






ORIGINAL ARTICLE

Outcomes and complications following ABO-incompatible kidney transplantation performed after desensitization by semi-selective immunoadsorption - a retrospective study

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SUMMARY

Because of the current organ shortage, ABO-incompatible (ABOi) transplantations have been increasingly performed in recent years. The results seem comparable to those of compatible transplantations, but there have also been reports of increased side effects possibly because of the desensitization therapy. To address an increase in severe infectious complications, we compared the outcomes of 48 ABOi transplant recipients to outcomes of 96 matched ABO-compatible (ABOc) controls transplanted at Heidelberg University Hospital from August 2005 to April 2018. Over a follow-up period of 8 years, ABOi transplant recipients had comparable graft and patient survival as well as graft function compared with ABOc patients. T-cell-mediated and antibody-mediated rejections were not different between groups. In ABOi transplant recipients, urosepsis (22.9% vs. 8.5%; $P = 0.019$) and pneumonia with opportunistic pathogens (8.3% vs. 1.0%, $P = 0.025$) appeared more frequently. As a consequence, a significantly higher number of deaths from infection have been observed after ABOi transplantations (6.3% vs. 0%, $P = 0.010$). High-titer recipients (isoagglutinin titer of $\geq 1:256$) showed a higher incidence of BK virus replication and postoperative bleeding complications. ABO-incompatible transplantations can be performed with results that are not different from results after ABOc transplantations. However, an increased rate of serious infectious complications must be taken into account.

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Key words

ABO incompatibility, desensitization, immunosuppression, kidney transplantation, side effects

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Introduction

ABO-incompatible (ABOi) kidney transplantation has long been considered a contraindication to successful transplantation because of an increased rate of early antibody-mediated rejections (ABMR). Since that time,

different desensitization protocols have been established to eliminate blood group anti-A/B antibodies from the recipient's circulation. The definite breakthrough in ABOi kidney transplantation was in 2003, when Tyden *et al.* [1] introduced a simplified and safe desensitization protocol that could easily be reproduced by others.

The current protocol used at many transplant centers consists of selective or semi-selective immunoadsorption (IA), or plasma exchange (PE) for blood group anti-A/B antibody elimination together with the administration of the anti-CD20 antibody rituximab (RTX) [2]. As previously illustrated by us, semi-selective IA features several advantages compared with selective IA, such as elimination of coexisting donor-specific HLA alloantibodies, reusability of IA devices, reduced cost, and less treatment-associated side effects [3,4]. According to the available literature, graft and patient survival rates in ABOi kidney transplant recipients seem to be comparable to survival in recipients of an ABO-compatible (ABOc) organ [2–4]. However, a recently published meta-analysis [5] revealed an increased incidence of death, mainly attributable to severe nonviral infections.

The aim of our current study was to address this increase in severe infectious complications and to further characterize the emergence and pathogen spectrum. For this purpose, we compared the long-term outcomes of 48 ABOi kidney transplant recipients, desensitized by different semi-selective IA devices, with a matched ABOc kidney transplantation cohort composed of 96 recipients transplanted within the same time period with an emphasis on infectious complications.

Materials and methods

Patients and study design

Eighty ABOi kidney recipients were desensitized from August 2005 to April 2018, and 48 recipients were eventually transplanted using a semi-selective IA device (Fig. 1). The characteristics of nontransplanted patients are given in detail in Table S1. ABOi kidney recipients desensitized by semi-selective IA devices were compared to a 1:2 matched ABOc standard-risk cohort with a follow-up of a maximum of 8 years until April 2018. The standard-risk cohort was composed of 96 recipients who were transplanted at Heidelberg Transplant Center in the same time period and were selected based on similar baseline demographic and clinical characteristics. Patients excluded from analysis are depicted in Fig. 1. The baseline characteristics of the ABOi or ABOc donors and recipients are given in Table 1.

Desensitization for ABO-incompatible transplantation with semi-selective IA devices

Rituximab (Roche, Basel, Switzerland) was administered at a single dose of 375 mg/m² in 46 patients either 2–

