Impact of donor ABH-secretor status in ABO-mismatched living donor kidney transplantation

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BACKGROUND: The ABO blood group is a major determinant in living donor kidney transplantation since AB antigens are expressed on renal tissue. Little attention has been directed to the ABH-secretor status of the donor kidney. As renal tissue is capable of secreting soluble ABH antigens in secretors, we examined the influence of the ABH-secretor status of kidney donors on outcome in ABO-mismatched living donor kidney transplantation.

STUDY DESIGN AND METHODS: We retrospectively analyzed all patients who underwent ABO-mismatched kidney transplantation at the University Hospital Basel from September 2005 to October 2013. The ABH-secretor status was determined in all donors by molecular genetic analysis.

RESULTS: Of all 55 patients who received transplants, we excluded all patients with donor-specific antibodies (n = 4). Forty-one donors were secretors (78%) and 11 were nonsecretors (22%). Recipients of ABH-secretor donor organs showed a significantly higher glomerular filtration rate throughout the first 6 months posttransplant, whereas no significant influence on posttransplant anti-A/B titers was found. Regression analysis revealed a significant impact on humoral rejection, whereas not on vascular or interstitial rejection in protocol kidney biopsies.

CONCLUSION: The donor ABH-secretor status may have an influence on early posttransplant renal function in patients undergoing ABO-mismatched living donor kidney transplantation. Further prospective studies with long-term follow-up are needed to elucidate involved pathomechanisms.

ABO-mismatched living donor kidney transplantation is increasingly performed for patients with end-stage kidney disease[1] and was demonstrated to improve survival.[2] Initial attempts of transplanting across the ABO blood group barrier were associated with high rates of early graft loss due to antibody-mediated rejection (AMR).[3] In recent years, progress in pretransplant antibody removal and immunosuppression has markedly improved outcome after ABO-mismatched living donor kidney transplantation[4] and therefore has become a standard procedure in many centers.

ABBREVIATIONS: AMR = antibody-mediated rejection; DSA = donor-specific anti-HLA; FUT2 = α1,2-l-fucosyltransferase 2; GFR = glomerular filtration rate; IA = immunoadsorption; IQR = interquartile range.

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However, the mechanisms of transplant accommodation and the early immunologic response in ABO-mismatched kidney transplantation is poorly understood so far. After pretransplant antibody removal by immunoadsorption and immunosuppressive treatment, isoagglutinin titers tend to stay low in the posttransplant period.[5] However, the exact mechanisms for this phenomenon remains unknown and little attention has been directed to the ABH-secretor status of the donor kidney. The term ABH secretor refers to the ability to secrete soluble ABH blood group substances into body fluids,[6] such as plasma or saliva. For example, group A secretors will secrete A substance. ABH secretion is controlled by two inherited alleles (*Se* and *se*), where *Se* is dominant and *se* is recessive. Approximately 80% of individuals are secretors (*SeSe* or *Sese*). The secretor gene (Se) encodes for the enzyme α1,2-L-fucosyltransferase 2 (FUT2) that converts the H-precursor substance in tissues to H-substance, which can be further converted to A- and/or B-substance according to the individual's personal blood group.[7] Interestingly, renal tissue is also able to secrete soluble A and B blood group substance according to their ABH-secretor status.[8-10] In consequence, we hypothesized that the secretion of ABH antigens by donor kidneys is capable of neutralizing circulating anti-A and/or anti-B of the recipient in vivo and therefore contributes to the transplant accommodation after ABO-mismatched kidney transplantation. In this respect, the objective of this study was to investigate the influence of the donor ABH-secretor status on outcome in ABO-mismatched living donor kidney transplantation.

MATERIALS AND METHODS

Study design and population

We retrospectively analyzed all patients who underwent major ABO-mismatched kidney transplantation at the University Hospital Basel from September 2005 to October 2013. Patients with donor-specific anti-HLA (DSA) were excluded. Patient, donor, and transplant characteristics were collected by chart review and through the electronic database of our institution. Donor-recipient pairs were classified according to the ABH-secretor status of the donor. The study was approved by the local ethics committee.

