher exact test.

As patient death is a competing event with AR, infection, and graft loss, instead of Kaplan-Meier estimates, we used competing risk analysis to estimate the cumulative incidence of AR, infection, and graft loss. The difference in the cumulative incidence was compared using the Fine and Gray method.[14,15] Univariate competing risk analysis was performed to explore the risk factors of AR and infection after ABOi KT. Then, covariates with $P < 0.10$ were used for multivariate analyses.

Several subgroup analyses were performed. To explore whether the stabilized individualized preconditioning scheme contributed to better transplant outcomes (cases 35–100, May 2017–Sep 2020), subgroup analysis was performed between the 66 ABOi KTRs and matched 132 ABOc KTRs. A second subgroup analysis was conducted to explore the impact of Rit on transplant outcomes in ABOi KTRs. The third subgroup analysis explored the impact of initial high IgM titer and high IgG titer on transplant outcomes in ABOi KTRs. The fourth subgroup analysis explored the impact of antibody titer rebound on transplant outcomes. In addition, we explored the impact of anti-A and anti-B antibodies on transplant outcomes. All statistical analyses were performed using the R software (version 4.0.0). *P* values <0.05 were considered statistically significant.

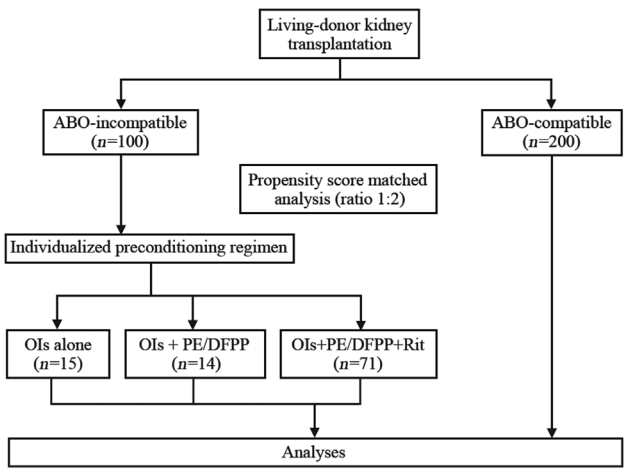

Figure 1: Flow chart of this study. DFPP: Double-filtration plasmapheresis; OIs: Oral Immunosuppressants; PE: Plasma exchange; Rit: Rituximab.

Results

Clinical characteristics

From September 2014,103 candidates received individualized preconditioning, whereas three did not receive ABOi KT. After propensity score analyses, 100 ABOi KTRs and 200 matched ABOc KTRs were selected [Figure 1]]. The baseline characteristics are shown in Table 1. Spouse donors were more common among ABOi KTRs ($P < 0.001$). Higher HLA mismatches were observed, and more patients had negative PRA ($P = 0.026$) in the ABOi group ($P = 0.037$). Among 100 ABOi KTRs, 15 received OIs alone, 14 received OIs plus PE/DFPP, and 71 received OIs plus PE/DFPP with Rit. The median follow-up period for ABOi KTRs was 25.8 months (range: 3.5–75.6 months).

Effect of individualized preconditioning protocol

The efficacy is indicated by a significant reduction in antidonor ABO antibody titers. Rit significantly reduced peripheral blood $CD19^{+}CD5^{+}$ B cell counts and percentages within the first year [Supplementary Figure 1, http://links.lww.com/CM9/B38].