4 weeks before the scheduled procedure (until October 2011, 15 patients) or on the day before surgery (from November 2011, 31 patients). Four different semi-selective IA devices were used at our transplant center. IA was accomplished by (i) the reusable antihuman Ig-coated Therasorb Ig-flex column on a Life 18 device ($N = 37$; Miltenyi Biotec, Bergisch Gladbach, Germany), (ii) the Therasorb Ig-omni5 column on a Life 18 device ($N = 5$; Miltenyi Biotec), the Immunosorba column on a Comtech centrifuge together with the Adasorb device ($N = 5$; Fresenius Medical Care AG & Co. KGaA, Bad Homburg, Germany), and the Globaffin column on a Comtech centrifuge together with the Adasorb device ($N = 1$; Fresenius Medical Care AG & Co. KGaA). During each IA treatment, 2.0–2.5 plasma volumes per patient were processed and anticoagulation was performed by a combination of heparin and sodium citrate. The selection of the number of preoperative IA treatments depended on the initial anti-A/B isoagglutinin titer. With a baseline isoagglutinin titer (Coombs or saline) of $\leq 1:8$, 4 IA treatments were scheduled and with every additional titer step, one additional IA treatment was added [3]. Since more than 50% of ABOi recipients required additional PE treatments to reach the target anti-A/B antibody titer, all patients received at least one additional preoperative PE from August 2012 onward. Preoperative PE ($N = 38$) was performed either with a Comtech centrifuge or with an Octo Nova apheresis device (Diamed Medizintechnik, Cologne, Germany) equipped with a Plasmaflo OP 0.2 plasma separator (Asahi Kasei Kuraray Medical Co., Tokyo, Japan). IVIGs (0.5 g/kg body weight; KIOVIG, Baxter, Vienna, Austria) were administered in seven patients on day -1 after the last preoperative apheresis treatment. Since IVIG contains anti-A/B isoagglutinins and led to an increase of one titer step upon administration [6], we stopped IVIG treatment from April 2010. The aim of the desensitization protocol was to achieve an anti-A/B antibody titer of $\leq 1:8$ (Coombs and saline) on the day of surgery (day 0). To address the isoagglutinin titer rebound after transplantation, we implemented additional postoperative IA or PE treatments to maintain isoagglutinin titers below a target titer of $\leq 1:16$ in the first or $\leq 1:32$ in the second week after surgery.

Immunosuppression

In ABOi kidney transplant recipients, immunosuppression was started before transplantation together with the initiation of IA therapy and consisted of tacrolimus (Astellas, Tokyo, Japan) with an initial target trough

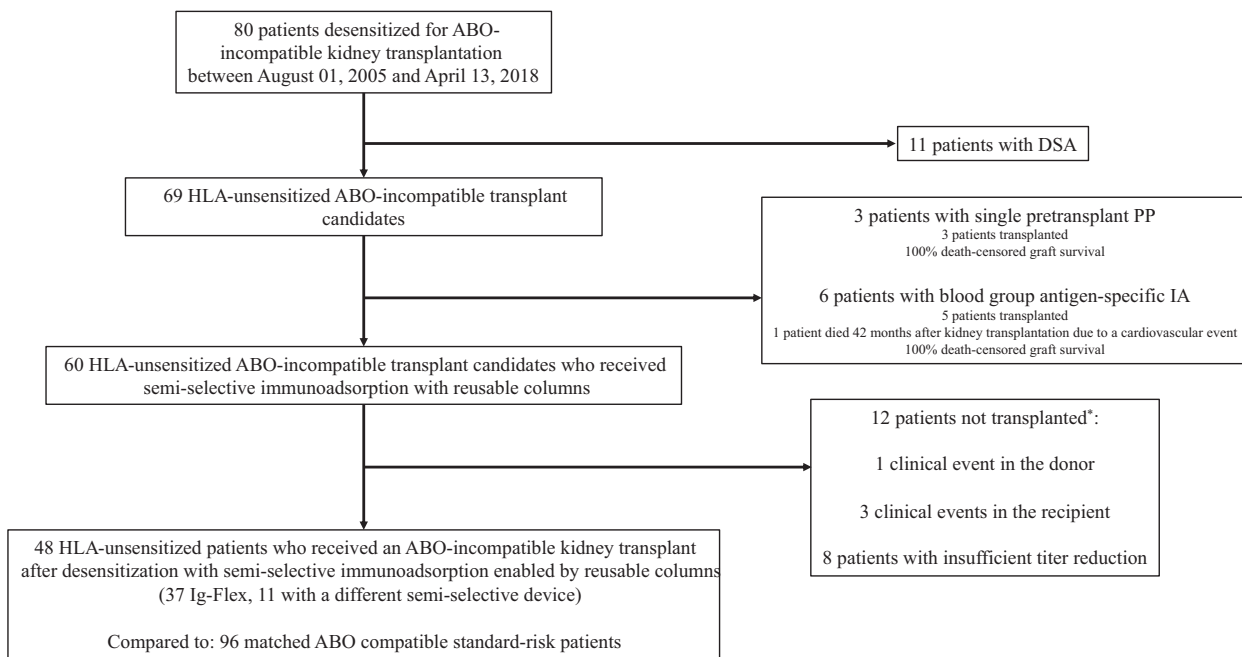


Figure 1 Patient selection with 48 HLA unsensitized patients who received an ABO-incompatible kidney transplantation after desensitization with semi-selective immunoadsorption enabled by reusable columns compared to 96 matched ABO-compatible standard-risk patients.

*Table S1.

level of 10–12 $\mu\text{g/l}$, enteric-coated mycophenolate sodium (720 mg twice daily; Novartis, Basel, Switzerland), and methylprednisolone (20 mg per day). At the time of transplantation, methylprednisolone was given at a dose of 250 mg, tapered to 20 mg/day by post-transplant day 9. A summary of the maintenance immunosuppression of ABOi kidney transplant recipients is given in Table 1.

Seventy-one percent of ABOc transplant recipients had cyclosporine (Novartis) with an initial target trough level of 150–180 $\mu\text{g/l}$, 27% received tacrolimus, and 2% had everolimus starting at the time of surgery. The doses of enteric-coated mycophenolate sodium and methylprednisolone were comparable to ABOi recipients. Immunosuppressive induction therapy was carried out with basiliximab (20 mg on days 0 and 4 after transplantation; Novartis) in 46 ABOi and in 93 ABOc standard-risk patients. Details of the induction and maintenance immunosuppressive therapy are listed in Table 1.