Molecular genetic analysis of secretor status

Pretransplant venous blood samples from the kidney donors were collected in tubes containing EDTA. Peripheral blood mononuclear cells were isolated with the Ficoll-Paque method. Samples were frozen at −80°C for later analysis. Genomic DNA was isolated with the use of a DNA isolation kit (MagnaPure LC, Roche Diagnostics). Wild-type (Se, 428G) and mutant (se, 428A) alleles of *FUT2* gene were detected by polymerase chain reaction (PCR) using sequence-specific priming technology in two independent reactions. Heterozygous individuals would give positive amplification in both reactions, homozygous individuals in one reaction only. Primers for the wild-type allele (428G) were FUT2-all+523R (CCGGCTCCCGTTCA CCTG-3′) and FUT2-Se+428G-F (CCGGCTACCCCTGCTC GTG-3′), and FUT2-all+523R and FUT-se+428A-F (ACC GGCTACCCCTGCTCGTA-3′) for the mutant allele (428A), respectively. Concentrations of the primers in the final reaction volume were 200 nmol/L, those of the control primers (GH1 + 96-F, TGCCTTCCCAACCATTCCCTTA-3′; and GH1 + 274-R, CCACTCACGGATTTCTGTTGTGTTTC-3′, resulting in a 434-bp PCR product) 90 nmol/L. All primers were provided by an oligonucleotide synthesizing service (Microsynth). Beside primers, the final PCR–sequence-specific priming reaction composition and cycling parameters were as described previously for KEL*01/KEL*02 genotyping.[11]

Patient preparation and desensitization protocol

All patients were prepared and desensitized as previously described.[12] In short, basic immunosuppressive therapy including tacrolimus (0.1 mg/kg body weight twice daily), mycophenolate mofetil (1000 mg twice daily, 500 mg twice daily if body weight was < 50 kg), and prednisone (30 mg once daily) was started 2 weeks before transplantation. A single dose of rituximab (375 mg/m^2) was given 4 weeks before transplantation in an outpatient setting. Selective blood group antibody removal by immunoadsorption (IA) was performed pretransplant with a low-molecular carbohydrate column containing A and/or B blood group antigens linked to a Sepharose matrix (Glycosorb, Glycorex Transplantation). IA was performed daily until the isoagglutinin titers were 8 or less. The transplantation was then carried out the following day. If one of the two titers remained higher than 8, additional IA was mandatory until the target titer was achieved. With each session, two plasma volumes, calculated with the formula of Kaplan[13] were processed. Additional 20 mg of intravenous (IV) basiliximab was administered on Days 0 and 4. Target tacrolimus trough levels were 10 to 12 ng/mL from Day −14 until Day 31, 8 to 10 ng/mL from Day 32 to Day 90, and 6 to 8 ng/mL from Day 91 to Day 365 and 4 to 6 ng/mL thereafter. Target mycophenolate mofetil trough level was more than 2 mg/mL. Steroids (IV methylprednisolone and prednisone orally) were tapered (500 mg IV on Day 0, 250 mg IV on Day 1, 100 mg IV on Day 2, 50 mg from Day 3 to Day 6, and 0.5 mg/kg body weight from Day 7 with a reduction by 5 mg every 2 weeks until 15 mg/day and then by 2.5 mg every 2 weeks until a maintenance dose of 0.1 mg/kg body weight). All complications of pretransplant preparation were prospectively recorded at each clinical visit.

Isoagglutinin titers

Serologic blood group typing and antibody screening was performed by gel test (Gel Test ID-system, Bio-Rad Laboratories DiaMed GmbH). The isoagglutinin titers were measured with the conventional tube method. Recipient serum was serially diluted and incubated with a 5% suspension of red blood cell (RBC) aliquots of the appropriate blood type in a test tube for approximately 15 minutes at room temperature. After centrifugation, macroscopic agglutinations of RBCs were evaluated for anti-A or anti-B immunoglobulin (Ig)M antibodies. For IgG detection, IgM in patient serum was inactivated (Neutr-AB II, Medion Grifols Diagnostics Ag) before testing for agglutination. Titers were determined as the highest dilution that still produced macroscopic agglutination. After transplantation, isoagglutinin titers (IgM and IgG anti-A/B) against the donor blood group were measured daily in the first week; weekly until Day 31; and then 3, 6, and 12 months thereafter.

