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Original Article

One hundred ABO-incompatible kidney transplantations between 2004 and 2014: a single-centre experience

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ABSTRACT

Background. ABO-incompatible kidney transplantation (ABOi KTx) expands the living donor transplantation options. However, long-term outcome data, especially in comparison with ABO-compatible kidney transplantation (ABOc KTx), remain limited. Since the first ABOi KTx in Germany on 1 April 2004 at our centre, we have followed 100 ABOi KTx over up to 10 years.
Methods. One hundred ABOi KTx and 248 ABOc KTx from 1 April 2004 until 28 October 2014 were analysed in this observational, single-centre study. Three ABOi KTx and 141 ABOc KTx were excluded because of cyclosporine A-based immunosuppression, and 1 ABOc KTx was lost to follow-up.
Results. Median estimated 10-year patient and graft survival in ABOi KTx was 99 and 94%, respectively, and surpassed ABOc-KTx patient and graft survival of 80 and 88%, respectively. The incidence rate of antibody-mediated rejections was 10 and 8%, and that of T-cell-mediated rejections was 17 and 20% in ABOi KTx and ABOc KTx, respectively. Infectious and malignant complications in ABOi KTx were not more common than in ABOc KTx. However, postoperative lymphoceles occurred more frequently in ABOi KTx. Subgroup analysis of ABOi-KTx patients revealed that patients with high-titre isohaemagglutinins before transplantation had equal long-term results compared with low-titre isohaemagglutinin patients.
Conclusion. Taken together, long-term outcome of ABOi KTx is not inferior to ABOc KTx. Incidences of rejection episodes, infectious complications and malignancies are not increased, despite the more vigorous immunosuppression in ABOi KTx. Our data provide further evidence that ABOi KTx with living donation is a safe, successful and reasonable option to reduce the organ shortage.

Keywords: ABO-incompatible kidney transplantation, complications, high-titre isohaemagglutinin patients, long-term outcome, rejections

INTRODUCTION

Facing the imperative need to enlarge the pool of donor organs, ABO-incompatible kidney transplantation (ABOi KTx) has been increasingly employed during the last decades [1, 2]. Successful protocols were developed mainly in Japan and the USA in the late 1980s and 1990s, before ABOi KTx was established in Sweden in 2001 and the rest of Europe thereafter [3–5]. In 2004, we adopted a modified version of the Swedish protocol, consisting

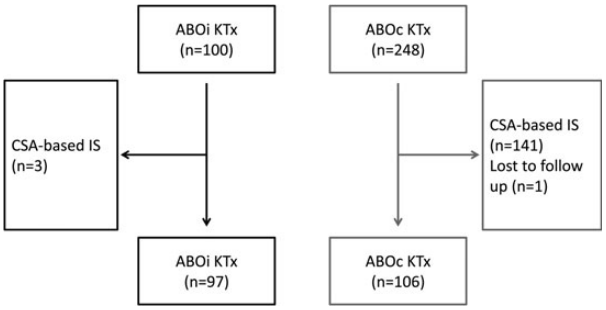

FIGURE 1: Study flowchart. CSA, cyclosporine A; IS, immunosuppression.

of an anti-CD20-treatment with rituximab (375 mg/m^2) and repetitive immunoadsorptions before transplantation. Since then, 100 ABOi KTx have been performed at our centre, and we prospectively gathered comprehensive outcome and follow-up data. Outcome results of our cohorts were reported in 2007 combining the data of 60 ABOi KTx of three centres including our centre and in 2010 evaluating 40 ABOi KTx of our single centre with follow-up data up to 5 years [6, 7].

This study now analyses the long-term outcome of the patients gathered at our centre in >10 years of our ABOi-KTx experience and compares them with an ABO-compatible kidney transplantation (ABOc KTx) cohort within the same time period and with an as similar as possible immunosuppressive protocol.

MATERIALS AND METHODS

Patients and study design

This study was an *ad hoc* analysis of ABO-compatible and ABO-incompatible adult living donor kidney recipients of our centre. The data were analysed from the prospectively conducted living kidney transplant registry from 1 April 2004 until 28 October 2014. All transplant recipients receiving basiliximab induction therapy and initial immunosuppression with tacrolimus, mycophenolic acid and prednisone were included. A total of 100 ABO-incompatible and 248 ABO-compatible living donor transplants were performed during the inclusion period. Of these, 3 patients with cyclosporine A-based immunosuppression of the ABO-incompatible cohort and 141 patients with cyclosporine A-based immunosuppression of the ABO-compatible cohort were excluded from analysis. One patient of the ABO-compatible cohort was lost to follow-up and therefore excluded from analysis. Thus, 97 ABO-incompatible kidney transplant recipients and 106 ABO-compatible kidney transplant recipients were compared (Figure 1). All patients of the Freiburg Medical Centre living donor kidney transplantation programme gave written consent to store and analyse their healthcare records and follow-up data. The study was approved by the ethics committee of the Freiburg Medical Centre.

Donor management and kidney graft preparation

In a 3-day check-up examination, the donor suitability was confirmed. An ethics committee of the District Medical Association Südbaden evaluated and approved all donor–recipient pairs. The kidney procurement was performed in supine position over an open anterior extraperitoneal minimal incision laparotomy. After retrieval, the kidney was immediately flushed with 5000 units of heparin in 150 mL of saline followed by 2 L of histidine–tryptophan–ketoglutarate solution. Of note, in our initial protocol (the first 26 ABOi KTx), the flush contained 20 000 units of heparin dissolved in 50 mL of saline. The kidney was stored on ice in the refrigerator at 6°C until transplantation. The recipient was scheduled for transplantation directly after the organ retrieval. The transplantation was performed in the established technique usually to the right iliac fossa of the recipient. All recipients received 12 500 units of heparin per day intravenously, starting 6 h after surgery until days 4–5. Both ABOi KTx and ABOc KTx were performed by the same surgical team.