Anti-infective prophylaxis

Antiviral prophylaxis with valganciclovir (Roche) was administered to all ABOi and ABOc kidney transplant recipients with a transplant from a cytomegalovirus-positive donor for 3 months. Fungal prophylaxis consisted of 10 ml of nystatin four times daily for

3 months. *Pneumocystis jirovecii* prophylaxis was conducted by alternate-day administration of trimethoprim (160 mg) and sulfamethoxazole (800 mg) for 6 months as well as a 1-time inhalation of pentamidine (300 mg) 24 h after transplantation.

Measurement of anti-A/B antibodies

Isoagglutinin titers were measured by serological methods using the gel column agglutination technique (Bio-Rad, Munich, Germany), both in the LISS/Coombs and in the neutral gel (saline) card techniques. The measurement of isoagglutinins in the LISS/Coombs and in the neutral gel (saline) card techniques corresponds to the IgG and IgM titration, respectively. For titration, commercially available test red blood cells of blood groups A and B were used (Reverse-Cyte, Medion Grifols, Duedingen, Switzerland). The highest dilution with a 1+ macroscopic agglutination was considered as the titer.

Biopsies and treatment of allograft rejection

Histological findings were graded and evaluated by an experienced nephropathologist according to the Banff criteria (Banff 2013). Immunohistochemical assay for C4d detection was applied on paraffin slides using a rabbit polyclonal antihuman C4d antibody (Biomedica Medizinprodukte GmbH & Co KG, Vienna, Austria).

Table 1. Baseline characteristics of transplanted patients.

	Standard risk (N = 96)	ABO-incompatible (N = 48)	P
Recipient characteristics			
Female sex, N (%)	44 (46)	20 (42)	0.72
Age, median (range), years	44 (18–71)	45 (18–65)	0.89
Caucasian race, N (%)	92 (96)	46 (96)	1.00
Cause of ESRD, N (%)			
Diabetes	7 (7)	1 (2)	0.27
Hypertension	7 (7)	3 (6)	1.00
Obstructive/recurrent pyelonephritis	7 (7)	5 (10)	0.53
ADPKD	26 (27)	19 (40)	0.13
Glomerulonephritis	40 (42)	15 (31)	0.28
Other	7 (7)	3 (6)	1.00
Unknown	2 (2)	2 (4)	0.60
Number of previous transplants, N (0/1/2)	89/5/2	46/2/0	0.72
Mode of pretransplant dialysis, N (%)			
Hemodialysis	61 (64)	35 (73)	0.35
Peritoneal dialysis	10 (10)	7 (14)	0.58
Preemptive transplantation	25 (26)	6 (13)	0.08
Waiting time for transplant, median (range), months	9 (0–104)	10 (0–109)	0.12
Donor characteristics			
Female sex, N (%)	55 (57)	28 (58)	1.00
Donor age, median (range), years	54 (31–77)	51 (28–72)	0.37
Related donor, N (%)	55 (57)	20 (42)	0.11
HLA A + B + DR mismatches, median (range)	3 (0–6)	4 (1–6)	0.19
Immunosuppression and follow-up			
Patients with preoperative anti-CD20 antibody, N (%)	0 (0)	46 (96)	<0.001
Patients with preoperative IVIg, N (%)	0 (0)	7 (15)	<0.001
Patients with anti-IL2 receptor antibody induction, N (%)	93 (97)	46 (96)	1.00
Initial maintenance immunosuppressive therapy			
Cyclosporine/EC-MPS/steroids	68 (71)	0 (0)	<0.001
Tacrolimus/EC-MPS/steroids	26 (27)	48 (100)	<0.001
Tacrolimus/everolimus/steroids	2 (2)	0 (0)	0.55
Postoperative hospital stay, median (range), days	13 (8–37)	15 (10–76)	<0.001
Clinical follow-up, median (range), months	57 (0–97)	41 (0–103)	0.14

ADPKD, autosomal-dominant polycystic kidney disease; EC-MPS, enteric-coated mycophenolate sodium; ESRD, end-stage renal disease.

Banff borderline changes and T-cell-mediated rejection episodes were treated with 125–250 mg methylprednisolone for 3 days, respectively.

Statistical analysis

Data are expressed as median and range or number and percent. In the graphs, the results are shown as mean \pm SEM. Analysis of continuous data was performed using the nonparametric *t*-test with Well's correction or the Mann–Whitney *U*-test. Statistical analysis of categorical data was performed using chi-square or Fisher tests. Kaplan–Meier graft and patient survival analysis as well as the time to the occurrence of severe bacterial and viral complications were calculated via the log-rank test.

Statistical significance was assumed at a *P*-value < 0.05. All statistical analyses were performed using GRAPHPAD PRISM version 7.0 (GraphPad Software, San Diego, CA, USA).