Renal function

After transplantation, renal function was evaluated by creatinine levels and glomerular filtration rate (GFR; according to the CKD-EPI formula[14]), which was measured respectively calculated on Days 7 and 14 and 1, 2, 3, 4, 5, 6, 9, and 12 months after transplantation.

Protocol biopsies

Protocol biopsies were taken after 1 week and after 3, 6, and 12 months. In general, two needle biopsy cores were obtained for morphologic workup and processed as previously described.[15,16] Core biopsy needles of 16-gauge were used, thus reducing the risk of sampling error. Immunofluorescence for detecting deposition of immunoglobulins and complement factors as well as expression of ABO blood group antigens was performed for each biopsy. Cryostat slides of 6 μm thickness were dried overnight before incubation for 60 minutes with the primary antibody (A antigen—Z-311-01-Y [Zytomed], dilution 1:30; B antigen—Z-312-01-Y [Zytomed], dilution 1:30). Afterward, the slides were incubated with the secondary antibody for 60 minutes (Antigen A—Molecular Probes A21151 [Thermo Fisher Scientific], dilution 1:100; Molecular Probes A21042 [Thermo Fisher Scientific], dilution 1:100). All slides were evaluated using a fluorescence microscope (Axioplan 2, Zeiss).

Statistical analysis

Categorical variables are expressed as numbers and percentages, continuous variables as means and standard deviation (SD), or medians and interquartile range (IQR). Categorical variables were compared with the use of the Pearson chi-square test and continuous variables with the use of the t test, Mann-Whitney U test, or Kruskal Wallis test. A multivariate regression analysis was generated to evaluate the impact of recipient sex, blood group, initial titer, plasma transfusion, and secretor status on the risk of rejection. All hypothesis testing was two-tailed, and p values of less than 0.05 were considered to indicate significance. All calculations were made using computer software (SPSS, SPSS, Inc.). All graphs were designed with computer software (GraphPad Prism 5 for Mac OS X, Version 5, GraphPad, Inc.).

RESULTS

Patient and donor characteristics

Between September 2005 and October 2013, a total of 55 patients underwent major ABO-mismatched kidney transplantation at our institution. Of these, four patients with DSA were excluded from the analysis. Thus, 51 patients were included in our study. Donors were predominantly females (n = 34, 67%), whereas recipients were mainly males (n = 41, 80%); there was a sex mismatch in 35 of 51 transplantations (69%). The mean age of recipients was 51 years and of the donor 52 years at transplantation; both values were not significantly different between secretors and nonsecretors. Regarding ABO blood group, donors were mainly blood group A (n = 38, 75%), and recipients blood group O (n = 34, 67%). The antibody screen test was negative in all donors and positive in one recipient, who had an anti-Lea alloantibody (missing data in 10 donors and three recipients). Ten patients (20%) received posttransplant IA and a total of 16 patients (31%) received peritransplant plasma infusion, whereas 35 patients (69%) of the cohort did not receive plasma infusion. All baseline characteristics of the study population are presented in Table 1; there was no significant difference between secretors and nonsecretors.

Secretor status

Forty-one donors (78%) were secretors, and 11 donors (22%) were nonsecretors. Of the secretors, 13 donors (33%) were homozygous (*Se*/*Se*) and 27 (68%) heterozygous (*Se*/*se*) in the secretor gene locus.

Renal function

The mean pretransplant renal function on Day −7, expressed by GFR, was 7 ± 2 mL/min in the entire cohort and there was no significant difference between recipients of kidneys from secretors and nonsecretors ($p = 0.601$). Posttransplant GFR, as displayed in Fig. 1, was significantly higher in cases transplanted with a secretor kidney ($p < 0.05$) in the first 6 months (Day 14 and Months 1, 2, 3, 4, 5, and 6), except on Day 7 ($p = 0.050$). At 9 months mean ± SD GFRs were 55 ± 15 mL/min in recipients of secretor kidneys and 45 ± 10 mL/min in recipients of nonsecretor kidneys ($p = 0.076$), and at 12 months