AR and infection

In total, 18 patients in the ABOi group experienced AR. Eleven were confirmed using biopsy, three showed cellmediated rejections and eight showed AMRs. Twenty AR events were observed in the ABOc group, and 12 were biopsy-proven, including six cell-mediated rejections and six AMRs. The onset of AR was earlier in the ABOi group compared to that in the ABOc group (median: 1.0 *vs.* 9.0 months, $P = 0.011$). In the competing risk analysis, the ABOi group had a higher cumulative incidence of AR than the ABOc group (21.9% *vs.* 15.8%, $P = 0.034$) [Supplementary Figure 2A, http://links.lww.com/CM9/B39. However, this difference was only observed within the first year (19.4% *vs.* 7.2%, $P = 0.002$) [Supplementary Figure 3A, C, http://links.lww.com/CM9/B40. Moreover, the recent 66 ABOi KTRs had a similar cumulative incidence of AR compared to that of ABOc KTRs (10.5% *vs.* 10.0%, $P = 0.346$) [Supplementary Figure 4A, C, http://links.lww.com/CM9/B41]. In the multivariate analysis, the first 34 cases had a higher risk of AR than the latter 66 cases (HR: 3.72, 95% CI: 1.40–9.86, $P = 0.005$) [Table 2].

Among 100 ABOi KTRs, 28 developed 30 post-operative infections, including 14 respiratory infections, seven urinary infections, four skin infections, three BK infections, and two CMV infections. Thirty patients developed 31 infections in the ABOc group, including 22 respiratory infections, four urinary infections, three BK infections, one EBV-associated hemophagocytic syndrome, and one B19 infection. Competing risk analysis showed a higher cumulative incidence of infection in the ABOi group (34.5% *vs.* 18.9%, $P = 0.003$) [Supplementary Figure 2B, http://links.lww.com/CM9/B39]. Of note, this difference was only observed within the first year (25.9% *vs.* 11.3%, $P = 0.001$) [Supplementary Figure 3B, D, http://links.lww.com/CM9/B40]. No significant difference was observed at

Table 1: Baseline characteristics of included patients who underwent living-donor kidney transplantations.

Characteristics	ABOc-KT ($N=200$)	ABOi-KT ($N=100$)	Statistics	*P* values
Donor BMI (kg/m^2)	24.1 (2.8)	24.5 (3.3)	1.098*	0.273
Donor gender			0.545†	0.460
Male	56 (28.0%)	24 (24.0%)		
Female	144 (72.0%)	76 (76.0%)		
Recipient age (years)	31.1 (8.3)	32.1 (9.5)	0.909*	0.364
Recipient BMI (kg/m^2)	20.6 (3.1)	21.1 (3.4)	1.314*	0.190
Recipient gender			0.200†	0.655
Male	139 (69.5%)	72 (72.0%)		
Female	61 (30.5%)	28 (28.0%)		
Dialysis			3.590†	0.309
Hemodialysis	166 (83.0%)	88 (88.0%)		
Peritoneal dialysis	19 (9.5%)	9 (9.0%)		
Both	4 (2.0%)	2 (2.0%)		
None	11 (5.5%)	1 (1.0%)		
Duration of dialysis (months)	17.8 (16.6)	20.8 (19.0)	1.413	0.159
HLA mismatch	3.1 (1.1)	3.5 (1.5)	2.303	0.037
Warm ischemic time (seconds)	204.4 (62.9)	196.4 (52.3)	-1.071*	0.285
Volume of kidney graft (mL)	172.5 (35.3)	172.2 (35.8)	-0.070*	0.944
Donor-recipient relationship			16.438†	<0.001
Spouse	0 (0)	8 (8.0%)		
Related	200 (100.0%)	92 (92.0%)		
PRA			4.929†	0.026
Positive	39 (19.5%)	31 (31.0%)		
Negative	161 (80.5%)	69 (69.0%)		
Anti-HLA antibody			7.083†	0.069
Both negative	161 (81.0%)	69 (69.0%)		
HLA-I positive	27 (14.0%)	18 (18.0%)		
HLA-II positive	5 (3.0%)	3 (3.0%)		
Both positive	7 (4.0%)	10 (10.0%)		
Induction therapy			16.642†	0.005
No	36 (18.0%)	11 (11.0%)		
ATG	56 (29.0%)	19 (20.0%)		
Basiliximab	106 (53.0%)	69 (69.0%)		
ABO match				
AB-A	/	10 (10.0%)		
AB-B	/	13 (13.0%)		
AB-O	/	1 (1.0%)		
A-B	/	9 (9.0%)		
B-A	/	10 (10.0%)		
A-O	/	33 (33.0%)		
B-O	/	24 (24.0%)		
Individualized preconditioning regimen				
OIs alone	/	15 (15.0%)		
OIs plus PE/DFPP	/	14 (14.0%)		
OIs plus PE/DFPP with Rit	/	71 (71.0%)		
OIs time (days)	/	21 (7–180)		
PE/DFPP times	/	2 (1–6)		
Dose of Rit (mg)	/	200 (100–500)		
Initial IgM titer	/	32 (1–256)		
Initial IgG titer	/	16 (0–512)		
IgM titer at transplantation	/	4 (0–32)		
IgG titer at transplantation	/	4 (0–32)		
Antibody titer rebound				
Rebound	/	11 (11.0%)		
Stabilized	/	89 (89.0%)		