Results

Elimination of anti-A/B antibodies by desensitization

Of the ABOi transplant recipients, 58% were blood group O, 23% blood group A, and 19% blood group B. The donor-recipient blood group mismatches were A to O (48%), A to B (19%), AB to A (15%), B to O (8%), B to A (6%), and AB to O (2%). Before desensitization, median baseline anti-A/B antibody titer was 1:64 (1:2–1:1024) in the Coombs technique and 1:32 (1:2–1:128) in the

saline technique. Of the ABOi transplant recipients, 19% were “high-titer” patients, defined as an initial IgG or IgM anti-A/B titer of $\geq 1:256$. In order to reach the target anti-A/B antibody titer of 1:8 before ABOi kidney transplantation, a median of 6 (2–13) preoperative IA treatments was necessary. In addition, 38 ABOi recipients had a median of 2 (2–6) preoperative PE treatments because of an inadequate anti-A/B IgM antibody depletion by commonly used IA columns. Fifty-four percent of our ABOi kidney transplant recipients either had IA or had PE treatments after surgery to maintain the isoagglutinin titers below the target titer.

Excellent patient and graft survival as well as graft function in ABO-incompatible transplant recipients

With a maximum follow-up of 8 years, overall graft survival (log-rank $P = 0.062$; Fig. 2a) and death-censored graft survival (log-rank $P = 0.13$; Fig. 2b) tended to be lower in ABOi compared with ABOc recipients, whereas patient survival (log-rank $P = 0.24$; Fig. 2c) was not different. However, when analyzing death from infectious complications, this rate was significantly higher in ABOi compared with ABOc recipients (log-rank $P = 0.010$; Fig. 2d). In the ABOi cohort, one patient died of sudden cardiac death and three patients of severe pneumonia (two patients with lobar pneumonia and one patient with *P. jirovecii* pneumonia). Death-censored graft failure appeared in three patients after ABOi living-donor kidney transplantation. One patient lost his graft due to chronic T-cell-mediated changes, and two ABOi recipients lost their transplant during severe pneumonia.

The incidence of delayed graft function was comparable in both groups (Table 2). Serum creatinine and glomerular filtration rate (eGFR) as estimated by the MDRD formula are given in Fig. 3. After 24 months, the graft function was slightly better in ABOc standard-risk patients compared with ABOi recipients (serum creatinine: 1.5 mg/dl vs. 1.3 mg/dl, $P = 0.026$, and MDRDGFR: 54.9 ml/min/1.73 m² vs. 59.1 ml/min/1.73 m², $P = 0.048$) while it was not significantly different during further follow-up (kidney function after 8 years: serum creatinine 1.4 mg/dl vs. 1.4 mg/dl, $P = 0.47$; eGFR 54.2 ml/min/1.73 m² vs. 63.6 ml/min/1.73 m², $P = 0.22$; or proteinuria 15.7 g/mole creatinine vs. 6.6 g/mol creatinine, $P = 0.43$).

Complications and rejections after ABO-incompatible transplantation

The frequency of complications such as lymphoceles, urinary leakages, or wound healing disturbances did not

differ between ABOi and ABOc patients. There was a trend toward increased postoperative bleeding complications in ABOi recipients, defined as the requirement of postoperative transfusion or additional operative interventions ($P = 0.13$), especially in high-titer patients (see below).

Rejection episodes of all BANFF categories were comparable in both groups. ABMR occurred in 2% and 3% ($P = 1.00$; Table 2) and T-cell-mediated rejection (TCMR) in 10% and 9% ($P = 1.00$; Table 2) in the ABOi and the ABOc cohort, respectively.

The frequency of uncomplicated urinary tract infections was not different between groups, whereas complicated urinary tract infections and urosepsis (23% vs. 8%; $P = 0.019$) occurred more often after ABOi living-donor kidney transplantation. The pathogen spectrum of urosepsis was different between groups with a predominance of multi-sensitive *Escherichia coli* ($P = 0.023$) in ABOc and *Enterococcus species* ($P = 0.012$) as well as *vancomycin-resistant Enterococcus (VRE)* ($P = 0.10$) in ABOi recipients. The incidence of pneumonia was also comparable between groups, but severe pneumonia leading to death occurred significantly more frequent in ABOi patients (6% vs. 0%; $P = 0.035$). In ABOi recipients, pneumonia was more often attributable to opportunistic pathogens compared with ABOc patients ($P = 0.025$). Central catheter infections were more common in ABOi patients (15% vs. 3%; $P = 0.016$). The incidence of multidrug-resistant bacteria such as vancomycin-resistant enterococci (VRE) and multidrug-resistant gram-negative bacteria (MRGN) was higher in ABOi recipients (VRE: 17% vs. 5%, $P = 0.032$, and MRGN: 8% vs. 1%, $P = 0.040$). As compared to ABOc recipients, ABOi kidney transplant recipients had no higher incidence of postoperative viral complications as BK virus replication or biopsy-proven BK virus nephropathy and cytomegalovirus (CMV) replication. The time to the occurrence of severe bacterial as well as viral complications is given in Fig. S1.

In this long-term follow-up, there were no differences concerning cardiovascular complications or malignancy, both known as long-time adverse events of applied immunosuppressive therapy.