Immunoadsorption protocols for ABO-incompatible patients

ABO-incompatible recipients were pre-treated with immunoadsorptions performed with commercially available apheresis devices (Octo Nova™, Diamed Medizintechnik, Köln, Germany), hollow-fibre plasma separators (P2™, Fresenius Medical Care, Bad Homburg, Germany or Microplas MPS 07™, Bellco, Italy) and antigen-specific adsorption columns (Glycosorb A/B™, Glycorex, Lund, Sweden) until November 2012, or non-specific immunoglobulin adsorption columns (Immunosorba™, Fresenius Medical Care) since December 2012. The transition from specific to non-specific immunoadsorption technique was made due to financial reasons. Immunoadsorption protocols were performed as described before [7]. If isohaemagglutinin target titres (≤1:4 for IgM and IgG) were not reached solely by non-specific immunoadsorption, additional plasma exchanges were performed. The amount of fresh frozen plasma (FFP) for plasma exchange depended on the individual blood coagulation status; ≤2 days before surgery, 100% blood type AB FFP was regularly used for replacement. Post-transplantation, isohaemagglutinin titres were monitored daily in the first week and every other day in the second week. Post-transplantation, immunoadsorption was scheduled immediately if titres exceeded 1:8 IgM/IgG in the first week or 1:16 IgM/IgG in the second week, respectively. Post-transplantation, plasmapheresis was only performed when initiated post-transplantation immunoadsorptions were not able to reach isohaemagglutinin target levels.

Isohaemagglutinin titre measurements, cross-matching and alloantibody detection

Isohaemagglutinin titres were measured as described before [7]. Briefly, until 2006 we used tube centrifugation method, and since 2007 the gel centrifugation technique was performed to quantify isohaemagglutinin IgG and IgM titres (anti-A/B titres). Human leukocyte antigen (HLA) antibodies were determined using the commercially available ELISA test (Lambda Antigen Tray™, One Lambda, Canoga Park, CA, USA) and microplates preloaded with frozen viable lymphocyte cell panels (Biotest, Dreieich, Germany).

Immunosuppression and anti-infective prophylaxis in ABO-incompatible recipients

ABO-incompatible recipients received a single dose of rituximab (375 mg/m^2 body surface) ~30 days before scheduled

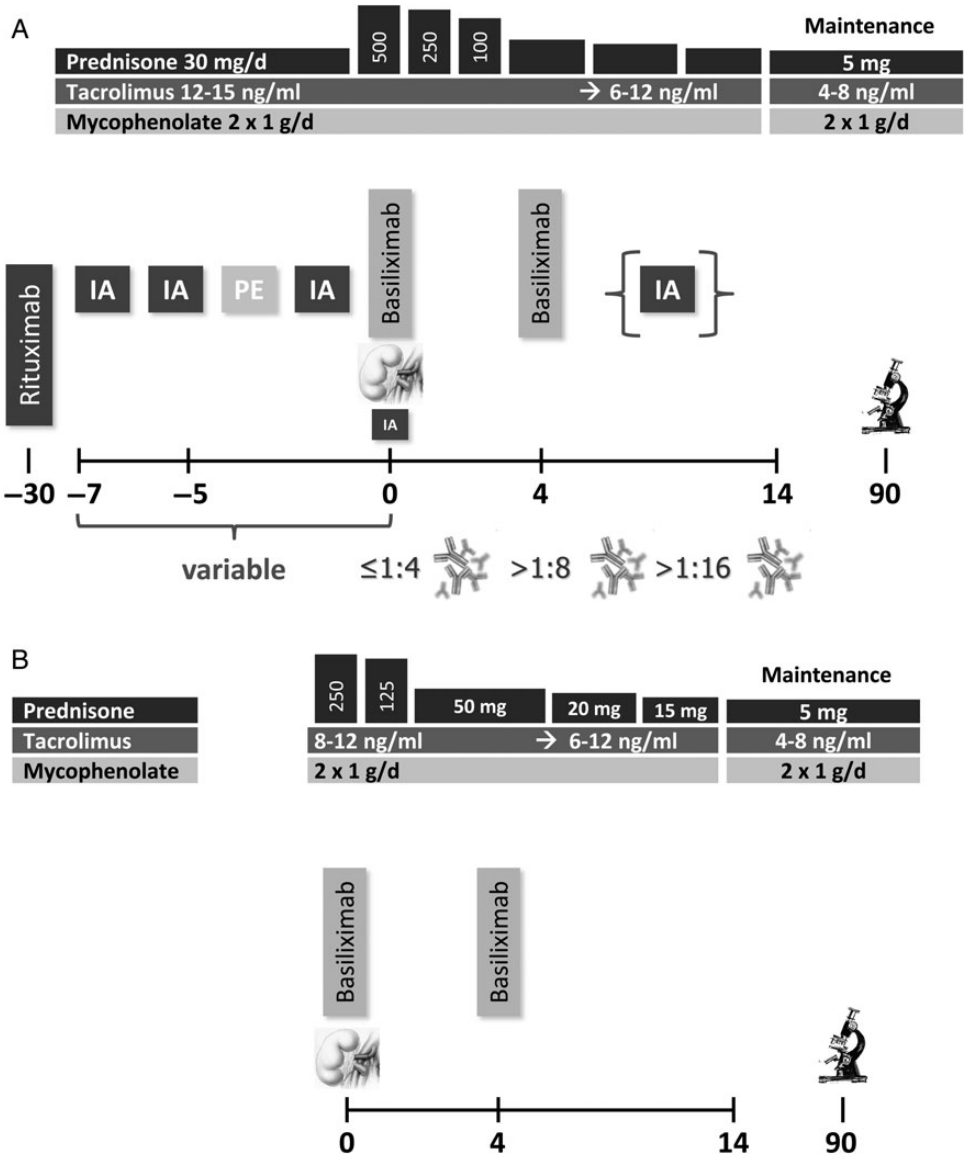

FIGURE 2: (**A**) ABOi-KTx protocol schematically illustrating timing (days) and dosage of immunosuppression, timing of rituximab administration, and anti-A/B titre cut-offs. IA, immunoadsorption; PE, plasma exchange. (**B**) ABOc-KTx protocol schematically illustrating timing and dosage of immunosuppression.