Data were shown as mean (standard deviation), *n* (percentage), or median (range). * Student's *t*-test. † Chi-squared test. ABOc-KT: ABO-compatible kidney transplantation; ABOi-KT: ABO-incompatible kidney transplantation; ATG: Anti-thymocyte globulin; BMI: Body mass index; DFPP: Double-filtration plasmapheresis; HLA: Human leukocyte antigen; OIs: Oral immunosuppressants; PE: Plasma exchange; PRA: Panel reactive antibody; Rit: Rituximab; IgM: Immunoglobulin M; IgG: Immunoglobulin G; /: Not applicable.

the time of the first infection between ABOi and ABOc groups (median: 3.0 vs 4.0 months, $P = 0.963$).

Patient survival, graft survival, and renal function

Three patients in the ABOi group experienced graft loss (post-transplant 2, 2, and 4 months) compared to five patients in the ABOc group (1.5,12,34,48, and 52 months). No significant difference was observed in the cumulative incidence of graft loss in the competing risk analysis (3.2% *vs.* 6.5%, $P = 0.787$). Three patients died from severe pneumonia in the ABOi group (1, 4, and 4 months post-transplant, respectively). Three patients died in the ABOc group, including two patients with severe pneumonia (1 and 14 months post-transplant, respectively), and one from EBV-associated hemophagocytic syndrome (12 months post-transplant). In the competing risks analysis, the ABOi group had similar patient survival compared to the patients in the ABOc group (3.2% *vs.* 1.8%, $P = 0.386$) [Supplementary Figure 2C, D, http://links.lww.com/CM9/B39].

Patients in the ABOi group had higher serum creatinine levels within the first 6 months and similar levels thereafter compared to those in the ABOc group (1-year: 105.03 ± 32.75 *vs.* 107.08 ± 25.74, 2-year: 103.18 ± 21.26 *vs.* 105.54 ± 38.39, 3-year: 112.88 ± 59.25 *vs.* 105.70 ± 31.38 μmol/L). Correspondingly, the ABOi group had lower eGFR levels within the first year and had comparable results thereafter compared to those of the ABOc group (1-year: 66.22 ± 24.68 *vs.*77.32 ± 20.24, 2-year: 70.70 ± 24.81 *vs.*75.82 ± 19.21, 3-year: 71.10 ± 16.92 *vs.* 72.17 ± 19.52 mL·min^{-1}·1.73 m^{-2}) [Supplementary Figure 5, http://links.lww.com/CM9/B42].

Subgroup analyses

Among the 100 ABOi KTRs, 71 received Rit. A similar cumulative incidence of AR was observed in the Rit-treated and Rit-free groups (20.3% *vs.* 24.7%, $P = 0.714$) [Figure 2]. However, the Rit-free group had a reduced cumulative incidence of infection (20.7% *vs.* 36.9%, $P = 0.018$) [Figure 2]. In the multivariate analysis, the Rit-free regimen was an independent protective factor for infection after transplantation (HR, 0.305; 95% CI: 0.120–0.781, $P = 0.013$) [Table 2].