Patients with high anti-A/B antibody titer

Patients in the “high-titer” anti-A/B antibody group (i.e., initial titer of $\geq 1:256$) required more preoperative IA ($P = 0.019$) and had a trend toward more PE ($P = 0.059$) treatments compared with patients in the “low-titer” anti-A/B antibody group (i.e., initial titer of

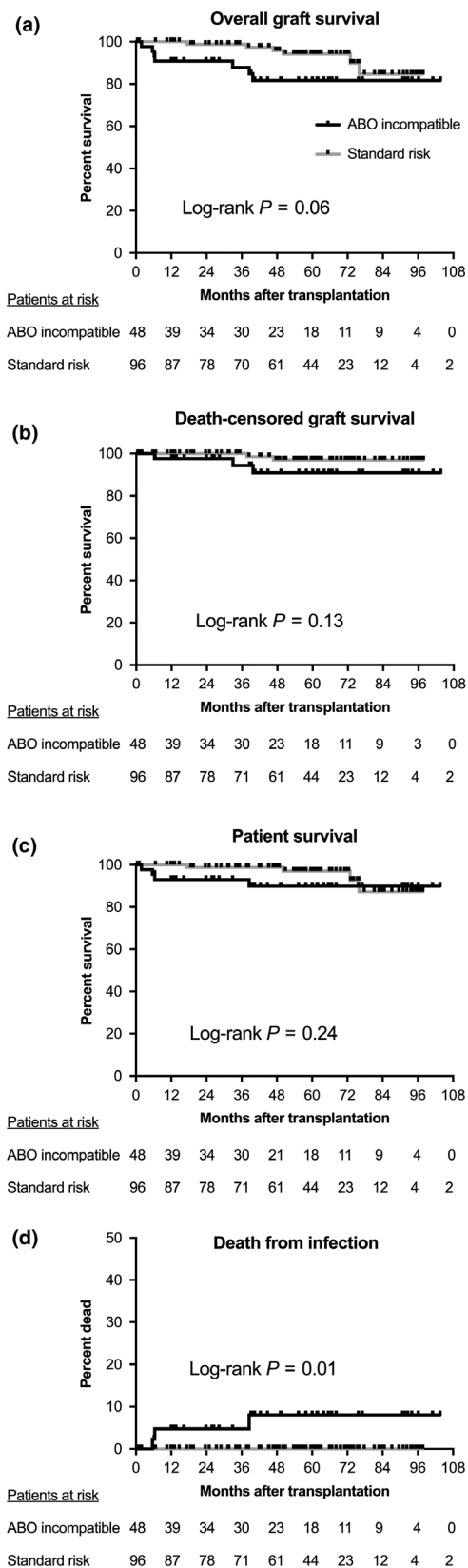


Figure 2 Overall graft survival (a), death-censored graft survival (b), patient survival (c), and death from infection (d) in 48 ABO-incompatible and in 96 matched control patients.

<1:256). After desensitization but before surgery, “high-titer” kidney transplant recipients had a significantly higher IgG anti-A/B antibody titer with a median of 1:4 (1:0–1:8) compared to “low-titer” patients with a median of 1:1 (1:0–1:8; $P = 0.014$). At postoperative day 30, the IgG and the IgM anti-A/B antibody titer in the “high-titer” group was at a median of 1:32 (1:4–1:256) and 1:8 (1:2–1:64), respectively, whereas patients in the “low-titer” group had significantly lower levels at a median titer of 1:4 (1:0–1:64; $P = 0.012$) and 1:2 (1:0–0:32; $P = 0.018$), respectively (Table 3).

Compared to ABOi patients with a “low-titer,” patients with a “high-titer” had comparable graft and patient survival, graft function, rejection rates, and rate of bacterial infections while they experienced a higher incidence of BKV replication (44% vs. 13%; $P = 0.049$). Graft biopsies were performed in 6 out of 9 (67%) ABOi living-donor kidney recipients. Four of these six patients had histologically confirmed BKV nephropathy, while two patients had BKV replication $>10^4$ /ml in the peripheral blood without histological signs of nephropathy. If BKV replication ($>10^4$ /ml) was detected in the peripheral blood of ABOi recipients, the immunosuppressive therapy was reduced or changed in all patients.

Surgical complications such as wound healing disturbances or lymphoceles were comparable between both groups, whereas postoperative bleeding complications occurred more frequent in high-titer compared with low-titer patients (44% vs. 8%; $P = 0.017$).

Discussion

Herein, we present our experience with a desensitization protocol consisting of semi-selective IA and administration of the anti-CD20 antibody rituximab. Forty-eight ABOi kidney transplant recipients were compared to 96 matched ABOc standard-risk recipients for a maximum of 8 years with a focus on infectious complications. Main findings were comparable graft survival, kidney graft function, and rejection rates between ABOi and ABOc patients with a higher frequency of severe infections and deaths from infection in ABOi transplant recipients.

Long-term outcomes and infection-associated death

Studies from Stockholm [7] and Freiburg [8] found comparable graft survival rates between ABOi and ABOc patients. In contrast, a study from the United Kingdom [9] showed reduced patient survival in ABOi recipients and this reduction was due to an increased

Table 2. Complications.