transplantation. Seven days before surgery, oral immunosuppression with tacrolimus (aimed trough level 12–15 ng/mL), mycophenolate mofetil (2 g daily) and prednisone (30 mg daily) was initiated. Basiliximab (20 mg) induction was given at surgery and postoperative day 4. Prednisone was started at 500 mg at surgery, 250 mg at day 1, 100 mg at day 2 after surgery and rapidly tapered to 15 mg at discharge around day 14. At day 7, tacrolimus target levels were lowered to 6–12 ng/mL. After 3 months, maintenance immunosuppression consisted of tacrolimus (trough level 4–8 ng/mL), mycophenolate mofetil (2 g daily) or mycophenolate sodium (1440 mg daily), and prednisone (5 mg daily; Figure 2A). Surveillance transplant kidney biopsy was performed 3 months after transplantation in every patient who consented. Individual modification of the immunosuppressive regimen was based on transplant kidney biopsy results or clinical side effects. Rejection treatment consisted of high-dose steroids and tacrolimus escalation in T-cell-mediated rejection (TCMR) and antibody-mediated rejection (ABMR), and additionally anti-thymocyte globulin plasma exchange in acute ABMR. All patients received valganciclovir cytomegalovirus (CMV) prophylaxis for 3 months and trimethoprim/sulfamethoxazole for 6 months post-transplantation. Additionally, fluconazole prophylaxis was administrated until postoperative day 28. Two patients' immunosuppression was switched to cyclosporine A or sirolimus due to the occurrence of post-transplant diabetes mellitus or calcineurin inhibitor toxicity, respectively.

Immunosuppression and anti-infective prophylaxis in ABO-compatible recipients

ABO-compatible recipients were treated with basiliximab induction therapy (20 mg, day 0 + 4) as well. Prednisone was administered starting with 250 mg at surgery, 125 mg at postoperative day 1 and 50 mg at postoperative day 2. Tacrolimus trough levels were aimed at 8–12 ng/mL and mycophenolate mofetil started with 2 g daily (Figure 2B). Subsequent

Table 1. Baseline characteristics of both study cohorts

Recipient and donor characteristics	ABOi KTx ($n = 97$)	ABOc KTx ($n = 106$)	P-value
Recipient age at Tx (years)	47 ± 11 (18–67)	49 ± 12 (18–73)	0.15
Donor age at Tx (years)	51 ± 9 (27–75)	52 ± 9 (29–73)	0.51
Recipient gender female/male	37/60 (38%/62%)	40/66 (38%/62%)	1.0
Donor gender female/male	62/35 (64%/36%)	62/44 (58%/42%)	0.47
Related donors	31 (32%)	30 (28%)	0.65
Unrelated donors	66 (68%)	76 (72%)	0.65
Pre-emptive Tx	21 (22%)	27 (27%)	0.62
Recipient's time on dialysis before Tx (months)	24 ± 29 (0–139)	21 ± 31 (0–145)	0.38
Recipient's hypertension at Tx	88 (91%)	97 (92%)	1.00
Recipient's diabetes at Tx	4 (4%)	11 (10%)	0.11
Recipient's coronary heart disease at Tx	8 (8%)	20 (19%)	0.04*
Recipient's history of prior malignancy	7 (7%)	11 (10%)	0.47

*$P < 0.05$.

tacrolimus target levels and maintenance immunosuppression were identical to ABOi KTx. Fluconazole and trimethoprim/sulfamethoxazole prophylaxis was identical, compared with ABO-incompatible recipients. Valganciclovir prophylaxis was applied for 3 months only in high-risk donor/recipient constellation (donor CMV+/recipient CMV−).

One hundred and forty-one cyclosporine A-based patients of the total ABOc-KTx cohort were primarily excluded from our analysis, but later on analysed in comparison with the selected tacrolimus-based ABOc-KTx cohort. Of those cyclosporine A-based patients, 38 were switched to tacrolimus in the follow-up, mostly because of rejection episodes, and 2 were switched to sirolimus because of skin tumours.

Clinical data, histological data and statistical analysis

Baseline characteristics were assessed from direct patient care and clinical records. Follow-up data were collected from post-kidney transplant care facilities throughout Germany. Estimated glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease (MDRD) formula. Proteinuria was determined mostly from spot urine collections. Infectious complications evaluated for comprised sepsis, CMV disease (defined by CMV replication and clinical symptoms), polyoma virus-associated nephropathy (proven by histology), pneumocystis pneumonia and recurrent urinary tract infections (more than three episodes). Non-infectious complications recorded included post-transplant lymphoproliferative disease, skin malignancy, other malignancies and surgical complications. Delayed graft function was defined by the need for at least one postoperative dialysis treatment. Graft failure was defined by the need to resume dialysis permanently. Transplant kidney biopsies were performed for surveillance and whenever indicated. Histology samples of transplant kidney biopsies were scored (retrospectively) according to Banff 2013 criteria [8].

Data are expressed as mean ± standard deviation (range), if not stated otherwise. Group comparison was performed with Student's *t*-test for continuous data (http://graphpad.com/quickcalcs/ttest1.cfm) and Fisher's exact test for discrete data (http://graphpad.com/quickcalcs/contingency1.cfm). Kaplan–Meier survival analysis was compared by the log-rank test. GraphPad™ Prism Version 6.02 software was used for diagram preparation. Statistical significance was assumed at a P-value of <0.05.

Table 2. Immunological risk factors of both study cohorts

Immunological risk factors	ABOi KTx ($n = 97$)	ABOc KTx ($n = 106$)	P-value
HLA mismatches (A/B/DR)	3.80 ± 1.37	3.57 ± 1.54	0.25
First Tx	83 (86%)	88 (83%)	0.70
Second or third Tx	14 (14%)	18 (17%)	0.70
HLA-identical sibling as donor	1 (1%)	3 (3%)	0.62
Panel reactive antibodies			
0–19%	94 (97%)	99 (93%)	0.34
20–79%	2 (2%)	6 (6%)	0.28
>80%	1 (1%)	1 (1%)	1.00

RESULTS

Baseline characteristics

Recipient and donor characteristics of the two cohorts did not significantly differ in age, gender distribution, proportion of related donors or proportion of pre-emptive transplantation. Recipients' time on dialysis before transplantation, naturally, differed widely within the cohorts, but was not statistically different between the two cohorts. Recipients' cardiovascular risk factors, such as hypertension, were very prevalent but equally distributed. Diabetes was found more often in ABOc-KTx recipients, but the difference to ABOi-KTx recipients was not significant. However, incidence of recipients' coronary heart disease was significantly higher in ABOc KTx. Recipients' history of prior malignancy was not significantly diverging (Table 1).