In ABOi KTRs receiving PE/DFPP, the initial high IgG group needed more sessions of PE/DFPP to achieve the target titer (median: 2.5 [range: 1–6] *vs.* 2.0 [1–6], $P = 0.020$). The initial high IgM group, but not high IgG, had a higher cumulative incidence of AR than that of the ABOc group ($P = 0.020$, $P = 0.658$) [Supplementary Figure 6A, B, http://links.lww.com/CM9/B43].

A total of 11 ABOi KTRs experienced antibody titer rebound, including five showing rebound of IgM alone, three showing rebound of IgG alone, and three showing rebound of both. These 11 ABOi KTRs had lower initial IgM (median: 16 [range: 1–64] *vs.* 64 [2–256], $P = 0.013$) and IgG titers (median: 0 [range: 0–128] *vs.* 16 [0–512], $P = 0.038$) compared with those without antibody rebound. The Rit-free group had a higher incidence of antibody titer rebound (7/29 [24.1%] *vs.* 5/71 [5.6%],

Table 2: Risk factors of AR and infection in the competing risks analyses among 100 ABOi-KTs.

	AR				Infection			
Characteristics	Unadjusted HR (95% CI)	*P* values	Adjusted HR (95% CI)	*P* values	Unadjusted HR (95% CI)	*P* values	Adjusted HR (95% CI)	*P* values
Rituximab *vs.* No	0.83 (0.32–2.19)	0.710			3.27 (1.28–8.33)	0.013	3.27 (1.28–8.33)	0.013
First 34 cases *vs.* latter 66 cases	3.72 (1.40–9.86)	0.008	3.65 (1.37–9.78)	0.010	0.94 (0.41–2.14)	0.880		
Donor gender	1.34 (0.48–3.72)	0.570			1.00 (0.44–2.30)	0.990		
Donor BMI	0.92 (0.80–1.05)	0.220			1.00 (0.89–1.13)	0.970		
Recipient age	0.99 (0.94–1.04)	0.670			0.98 (0.99–1.03)	0.490		
Recipient gender	0.79 (0.30–2.10)	0.620			1.25 (0.55–2.83)	0.600		
Recipient BMI	1.05 (0.92–1.21)	0.450			1.01 (0.92–1.10)	0.880		
Peritoneal dialysis vs. hemodialysis	0.97 (0.24–3.97)	0.970			0.62 (0.15–2.6)	0.510		
Duration of dialysis	0.99 (0.96–1.03)	0.770			1.01 (0.99–1.02)	0.260		
HLA (>3 *vs.* <3)	1.01 (0.41–2.48)	0.990			1.42 (0.68–2.96)	0.350		
PRA (positive *vs.* negative)	1.73 (0.69–4.36)	0.240			1.13 (0.53–2.37)	0.750		
Pretransplant anti-HLA antibody								
Both negative	Ref				/	/		
HLA-I positive	1.47 (0.45–4.76)	0.520			/	/		
HLA-II positive	2.56 (0.40–16.6)	0.320			/	/		
Both positive	2.00 (0.58–6.90)	0.270			/	/		
Induction therapy								
Basiliximab	Ref				Ref			
No	2.56 (0.78–8.40)	0.120			0.82 (0.24–2.84)	0.750		
ATG	1.16 (0.33–4.06)	0.810			0.69 (0.25–1.91)	0.480		
Warm ischemic time (per 10 s)	0.98 (0.9–1.08)	0.640			0.98 (0.90–1.06)	0.590		
Initial antibody titer (<1:16 *vs.* >1:16)	0.87 (0.28–2.69)	0.800			0.68 (0.26–1.78)	0.440		
Preoperative Hb level (per 10 g)	0.96 (0.86–1.08)	0.530			0.94 (0.84–1.05)	0.270		
Preoperative lymphocyte level (per 10 g)	1.38 (0.68–2.80)	0.380			0.95 (0.46–1.95)	0.890		
Antibody titer rebound	2.84 (0.99–8.19)	0.053	2.72 (1.01–7.31)	0.048	0.68 (0.16–2.86)	0.600		
Anti-A *vs.* B type antibody	1.20 (0.47–3.05)	0.700			1.52 (0.72–3.22)	0.280		

ABOi-KTs: ABO-incompatible kidney transplantations; AR: Acute rejection; ATG: Anti-thymocyte globulin; BMI: Body mass index; HLA: Human leukocyte antigen; PRA: Panel reactive antibody; /: Not applicable.