	Standard risk (N = 96)	ABO-incompatible (N = 48)	P
Allograft rejection			
Patients with rejection, without BL changes, N (%)	12 (13)	6 (13)	1.00
BL changes, N (%)	26 (27)	18 (38)	0.25
T-cell-mediated rejection, N (%)	9 (9)	5 (10)	1.00
Antibody-mediated rejection, N (%)	3 (3)	1 (2)	1.00
Delayed graft function*, N (%)	1 (1)	1 (6)	0.11
Surgical complications†			
Wound healing disturbance, N (%)	3 (3)	2 (4)	1.00
Hemorrhage, N (%)	6 (6)	7 (15)	0.13
Lymphocele, N (%)	13 (14)	2 (4)	0.15
Urinary leakage, N (%)	2 (2)	1 (2)	1.00
Infectious complications			
Viral			
BK virus replication‡, N (%)	9 (9)	9 (19)	0.12
BK virus nephropathy, N (%)	2 (2)	4 (8)	0.10
Cytomegalovirus replication§, N (%)	13 (14)	6 (13)	1.00
Bacterial and opportunistic germs			
Patients with UT infection, uncomplicated, N (%)	43 (45)	21 (44)	1.00
UT infection episodes, uncomplicated, N	108	55	0.83
Patients with UT infection, complicated, N (%)	8 (8)	11 (23)	0.019
<i>Escherichia coli</i> , N	6	2	0.023
<i>Enterococcus species</i> , N	0	7	0.012
VRE, N	0	4	0.10
UT infection episodes, complicated, N	13	12	0.019
Patients with pneumonia, N (%)	19 (20)	9 (19)	1.00
Opportunistic, N (%)	1 (1)	4 (8)	0.025
Pneumonia episodes, N	20	10	1.00
Death from pneumonia, N (%)	0 (0)	3 (6)	0.035
Central venous catheter infection, N (%)	3 (3)	7 (15)	0.016
Multidrug-resistant bacteria			
VRE, N (%)	5 (5)	8 (17)	0.032
MRGN, N (%)	1 (1)	4 (8)	0.040
Immunosuppression-associated skin carcinoma, N (%)	3 (3)	2 (4)	1.00
Cardiovascular complications			
NSTEMI or STEMI, N (%)	0 (0)	1 (2)	0.33
Stroke, N (%)	0 (0)	1 (2)	0.33

BL, borderline; MRGN, multidrug-resistant gram-negative; (N)STEMI, (non) st-elevated myocardial infarction; UT, urinary tract; VRE, vancomycin-resistant enterococcus.

*Defined by dialysis within the first week after transplantation.

†Requiring intervention.

‡Replication >10⁴ copies/ml plasma.

§pp65 ≥ 1.

frequency of infectious complications, mostly *P. jirovecii* pneumonia (91% vs. 98%; $P = 0.01$). An analysis from the Collaborative Transplant Study (CTS) [2], comparing 1420 ABOi kidney transplant recipients with matched ABOc standard-risk recipients, revealed also reduced early patient survival ($P = 0.03$) most likely attributable to an increased rate of early deaths by infectious complications. Only recently, a large meta-analysis of 1346 ABOi compared to 4943 ABOc recipients

confirmed these findings [5]. Forty-nine percent of patient deaths after ABOi transplantation were of infectious origin, while the same number was only 13% in ABOc patients ($P = 0.02$). Infection-associated deaths were most often the consequence of nonviral infections. In line with these data, our study shows a trend to reduced overall graft survival that was the consequence of severe pneumonia, leading to either graft loss or death with functioning allografts. Death from infection