Immunological risk factors comprising HLA (A/B/DR) mismatches, preceding kidney transplantation or level of recipient's sensitization measured by panel reactive antibodies were not significantly different in both cohorts (Table 2).

Preoperative and postoperative removal of isohaemagglutinins

To reach the isohaemagglutinin target titres (≤1:4 for IgM and IgG), recipients received at least one pre-transplantation treatment: immunoadsorption was repeated on average 5.72 ± 3.64 (0–18) times and plasma exchange was carried out in 26

Table 3. Study short- and long-term results

Results	ABOi KTx ($n = 97$)	ABOc KTx ($n = 106$)	P-value
Median follow-up (months)	58 ± 36 (3–128)	48 ± 29 (2–122)	0.03*
Median patient survival (%)	99	80	0.007**
Deaths	1	9	0.02*
Median death-censored graft survival (%)	94	88	0.24
Graft loss	4	8	0.38
Serum creatinine at discharge from the hospital (mg/dL)	1.52 ± 0.47 (0.7–3.7)	1.50 ± 0.4 (0.7–2.8)	0.75
eGFR (MDRD) at discharge from the hospital (mL/min/1.73 m^2)	51 ± 15 (22–100)	51 ± 15 (19–101)	0.77
Serum creatinine at the last follow-up (mg/dL)	1.48 ± 0.5 (0.7–3.86)	1.48 ± 0.42 (0.6–2.6)	0.90
eGFR (MDRD) at the last follow-up (mL/min/1.73 m^2)	53 ± 15 (18–92)	52 ± 15 (23–115)	0.74
Proteinuria at the last follow-up (g protein/g creatinine)	0.22 ± 0.52 (0.03–3.87)	0.15 ± 0.13 (0.02–0.88)	0.22

*P < 0.05, **P < 0.01.

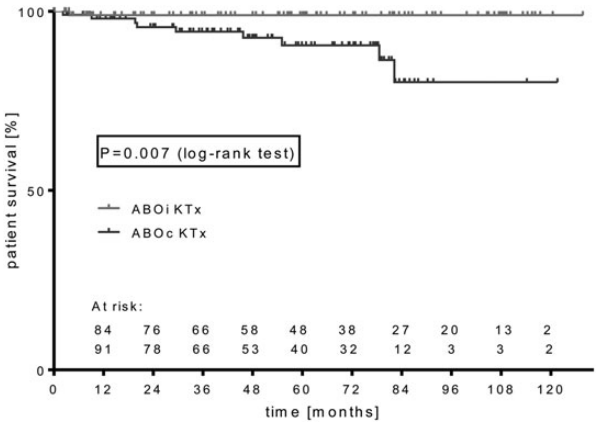

FIGURE 3: Kaplan–Meier graph of patient survival. Number of recipients at risk (upper row: ABOi KTx, lower row: ABOc KTx).

patients (27% of all ABOi-KTx recipients) for 2.23 ± 1.07 (1–7) times. Post-transplantation immunoadsorption was necessary in 22 cases (23% of all ABOi-KTx recipients) and carried out 2.67 ± 1.98 (1–8) times. Post-transplantation plasmapheresis was only performed when beforehand initiated post-transplantation immunoadsorptions were not able to reach isohaemagglutinin target levels.

Patient survival and death-censored graft survival

The median follow-up of the ABOi-KTx cohort versus ABOc-KTx cohort was 58 ± 36 (3–128) and 48 ± 29 (2–122) months, respectively. The significantly shorter observation period in ABOc KTx resulted from exclusion of recipients on cyclosporine A-based immunosuppression, which was the commonly used regimen for ABOc KTx until June 2007 at our centre. Kaplan–Meier estimated median patient survival differed highly significantly, reaching 99% in ABOi KTx at 1, 3, 5 and 10 years versus 98, 94, 91 and 80% in ABOc KTx at 1, 3, 5 and 10 years, respectively, resulting from one death in the ABOi-KTx cohort and nine deaths in the ABOc-KTx cohort, respectively (P = 0.007; Table 3 and Figure 3). Causes of death compromised severe infectious complications, progressive malignancies, and one confirmed as well as one suspected cardiovascular event (Supplementary data, Table S1). Of note, patient survival of the total ABOc-KTx cohort (including the cyclosporine A-treated subgroup) was 97, 96, 94 and 89% at 1, 3, 5 and 10 years, respectively, and therefore higher as in the selected ABOc-KTx cohort, not significantly different from the ABOi-KTx cohort (P = 0.057; Supplementary data, Figure S1).

Estimated death-censored graft survival was 98, 97, 97 and 94% in ABOi KTx at 1, 3, 5 and 10 years, respectively, and 96, 94, 94 and 88% in ABOc KTx at 1, 3, 5 and 10 years, respectively (P = 0.24; Table 3 and Figure 4). Causes of graft loss were mainly long-term chronic antibody-mediated allograft rejections in both groups. Acute rejection, bleeding and arterial graft thrombosis were less frequent and occurred early in follow-up (Supplementary data, Table S2). Of note, graft survival of the total ABOc-KTx cohort was 97, 96, 95 and 90% at 1, 3, 5 and 10 years, respectively, and therefore higher as in the selected takrolimus-based ABOc-KTx cohort, not significantly different from the ABOi-KTx cohort (P = 0.49; Supplementary data, Figure S2).

Graft function

The course of eGFR in both cohorts is depicted in Figure 5. At discharge, serum creatinine in the ABOi-KTx cohort was 1.52 ± 0.47 (0.7–3.7) mg/dL corresponding to an eGFR of 51 ± 15 (22–100) mL/min. Very similar values were measured in the A BOc-KTx cohort, namely a serum creatinine of 1.50 ± 0.4 (0.7–2.8) mg/dL (P = 0.75) and corresponding eGFR of 51 ± 15 (19–101) (P = 0.77). Graft function remained the same in both cohorts, with the mean last follow-up values of serum creatinine 1.52 ± 0.47 (0.7–3.7) mg/dL and eGFR 51 ± 15 (22–100) mL/min in the ABOi-KTx group and serum creatinine 1.48 ± 0.42 (0.6–2.6) mg/dL (P = 0.9) and eGFR 52 ± 15 (23–115) mL/min (P = 0.74) in the ABOc-KTx group. Increasing proteinuria was rarely recorded in both groups with mean protein/creatinine ratios of 0.22 ± 0.52 (0.03–3.87) in the ABOi-KTx cohort and 0.15 ± 0.13 (0.02–0.88) in the ABOc-KTx cohort (Table 3). One recurrence of membranoproliferative glomerulonephritis (C3GN) with overt proteinuria was recorded in the ABOi-KTx cohort, which did not respond to additional rituximab, but meanwhile responded well to eculizumab (for more details, see Supplementary data).