$P = 0.013$). Six patients received post-transplant PE/DFPP (median 2, range: 1–4) and all fell to ≤16 within 2 weeks. Antibody titer rebound had a higher incidence of AR than the stable antibody (63.6% *vs* 18.2%, $P = 0.062$) [Supplementary Figure 6C, D, http://links.lww.com/CM9/B43]. In the multivariate analysis, antibody titer rebound increased the risk of AR 1.72-fold (HR: 2.72, 95% CI: 1.01–7.31, $P = 0.048$).

For recipients with anti-A-type antibodies, more received Rit ($P = 0.031$). In addition, patients with anti-A-type antibodies had higher IgM and IgG levels initially ($P = 0.012$) and transplantation ($P = 0.012$). Despite a higher trend of cumulative incidence of AR (25.7% *vs*. 16.2%, $P = 0.704$) and infection in those with anti-A-type antibody (37.4% *vs*. 31.1%, $P = 0.282$), significant differences were not observed [Supplementary Figure 6E, F, http://links.lww.com/CM9/B43].

Discussion

This study showed that an individualized preconditioning regimen based on the initial antidonor blood group antibody can successfully prevent AR and achieve excellent graft and patient survival in ABOi KTRs. For patients with lower antibody titers, minimizing the immunosuppression burden using oral immunosuppressives alone can reduce infections; however, antibody titer rebound should be monitored. For patients with higher titers, lymphocyte B cell elimination can successfully reduce the risk of rejection; however, more infection events may develop within the first post-transplant year. Overall, compared to the more intensive immunosuppression in previous studies, this simplified individualized scheme reduced the risk of infections, and was more cost-effective.

Removal of ABO blood-group antibody and avoidance of antibody titer rebound were considered essential in successful ABOi KTs. In Barnett's study, the authors reported that among the patients who did not undergo desensitization ($n = 7$) or Rit alone ($n = 6$), none experienced rejection or allograft loss. Their findings showed that individualized use of preconditioning may contribute to comparable res in ABOi KTRs.[16] Furthermore, Masterson et a1[17] suggested that initial antibody titer was a reliable predictor of AR, reporting that ABOi KT could be successfully performed without Rit or antibody removal in recipients with sufficiently low baseline ABO antibody titers (≤1:16). In our previous study, we proposed an individualized preconditioning regimen in a Chinese cohort. Further, the current study reports the efficacy and safety of an individualized preconditioning regimen using a larger sample size and longer follow-up.

Although the reported preconditioning scheme prevented rejections and improved graft survival effectively, increased rates of post-transplant infections were observed, resulting in higher mortality.[9,10,18-20] An analysis from a Collaborative Transplant Study, comparing 1420 ABOi KTRs with matched ABOc recipients, showed that early patient survival was lower in ABO-incompatible kidney transplantation ($P = 0.006$) because of a higher rate of early infectious deaths ($P = 0.03$).[17] A recent large metaanalysis of 1346 ABOi KTRs reported that 59% of patients died of infections after A ABOi KT, compared to 13% in ABOc patients ($P = 0.02$).[20] Our results showed that Rit use was an independent risk factor for post-transplant infections. The higher incidence of infection can be explained by the elimination of lymphocyte B cells, which subsequently reduces antigen presentation and antibody secretion. Similar results have been reported in previous studies.[21,22] In Okada's retrospective study including 205 ABOi KTRs, the Rit-treated group had a higher incidence of infection than the Rit-free group (28.2% [37/131] vs. 9.4% [5/53], $P = 0.006$).[21] To reduce the risk of serious infection, minimizing immunosuppression has been attempted, including fewer sessions of antibody removal and lowering the Rit dose. In Lee's study, all ABOi KTRs were divided into two groups receiving standard Rit (375 mg/m^2, $n = 76$) or reduced dose 200 mg, $n = 19$) within 7 days before transplantation.[23] A total