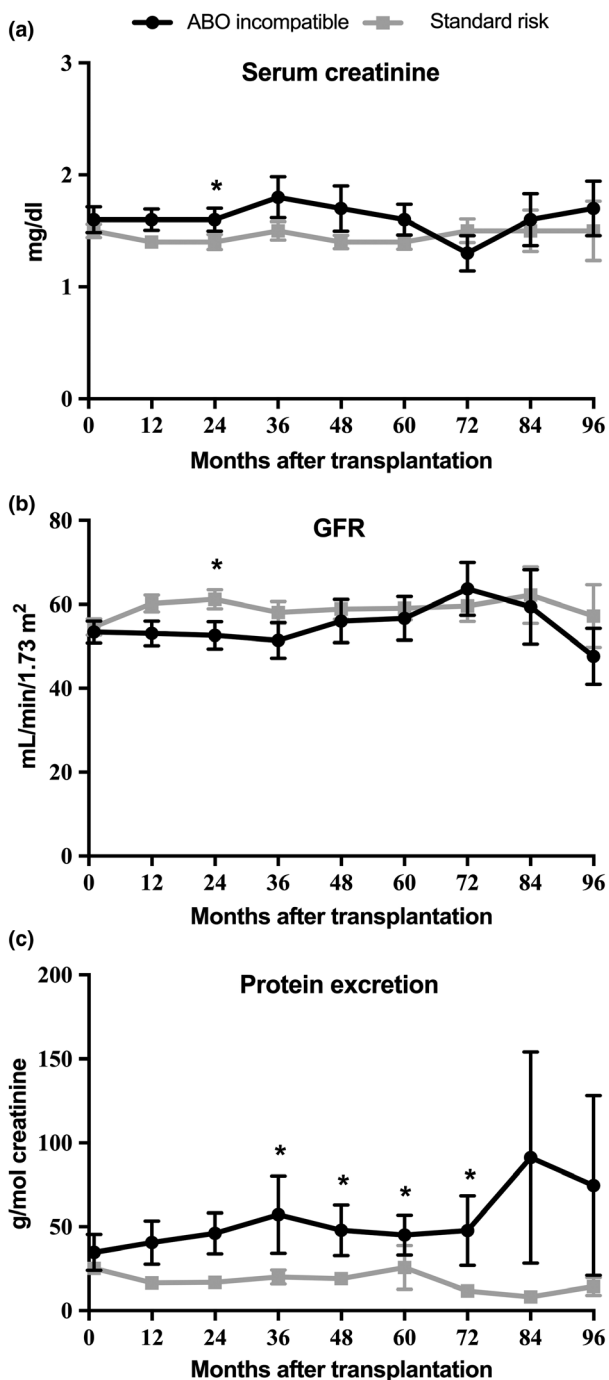


Figure 3 Serum creatinine (a), MDRDGFR (b), and urine protein-to-creatinine ratio (c) in 48 ABO incompatible and in 96 matched control patients. MDRDGFR, glomerular filtration rate as estimated by the MDRD formula. * indicates significance value $P < 0.05$

was also significantly higher in ABOi compared with ABOc transplantations in our study.

ABOi recipients had good graft function up to 8 years after transplantation that was not significantly different from graft function in ABOc patients. These

outcomes are also compatible with recently published data from Japan [10] and Freiburg [8].

Infectious and noninfectious complications

Despite more intensive immunosuppressive protocols and repeated IA treatments, we did not observe a general increase in infectious complications in ABOi patients during the observation period. However, complicated infections such as urosepsis and opportunistic pneumonia appeared more frequently in ABOi compared with ABOc kidney recipients as it has also been described by Barnett and colleagues from the UK [9]. We recorded a shift of the pathogen spectrum in urosepsis with more *Enterococcus species* and VRE in ABOi recipients, while multisensitive *E. coli* was the predominant germ in ABOc patients. The same was found for pneumonia with a higher incidence of opportunistic pathogens in ABOi compared with ABOc patients. Of nine pneumonia episodes after ABOi kidney transplantation, one patient had *P. jirovecii* pneumonia, two patients had fungal pneumonia, and one patient had a pneumonia caused by *respiratory syncytial virus (RSV)*. These findings are backed by data from a US study [11] where ABOi compared to ABOc transplantation was associated with a doubled risk of pneumonia. The increased incidence of severe infectious complications cannot definitely be attributed to a specific measure of our desensitization protocol. Of note, ABOi patients had tacrolimus as preferred calcineurin inhibitor, while ABOc patients were preferentially on cyclosporine therapy. However, ABOc living-donor kidney transplant recipients had comparable severe infectious adverse events independent of the use of tacrolimus or cyclosporine maintenance therapy. Infectious complications in ABOi patients may be the result of a general increase in the immunosuppressive burden or be linked to rituximab administration and/or immunoabsorption [12,13]. We are aware that reduced-dose rituximab might be sufficient for induction therapy in ABOi living-donor kidney transplantation at equal efficacy but with less side effects, although long-term data are pending. The finding of comparable infection rates in “high-titer” and “low-titer” patients may point to rituximab as the causative agent for the higher rate of infectious complications, but this remains speculative. The therapeutic procedures such as semi-selective IA not only result in a decrease in isoagglutinin titers but may also result in hypogammaglobulinemia which may predispose ABOi recipients to the risk of an increased rate of infectious complications [14]. We stopped IVIG substitution

Table 3. High-titer versus low-titer isoagglutinins

	High titer* (N = 9)	Low titer (N = 39)	P
Isoagglutinin titer			
day of KTx—Coombs technique, median (range), 1:X	4 (0–8)	1 (0–8)	0.014
day of KTx—saline technique, median (range), 1:X	1 (0–4)	0 (0–8)	0.66
day 7 after KTx—Coombs technique, median (range), 1:X	16 (8–128)	4 (0–64)	<0.001
day 7 after KTx—saline technique, median (range), 1:X	6 (0–16)	1 (0–16)	0.08
day 30 after KTx—Coombs technique, median (range), 1:X	32 (4–256)	2 (0–64)	0.012
day 30 after KTx—Saline technique, median (range), 1:X	8 (2–64)	1 (0–32)	0.018
Allograft function at last follow-up			
Serum creatinine (mg/dl), median (range)	1.7 (1.0–5.2)	1.4 (0.9–2.7)	0.13
MDRDGFR (ml/min/1.73 m ²), median (range)	45.6 (8.1–88.6)	54.6 (27.5–90.2)	0.51
Protein excretion (g/mol creatinine), median (range)	17.2 (2.8–38.0)	14.3 (2.9–337.1)	0.68
Allograft rejection			
Patients with rejection, without BL changes, N (%)	1 (11)	5 (13)	1.00
BL changes, N (%)	5 (55)	13 (33)	0.27
T-cell-mediated rejection, N (%)	1 (11)	4 (10)	1.00
Antibody-mediated rejection, N (%)	0 (0)	1 (3)	1.00
Surgical complications†			
Wound healing disturbance, N (%)	0 (0)	2 (5)	1.00
Hemorrhage, N (%)	4 (44)	3 (8)	0.017
Lymphocele, N (%)	1 (11)	1 (3)	0.34
Infectious complications			
Viral			
BK virus replication‡, N (%)	4 (44)	5 (13)	0.049
BK virus nephropathy, N (%)	2 (22)	2 (5)	0.15
Cytomegalovirus replication§, N (%)	1 (11)	5 (13)	1.00
Bacteria			
Patients with UT infection, uncomplicated, N (%)	5 (55)	16 (41)	0.48
UT infection episodes, uncomplicated, N	13	42	0.46
Patients with UT infection, complicated, N (%)	1 (11)	10 (26)	0.66
UT infection episodes, complicated, N	1	11	0.43
Patients with pneumonia, N (%)	3 (33)	6 (15)	0.34
Pneumonia episodes, N	3 (33)	7 (18)	0.34
Death from pneumonia, N (%)	1 (11)	2 (5)	0.47
Central venous catheter infection, N (%)	1 (11)	6 (15)	1.00