Graft rejections

Within the observation period of both cohorts, graft rejections occurred in 26 (27%) of ABOi-KTx and 29 (27%) of ABOc-KTx patients. Acute TCMR accounted for 16 (17%) and 21 (20%), and acute ABMR accounted for 10 (10%) and 8 (8%) in the ABOi-KTx cohort and the ABOc-KTx cohort,

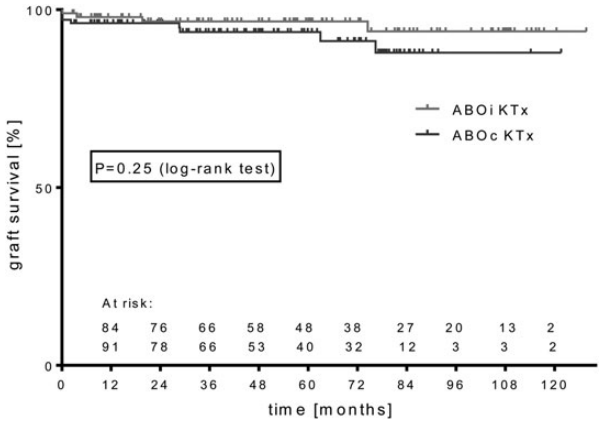

FIGURE 4: Kaplan–Meier graph of transplant survival. Number of recipients at risk (upper row: ABOi KTx, lower row: ABOc KTx).

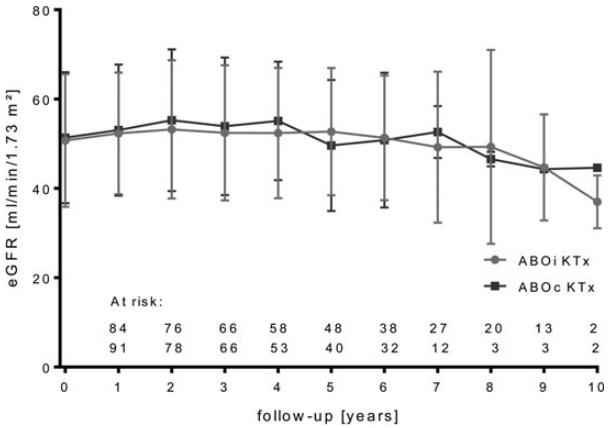

FIGURE 5: Ten-year follow-up time course of eGFR of ABOi-KTx and ABOc-KTx recipients. Number of recipients at risk (upper row: ABOi KTx, lower row: ABOc KTx).

respectively. These events were not statistically different, although there was a trend of less TCMR, especially TCMR Banff class IA, and a trend of more borderline changes in ABOi KTx (Table 4). In ABMR, there was no significant difference in episodes of rejections, as tested in very early (<14 days after KTx), early (<1 year after KTx) and late (>1 year after KTx) onset in time (data not shown). Furthermore, in ABOi-KTx recipients, occurrence of very early, early and late ABMR did not correlate with rebound of isohaemagglutinins beyond cutoff. In fact, no ABOi-KTx recipient with very early or early ABMR showed titre rebound requiring additional postoperative immunoadsorptions or plasmapheresis per protocol before ABMR diagnosis (data not shown).

There was no statistical significant difference in rejection rates of the tacrolimus-based ABOc-KTx cohort from the total ABOc-KTx cohort reporting 57 (23%) TCMR and 25 (10%) ABMR (P = 0.58 and 0.55, respectively).

Infectious complications

Infectious complications requiring hospitalization occurred in 38 versus 35% of ABOi-KTx and ABOc-KTx patients, respectively. Common complications were recurrent urinary tract infections, sepsis and CMV disease, and the latter with a trend to more frequent episodes in the ABOc-KTx cohort. Polyoma virus nephropathy, varicella infections and herpes simplex infections were recorded, but also did not differ between both groups. Sepsis and pneumocystis infections contributed to morbidity and mortality in both cohorts (Table 5 and Supplementary data, Table S1). There was no significant difference in hospitalization rates (35 versus 38%, P = 0.63) or specific infectious complications between the tacrolimus-based ABOc-KTx cohort and the total ABOc-KTx cohort.

Table 4. Rejections and borderline changes of both study cohorts

Biopsy results according to Banff Classification 2013	ABOi KTx ($n = 97$)	ABOc KTx ($n = 106$)	P-value
Total patients with rejections or borderline changes	44 (45%)	43 (41%)	0.67
Patients with rejections	26 (27%)	29 (27%)	1.00
Patients with borderline changes	18 (19%)	14 (13%)	0.34
Patients with ABMR	10 (10%)	8 (8%)	0.62
ABMR I	0 (0%)	0 (0%)	1.00
ABMR II	10 (10%)	8 (8%)	0.62
ABMR III	0 (0%)	0 (0%)	1.00
Patients with TCMR	16 (17%)	21 (20%)	0.59
TCMR IA	1 (1%)	7 (7%)	0.067
TCMR IB	2 (2%)	0 (0%)	0.23
TCMR IIA	8 (8%)	8 (8%)	1.00
TCMR IIB	5 (5%)	5 (5%)	1.00
TCMR III	0 (0%)	1 (1%)	1.00

Table 5. Infectious complications of both study cohorts

Infectious complications	ABOi KTx ($n = 97$)	ABOc KTx ($n = 106$)	P-value
Infectious complications requiring hospitalization	37 (38%)	37 (35%)	0.66
Sepsis	8 (8%)	8 (8%)	1.00
Recurrent UTI requiring antibiotical prophylaxis	17 (18%)	14 (13%)	0.44
CMV disease	5 (5%)	11 (10%)	0.20
Polyoma virus nephropathy	7 (7%)	5 (5%)	0.56
Varicella infections	1 (1%)	4 (4%)	0.37
Herpes simplex infections	1 (1%)	2 (2%)	1.00
Pneumocystis jiroveci pneumonia	1 (1%)	2 (2%)	1.00

Surgical complications

Strikingly, unscheduled operative revision procedures were needed more often in ABOi-KTx patients than in ABOc-KTx patients. This resulted from a highly significantly raised incidence of lymphoceles in the ABOi-KTx cohort. Bleeding or haematoma complications also occurred more often in ABOi-KTx patients—this finding, however, was not statistically different from ABOc-KTx patients (Table 6).