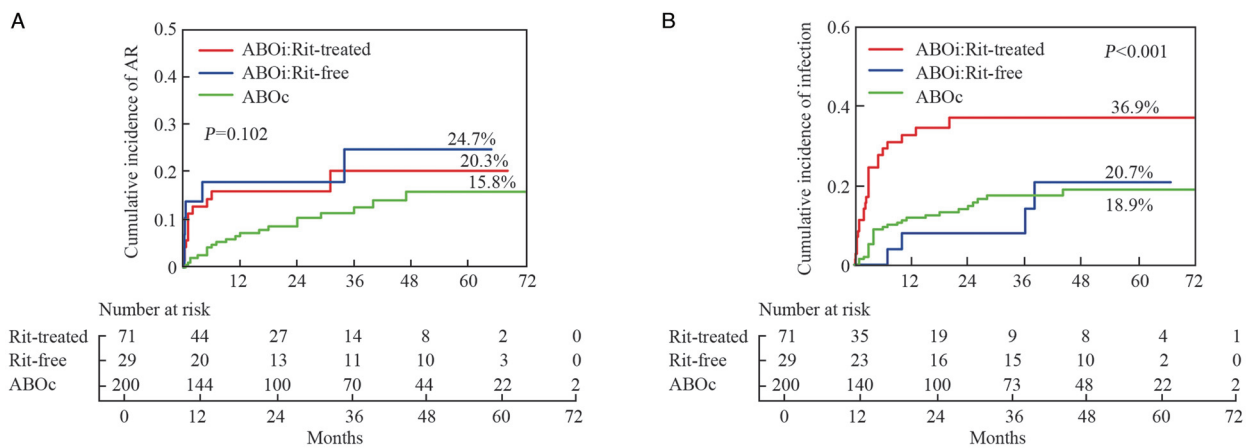

Figure 2: Cumulative incidence of AR (A: Rit-treated *vs.* Rit-free, $P = 0.714$; Rit-treated *vs.* ABOc, $P = 0.072$; Rit-free *vs.* ABOc, $P = 0.107$) and infection (B: Rit-treated *vs.* Rit-free, $P = 0.794$; Rit-treated *vs.* ABOc, $P = 0.001$; Rit-free *vs.* ABOc, $P = 0.018$) in 100 ABOi kidney transplant recipients who did and did not receive Rit. ABOc: ABO-compatible; ABOi: ABO-incompatible; AR: Acute rejection; Rit: Rituximab.

of 118 ABOc KTRs were referred to as the control group. There were 29 patients (38.2%) in the standard Rit group, five patients (26.3%) in the reduced Rit group, and 27 patients (22.9%) in the ABOc group, diagnosed with an infection during the 12-month followup period ($P = 0.069$). Differently, in our study, patients with a lower antibody titer did not receive Rit and thus had a lower risk of infection.

However, despite no additional risk of AR, we observed a higher incidence of antibody titer rebound within the first 2 weeks in these ABOi KTRs not receiving B cell-eliminated therapy. In contrast, Baek *et al*[24] reported that a high antibody titer (>1:128) was associated with titer rebound (35.8% *vs*. 15%, $P = 0.002$). In addition, no association was established between titer rebound (>1:16) and AR ($P = 0.68$). However, a significant difference from our study was that all patients received Rit (200 mg) in their study. A recent study by Tobian et a1[25] reported that the risk of rejection was significantly higher in those with an antibody titer ≥64 within the first 2 weeks post-transplant (P = 0.006). Notably, the positive predictive value was 33.3% and the negative predictive value was 91.2%, which means that most individuals with AMR had an elevated titer, whereas the positive predictive value of a high titer for rejection was poor. However, in the study by Ishida *et al*[26], they included 191 ABOi recipients, who were divided into two groups: group 1 consisted of low rebound ([≤1:32], $N = 170$) and group 2 consisted of high rebound ([>1:64], N = 21) within the first-year post-transplant. Although no prophylactic treatment for rejection was administered for all elevated anti-blood type antibodies, they showed that both T-cell-mediated rejection and AMR did not differ between the two groups (8% *vs*. 10%, $P = 0.432$; 9% *vs*. 10%, $P = 0.898$). Hence, the timing of the antibody rebound may also be important. Further studies are needed to explore whether an active intervention using PE or DFPP is required after rebound.