BL, borderline; IA, immunoadsorption; KTx, kidney transplantation.

*Defined as an isoagglutinin titer $\geq 1:256$.

†Requiring intervention.

‡Replication $>10^4$ copies/ml plasma

§pp65 ≥ 1

during desensitization for ABOi transplantation in 2010 since IVIG contain anti-A/B isoagglutinins and led to an increase of one titer step upon administration [6]. Central venous catheter-associated infections occurred more often in ABOi patients which is most likely the result of a longer dwell time of the catheter for IA treatment. ABOi recipients were colonized more often with multidrug-resistant bacteria which may either be the result of intensified immunosuppression or be the longer hospital stay in ABOi patients.

Surgical complications such as hemorrhages, lymphoceles, and wound healing disturbances were not observed

more frequently in ABOi compared with ABOc recipients in the current analysis, while an increased frequency had been described before by others and us [4,5].

ABMR and TCMR (including borderline changes) were comparable between ABOi and ABOc patients. Results in the literature are conflicting where higher rejection rates in ABOi patients had been described, for example, in the United States [11]. In the recent meta-analysis of 1346 ABOi transplant recipients, the relative risk for biopsy-proven acute rejection was 1.39 ($P < 0.001$) and for ABMR a striking 3.86 ($P < 0.001$). While there is a slightly increased ABMR rate in some

centers, the large difference in ABMR between ABOi and ABOc patients in this meta-analysis was driven by data collected from outside Europe (US, Korea) where different desensitization procedures and anti-A/B antibody cutoffs were applied [2,3,5,10,15,16]. Differences in the indication for biopsies as well as differences in the immunosuppressive regimens may also bias these results.

Results in high-titer patients

As compared to low-titer patients, high-titer ($\geq 1:256$) patients showed a significantly higher IgG and IgM anti-A/B titer rebound on day 7 and day 30 after transplantation resulting in a higher number of postoperative IA and PE treatments. Long-term graft survival and function, however, were not significantly affected. Outcome results in high-titer patients in the literature are inconclusive. While there was no correlation between preoperative anti-A/B antibody titer and incidence of acute rejection in a study by Ishida *et al.* [10], other studies found higher incidences of ABMR [17–19] or borderline changes [8] in ABOi recipients. Most importantly, desensitization fails more often in high-titer patients and patients have to continue on dialysis ($P = 0.0095$).

Bacterial infections as well as the incidence of CMV replication were comparable between high-titer and low-titer recipients, whereas the incidence of BKV replication was significantly higher in high-titer recipients which may be the consequence of an even more intensified immunosuppressive therapy. Another explanation would be that different blood group antigens may influence binding capacity of viral pathogen receptors to sialic acid on renal tubular cells [20].

Postoperative hemorrhage also occurred more frequently in the high-titer compared with low-titer recipients. This increased bleeding tendency has been shown by others and us [21–23], but the current data suggest that among the ABOi cohort high-titer recipients are

especially at risk as a consequence of the more intensified desensitization therapy.

Conclusions

ABOi kidney transplant recipients may be safely transplanted, even when they have a high anti-A/B antibody titer before surgery. However, particular attention has to be paid to severe infectious complications. Especially, pneumonia causes an increased frequency of deaths from infections in ABOi kidney transplant recipients during early follow-up.

Authorship

CS and CM: designed and performed the study, analyzed the data, and wrote the article. FK, CN, and LP: contributed to obtaining and analyzing data. MS, LB, KK, CS, and JB: contributed to obtaining the data and wrote the article. AL, RW, CS, AM, and MZ: reviewed the article.

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SUPPORTING INFORMATION

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

Figure S1. Time to the occurrence of pneumonia (A), opportunistic pneumonia (B), urosepsis (C), BKV replication (D), and CMV infection (E) in 48 ABO-incompatible and in 96 matched control patients.

Table S1. Non-transplanted patients.

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