High-titre isohaemagglutinin ABOi-KTx patients

High-titre isohaemagglutinin patients were defined by anti-A/B titres ≥1:256 (as in ref. [9]) and documented in 27 of the 97 ABOi-KTx with 1:2048 being the upper accepted

limit for transplantation. Baseline characteristics of the high- and low-titre ABOi-KTx subgroups differed: the high-titre subgroup showed more blood group A1 and A2 to 0 transplantations, and a tendency towards a shorter timespan on dialysis before transplantation (presumably caused by more pre-emptive transplantations), the blood group constellation was almost exclusively A1 to 0 ($n = 18$) or A2 to 0 ($n = 5$), with three cases of B to 0 ($n = 3$) and only one A1B to 0 ($n = 1$), and the donors were younger compared with the ones in the low-titre group (Supplementary data, Table S3).

The number of pre-transplantation isohaemagglutinin removal procedures (immunoadsorption/plasma exchange) was 10.28 ± 4.23 in the high-titre group, with plasma exchange being necessary in 12 of 27 recipients in case the titres stopped declining after two consecutive procedures. However, in the low-titre group, the number of pre-transplantation isohaemagglutinin removal procedures (immunoadsorption/plasma exchange) was 4.81 ± 2.64 ($P < 0.001$), with plasma exchange performed in 14 of 70 patients ($P < 0.05$). The minimum platelet count during preparation was $139 \pm 34 \times 10^3/\mu L$ versus $161 \pm 34 \times 10^3/\mu L$, but not significantly different in the high- and low-titre group, respectively ($P = 0.09$). The number of post-transplantation immunoadsorptions (if titres above target, see protocol) was 1.44 ± 2.17 versus 0.24 ± 0.80 in the high- and low-titre group, respectively ($P < 0.001$).

Long-term patient and transplant survival and kidney function did not differ between high- and low-titre ABOi KTx. There was also no difference in post-transplant infection rate and humoral or T-cell-mediated rejection. But borderline changes, 'suspicious' for acute T-cell mediated rejection, were significantly more frequent in the high-titre group (Table 7).

Table 6. Surgical complications of both study cohorts

Surgical complications	ABOi KTx ($n = 97$)	ABOc KTx ($n = 106$)	P-value
Operative revisions yes/no	37 (38%)	25 (24%)	0.032*
Bleeding/haematoma requiring surgery	20 (21%)	14 (13%)	0.19
Lymphocele total	32 (33%)	16 (15%)	0.003**
Lymphocele requiring surgery	19 (20%)	8 (8%)	0.013*
Ureteral complications	11 (11%)	9 (9%)	0.64

*$P < 0.05$, **$P < 0.01$.

DISCUSSION

For many end-stage renal disease patients and their living donors, ABOi KTx has become a safe and successful procedure to shorten waiting time for a renal graft.

With 97 ABOi-KTx patients being included, this study analyses one of the largest 10-year follow-up single-centre cohorts using a preparation protocol based on rituximab and immunoadsorption for removal of isohaemagglutinins, adding substantial support to the overall promising results of ABOi KTx reported so far.

The baseline characteristics of both cohorts did not show any exceptional differences either between our ABOi-KTx and ABOc-KTx cohort, or compared with other study cohorts published [10, 11]. Whether the elevated incidence of coronary heart disease in our ABOc-KTx patients contributed to the higher mortality of this cohort remains unclear, but seems unlikely, since there was only one suspected cardiovascular death attributable to coronary heart disease. Moreover, there is the possibility of selection bias since the results of the ABOi-KTx cohort showed less differences versus the total ABOc-KTx cohort (including cyclosporine A) compared with the tacrolimus-based ABOc-KTx group.

Ten years median patient survival of 99% and death-censored graft survival of 94% of the ABOi KTx document excellent survival rates. In a cohort of 74 ABOi KTx from 2005 to 2010 Tokyo Women's Medical University divided into two groups with high- and low-dose rituximab protocols reported a 5-year patient survival of 100 and 100%, and an 1-year graft survival of 95.7 and 98%, respectively [10]. In a recent study with 191 ABOi-KTx recipients divided into two cohorts of high versus low rebound isohaemagglutinin titres, the 10-year patient survival rates reached 96–97%, and the 10-year graft survival rates were 86–93% in both groups [12].

Graft function in ABOi KTx appears to be very stable over 10 years of follow-up—similar to the most recent Japanese data [12]. The slightly inferior results in the USA and of the Collaborative Transplant Study (CTS) might be explained by a wide variation in preconditioning protocols of the participating centres [13, 14]. However, our results strongly support ABOi KTx as being equivalent in long-term results compared with ABOc KTx [6, 7, 14–17].

We did not record an increased incidence of infectious complication, despite the intensified immunosuppression including

Table 7. Short- and long-term results of low-titre versus high-titre ABOi KTx

	ABOi ≤1:128 ($n = 70$)	ABOi ≥1:256 ($n = 27$)	P-value
Serum creatinine (mg/dL) at discharge	1.52	1.51	0.92
eGFR (MDRD) (mL/min/1.73 m^2) at discharge	50 (23–100)	52 (27–78)	0.57
Median death-censored graft survival	94%	100%	0.57
Serum creatinine (mg/dL) at the last follow-up	1.51	1.41	0.45
eGFR (MDRD) (mL/min/1.73 m^2) at the last follow-up	52 (11–92)	54 (31–82)	0.50
Proteinuria (g/g) at the last follow-up	0.19	0.30	0.77
ABMR	5 (7%)	4 (15%)	0.26
TCMR	9 (13%)	4 (15%)	0.75
Borderline changes	7 (10%)	8 (30%)	0.03*

*$P < 0.05$.

administration of rituximab in ABOi KTx. In contrast, the CTS also reported a small but significant difference in patient survival, because of a significantly higher rate of early death from infection in recipients of ABO-incompatible grafts versus the matched ABO-compatible recipients [14]. In a study of 119 Medicare-insured recipients in the USA, there were higher incidences, especially urinary tract infections in the first 90 days but not in the first years thereafter [18]. Smaller sample-sized reports differed in finding an increased, equal or even lowered incidence of infectious complications [7, 15, 19–22]. Whereas infectious complications could not be correlated with rituximab administration [23, 24], they seem rather to correlate with the total immunosuppressive burden of the individual patient.