For patients with higher antibody titers, both B cell elimination and antibody removal treatment were required in our study. A pre-KT titer of 16 or less has been established as agoal in many centers, such as Johns Hopkins Hospital and the Mayo Clinic.[27,28] However, which type of ABO antibody, IgM or IgG, is significantly involved in AR is unclear. Previous studies reported that ABO antigen is carbohydrate-based, which mainly increases the IgM production in antibody response. Kim *et al*[29] reported comparable 5-year graft survival in the high IgG group (median: 32, range: 16–64) and low IgG group (median: 2, range: 1–4) in 120 KTRs (100% *vs*. 97.4%, $P = 0.314$) with IgM antibody titer was ≤4. This indicated that IgM anti-ABO antibodies may have an important influence on ABOi KT outcomes. In our study, initial high IgM, but not high IgG, seemed to be associated with a higher risk ofAR. However, studies with larger sample size and longer follow-up are still needed to clarify the relationship.

Diagnostic criterion of AR is still controversial in the ABOi KTs. As previously reported,[30,31] the deposition of C4d is commonly observed in Ai ABOi KTs. In 9 biopsies from ABOi KTs, Setoguchi et a1[30] showed that the deposition of C4d in 94% of the studied patients while AMR occurs for only in 27% of these patients. In the context of ABOi KT, the value of the deposition of C4d in predicting AMR remains unclear. Ishihara et a1[32] investigated the role of microvascular inflammation (MVI) score by including 148 ABOi KTRs without preformed and *de novo* anti-HLA antibody. They found that MVI score≥2 was associated with lower 5-year graft survival and worse graft function. Parajuli *et al*[33] compared the transplant outcomes of 25 patients with HLA donor-specific antibody negative (DSA–) MVI with 155 with HLA donor-specific antibody positive (DSA_+) MVI. They reported that transplant outcomes and response to treatment with donor-specific antibody negative (DSA–) MVI patients are similarly poor to those with DSA_+ MVI patients. These results suggest that high MVI score may be used to diagnose AMR.

This study has several strengths and limitations. First, the current research involves a very large Chinese cohort that has been studied recently, to explore the efficacy and safety of ABOi KTs. Our study used propensity score analysis to establish well-balanced baseline characteristics, and a competing risk analysis was used to increase the reliability. However, this was a retrospective study. Additionally, some rejections were not biopsy-proven. In addition, although we did not set an upper limit for ABO titer for participation in our ABOi program, the highest ABO antibody titer in our study was 1:512. This means that extrapolating our results to higher titer transplants candidates should be done with caution. Finally, a longer follow-up was more convincing.

In conclusion, ABOi KT using an individualized pre-conditioning regimen had comparable graft survival and patient survival with ABOc KT. For ABOi KTRs receiving Rit due to high antidonor ABO antibody titers, more attention should be paid to infections. Rit-free preconditioning regimen was effective in preventing AR without increasing the risk of infections in patients with low titers, but antibody titer rebound should be monitored.

Data availability

After publication, data are available upon reasonable request. A proposal with a detailed description of study objectives and statistical analysis plan will be needed for evaluation of the reasonability of requests. Additional materials might also be required during the process of evaluation. Participant data will be provided after approval from the corresponding author.

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Conflicts of interest

None.

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