In the very recently published large cohort ($n = 191$, 2001–2013) of ABOi KTx in Japan, Ishida *et al.* reported a total of 15 (8%) TCMR and a total of 17 (9%) ABMR [12]. Tokyo Women's Medical University centre performed low-dose rituximab (200 mg at day 7 before surgery) combined with double filtration plasmapheresis for isohaemagglutinin elimination (pre-transplantation titre cutoff ≤1:32). Patients with pre-transplantation splenectomy instead of rituximab had a higher incidence of TCMR [12]. Having only few differences in immunosuppression protocols we observed comparable rates of ABMR but twice as many TCMR in our ABOi-KTx cohort (16.5%) compared with the Japanese cohort ABOi KTx (8–10%) with similar tacrolimus levels aimed for [12]. However, within our observational study, the ABOc-KTx cohort had a trend to more TCMR and our ABOi-KTx cohort more borderline changes. This effect, we speculate, might be due to rituximab administration in the ABOi-KTx cohort.

The bleeding complications in the ABOi-KTx cohort were not significantly elevated, although reported in the preceding study [7] and analysed in a recent report of the Rotterdam transplantation centre [25]. Renner *et al.* [26] suspected high heparin doses for graft artery perfusion after donor nephrectomy as a leading cause for postoperative bleeding. This hypothesis is in line with our data. In the beginning of the programme (first 26 patients), we used higher doses of heparin to flush the kidneys, with increased bleeding complications. After reducing the heparin dose, the incidence of postoperative bleeding has decreased significantly resulting in an overall similar incidence of bleeding complications compared with ABOc KTx.

The incidence of postoperative lymphoceles was significantly higher in the ABO-incompatible recipients. Conceivably, this is due to preoperative immunosuppressive medication. Lopau *et al.* [27] showed an impact of mycophenolate mofetil on the development of lymphoceles. It is speculated that the preoperatively administrated immunosuppressive medication in ABOi KTx even increases this effect. The majority of lymphocytes was drained laparoscopically into the abdominal cavity.

The preparation of patients with high titres of isohaemagglutinins can be demanding for the healthcare team and the patient. Applying our protocol, we needed ∼10 antibody removal procedures compared with less than half the number of procedures in the low-titre group. Especially when using protein-A-based semi-selective columns for immunoadsorption that bind IgM isohaemagglutinins less well [28], combining additional plasma exchange with immunoadsorption provided an effective strategy to overcome high antibody titres and/or significant rebound after an immunoadsorption session. Still, a matter of debate is the target titre of isohaemagglutinins that should be aimed for at the time of transplant surgery for successful accommodation process. The rather low target titre of ≤1:4 is still applied by our centre, but titres of ≤1:8 or probably even ≤1:16 before surgery might be safe as well [4, 11, 29, 30]. However, when comparing the titres between transplant centres, the different methods being used for titre measurement and their variability have to be taken into account. After transplantation, titre rebound is physiological and discussed not to be immunologically relevant for the graft after the first 14 days of 'accommodation' [2]. Whether postoperative isohaemagglutinin rebound should be treated at all is under current debate: a recent large single-centre study concluded that absolute titre rebound does not correlate with poor outcome [31]. However, former studies—although statistically underpowered for this specific question—showed incidences of ABMR after high-titre rebound [11, 32, 33]. In this study, we could not attribute ABMR, especially early ABMR, to titre rebound. However, data on this issue are still scarce and well-powered trials are needed. Therefore, we currently continue the on-demand strategy of postoperative titre rebound treatment [9].

Interestingly, we did not find any disadvantage in long-term results of high-titre ABOi-KTx patients regarding mortality, graft survival, transplant function or infectious complications. The higher rate of borderline changes in the high-titre group points towards the higher immunological risk in this group. The transplant centre at Seoul St Mary's Hospital using a very similar preconditioning protocol reported an increase in acute humoral rejection and infection. They defined the high-titre group by anti-A/B ≥1:512, however [29]. But, even an analysis of a subgroup of our patients with anti-A/B ≥1:512 did not reveal significant differences compared with the low-titre group. Whether our results are a consequence of the low target titres aimed at in the pre- and early post-transplantation period (see above) remains uncertain.

These results in patients with high anti-A/B titres consolidate data on shorter follow-up periods reported previously by our centre. To successfully pre-treat recipients with very high titre (≥1:1024), we meanwhile consider the application of rituximab earlier on, up to 6 months in advance of planned transplant surgery (one patient in our cohort). Furthermore, starting oral immunosuppressive medication some weeks before transplantation will be considered in future. We nowadays accept patients with starting anti-A/B titres of up to 1:2048 at our centre: this implies more frequent pre-transplant isohaemagglutinin removal procedures and higher vigilance with respect to changes in blood coagulation during the preparation period, but so far elimination of isohaemagglutinins could be achieved in all cases with comparable long-term results during the past 5 years.

Altogether, our data further substantiate living donor ABOi KTx as a safe and efficient procedure with at least equivalent results compared with ABOc KTx.

SUPPLEMENTARY DATA

Supplementary data are available online at http://ndt.oxfordjournals.org.

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CONFLICT OF INTEREST STATEMENT

The results presented in this paper have not been published previously in whole or part, except in abstract form.

REFERENCES

1. Council of Europe - Newsletter Transplant. International Figures on Donation and Transplantation – 2013. Strasbourg: European Directorate for the Quality of Medicines & Health Care, 2014
2. Zschiedrich S, Kramer-Zucker A, Janigen B *et al.* An update on ABO-incompatible kidney transplantation. Transpl Int 2015; 28: 387–397
3. Tanabe K. Japanese experience of ABO-incompatible living kidney transplantation. Transplantation 2007; 84: S4–S7
4. Montgomery RA, Locke JE, King KE *et al.* ABO incompatible renal transplantation: a paradigm ready for broad implementation. Transplantation 2009; 87: 1246–1255
5. Tyden G, Kumlien G, Fehrman I. Successful ABO-incompatible kidney transplantations without splenectomy using antigen-specific immunoadsorption and rituximab. Transplantation 2003; 76: 730–731
6. Tyden G, Donauer J, Wadstrom J *et al.* Implementation of a protocol for ABO-incompatible kidney transplantation—a three-centre experience with 60 consecutive transplantations. Transplantation 2007; 83: 1153–1155
7. Wilpert J, Fischer KG, Pisarski P *et al.* Long-term outcome of ABO-incompatible living donor kidney transplantation based on antigen-specific desensitization. An observational comparative analysis. Nephrol Dial Transplant 2010; 25: 3778–3786
8. Haas M, Sis B, Racusen LC *et al.* Banff 2013 meeting report: inclusion of c4d-negative antibody-mediated rejection and antibody-associated arterial lesions. Am J Transplant 2014; 14: 272–283
9. Wilpert J, Geyer M, Pisarski P *et al.* On-demand strategy as an alternative to conventionally scheduled post-transplant immunoadsorptions after ABO-incompatible kidney transplantation. Nephrol Dial Transplant 2007; 22: 3048–3051
10. Shirakawa H, Ishida H, Shimizu T *et al.* The low dose of rituximab in ABO-incompatible kidney transplantation without a splenectomy: a single-centre experience. Clin Transplant 2011; 25: 878–884
11. Genberg H, Kumlien G, Wennberg L *et al.* The efficacy of antigen-specific immunoadsorption and rebound of anti-A/B antibodies in ABO-incompatible kidney transplantation. Nephrol Dial Transplant 2011; 26: 2394–2400
12. Ishida H, Kondo T, Shimizu T *et al.* Postoperative rebound of anti-blood type antibodies and antibody-mediated rejection after ABO-incompatible living related kidney transplantation. Transpl Int 2015; 28: 286–296
13. Montgomery JR, Berger JC, Warren DS *et al.* Outcomes of ABO-incompatible kidney transplantation in the United States. Transplantation 2012; 93: 603–609
14. Opelz G, Morath C, Susal C *et al.* Three-year outcomes following 1,420 ABO-incompatible living-donor kidney transplants performed after ABO antibody reduction: results from 101 centres. Transplantation 2015; 99: 400–404
15. Genberg H, Kumlien G, Wennberg L *et al.* ABO-incompatible kidney transplantation using antigen-specific immunoadsorption and rituximab: a 3-year follow-up. Transplantation 2008; 85: 1745–1754
16. Tanabe K, Ishida H, Inui M *et al.* ABO-incompatible kidney transplantation: long-term outcomes. Clin Transpl 2013; 307–312
17. van Agteren M, Weimar W, de Weerd AE *et al.* The first fifty ABO blood group incompatible kidney transplantations: the Rotterdam experience. J Transplant 2014; 2014: 913902
18. Lentine KL, Axelrod D, Klein C *et al.* Early clinical complications after ABO-incompatible live-donor kidney transplantation: a national study of Medicare-insured recipients. Transplantation 2014; 98: 54–65
19. Hwang JK, Kim SI, Choi BS *et al.* Short-term results of ABO-incompatible living donor kidney transplantation: comparison with ABO-compatible grafts. J Korean Surg Soc 2011; 81: 10–18
20. Habicht A, Broker V, Blume C *et al.* Increase of infectious complications in ABO-incompatible kidney transplant recipients—a single centre experience. Nephrol Dial Transplant 2011; 26: 4124–4131
21. Flint SM, Walker RG, Hogan C *et al.* Successful ABO-incompatible kidney transplantation with antibody removal and standard immunosuppression. Am J Transplant 2011; 11: 1016–1024
22. Schwartz J, Stegall MD, Kremers WK *et al.* Complications, resource utilization, and cost of ABO-incompatible living donor kidney transplantation. Transplantation 2006; 82: 155–163
23. Zarkhin V, Li L, Kambham N *et al.* A randomized, prospective trial of rituximab for acute rejection in pediatric renal transplantation. Am J Transplant 2008; 8: 2607–2617
24. Tyden G, Genberg H, Tollemar J *et al.* A randomized, double-blind, placebo-controlled, study of single-dose rituximab as induction in renal transplantation. Transplantation 2009; 87: 1325–1329
25. de Weerd AE, van Agteren M, Leebeek FW *et al.* ABO-incompatible kidney transplant recipients have a higher bleeding risk after antigen-specific immunoadsorption. Transpl Int 2015; 28: 25–33
26. Renner FC, Czekalinska B, Kemkes-Matthes B *et al.* Postoperative bleeding after AB0-incompatible living donor kidney transplantation. Transplant Proc 2010; 42: 4164–4166
27. Lopau K, Syamken K, Rubenwolf P *et al.* Impact of mycophenolate mofetil on wound complications and lymphoceles after kidney transplantation. Kidney Blood Press Res 2010; 33: 52–59
28. Wahrmann M, Schiemann M, Marinova L *et al.* Anti-A/B antibody depletion by semiselective versus ABO blood group-specific immunoadsorption. Nephrol Dial Transplant 2012; 27: 2122–2129
29. Chung BH, Lim JU, Kim Y *et al.* Impact of the baseline anti-A/B antibody titre on the clinical outcome in ABO-incompatible kidney transplantation. Nephron Clin Pract 2013; 124: 79–88
30. Tanabe K, Ishida H, Shimizu T *et al.* Evaluation of two different preconditioning regimens for ABO-incompatible living kidney donor transplantation. A comparison of splenectomy vs. rituximab-treated non-splenectomy preconditioning regimens. Contrib Nephrol 2009; 162: 61–74
31. Ishida H, Kondo T, Shimizu T *et al.* Postoperative rebound of antiblood type antibodies and antibody-mediated rejection after ABO-incompatible living-related kidney transplantation. Transpl Int 2015; 28: 286–296
32. Won D, Choe W, Kim HJ *et al.* Significance of isoagglutinin titre in ABO-incompatible kidney transplantation. J Clin Apher 2014; 29: 243–250
33. Gloor JM, Lager DJ, Moore SB *et al.* ABO-incompatible kidney transplantation using both A2 and non-A2 living donors. Transplantation 2003; 75: 971–977

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