TABLE 1. Baseline characteristics*

Characteristics	All (n = 51)	Secretors (n = 40)	Nonsecretors (n = 11)	p values
Donor				
Age (years)	51 (±9.9)	50 (±9.9)	53 (±10.0)	0.507
Male sex	17 (33)	14 (35)	3 (27)	0.150
Blood group				0.212
A	38 (75)	30 (75)	8 (73)	
B	7 (14)	6 (15)	1 (9)	
AB	6 (12)	4 (10)	2 (18)	
O	0 (0)	0 (0)	0 (0)	
Recipient				
Age (years)	51 (±12.3)	50 (±11.9)	53 (±13.6)	0.558
Male sex	41 (80)	30 (75)	11 (100)	0.064
Blood group				0.134
A	9 (18)	8 (20)	1 (9)	
B	8 (16)	8 (20)	0 (0)	
AB	0 (0)	0 (0)	0 (0)	
O	34 (67)	24 (60)	10 (91)	
Pretransplant IgG titer	1 (0-1:4)	0 (0-1:4)	1:2 (0-1:8)	0.127
Pretransplant IgM titer	4 (2-5)	4 (1-4)	6 (2-8)	0.190
Posttransplant IA	10 (20)	9 (23)	1 (9)	0.302
Plasma infusion	16 (31)	13 (33)	3 (27)	0.481
ABO blood group mismatch (donor/recipient)				0.509
A/O	33 (65)	24 (60)	9 (82)	
A/B	6 (12)	6 (15)	0 (0)	
B/A	7 (14)	6 (15)	1 (9)	
AB/O	2 (4)	1 (3)	1 (9)	
AB/A	1 (2)	1 (3)	0 (0)	
AB/B	2 (4)	2 (5)	0 (0)	

* Data are presented as mean ± SD, number of patients (%), or median (IQR).

mean ± SD GFRs were 58 ± 16 and 48 ± 13 mL/min, respectively ($p = 0.097$). Overall, the mean GFR ranged between 52 and 60 ± 21 mL/min and remained substantially stable over time. In the subgroup of recipients kidneys from homozygous secretors' GFR was also significantly better than in those transplanted with a kidney from heterozygous secretors in the first 6 months (mean ± SD GFR 62-75 ± 15-21 mL/min vs. mean ± SD GFR 49-57 ± 11-20 mL/min, all $p < 0.05$). At Months 9 and 12 there was no significant difference between the homozygous secretors and heterozygous secretors ($p = 0.104$ and $p = 0.142$).

Isoagglutinin titers

The pretransplant isoagglutinin IgM and IgG titers (Day −1) were not significantly different between recipients of kidneys from secretors and nonsecretors (median [IQR] IgM 4 [1-4] vs. median [IQR] IgM 6 [2-8], $p = 0.190$; median [IQR] IgG 0:0 [0-4] vs. median [IQR] IgG 2 [0-8], $p = 0.127$). Posttransplant isoagglutinin titers of IgG and IgM were even lower than pretransplant titers, as shown in Fig. 1. Overall, titers remained stable during the observation period and no significant difference at all time points posttransplant between recipients of kidneys from secretors and nonsecretors was observed ($p > 0.05$).

Protocol biopsies

In a model accounting for the impact of the secretor status, recipient sex, recipient blood group, initial isoagglutinin titers, and number of plasma transfusions on the risk of rejection in the protocol biopsies (1 week and after 3, 6, and 12 months) there was a significant difference regarding humoral rejection, but not regarding vascular, interstitial, and rejection in total (Table 2). Besides, the expression of A and B blood group antigens in the transplanted kidney tissue was not significantly different between the secretors and nonsecretors ($p > 0.05$).

DISCUSSION

Due to organ shortage ABO-mismatched living donor kidney transplantation is increasingly performed for patients with end-stage kidney disease.[1] In this respect, little attention has been directed to the ABH-secretor status of the donor kidney. To the best of our knowledge, this is the first study investigating the influence of the donor ABH-secretor status on outcome of ABO-mismatched living donor kidney transplantation. We can report three major findings from our cohort:

First, early posttransplant renal function was significantly better in patients who received a kidney from a secretor-positive donor. This especially held true for the subgroup of patients receiving a kidney from homozygous secretors. Additionally, in posttransplant protocol biopsies a significant impact was shown on humoral rejection. However, posttransplant hemagglutinin titers were not significantly influenced by the donor ABH-secretor status.

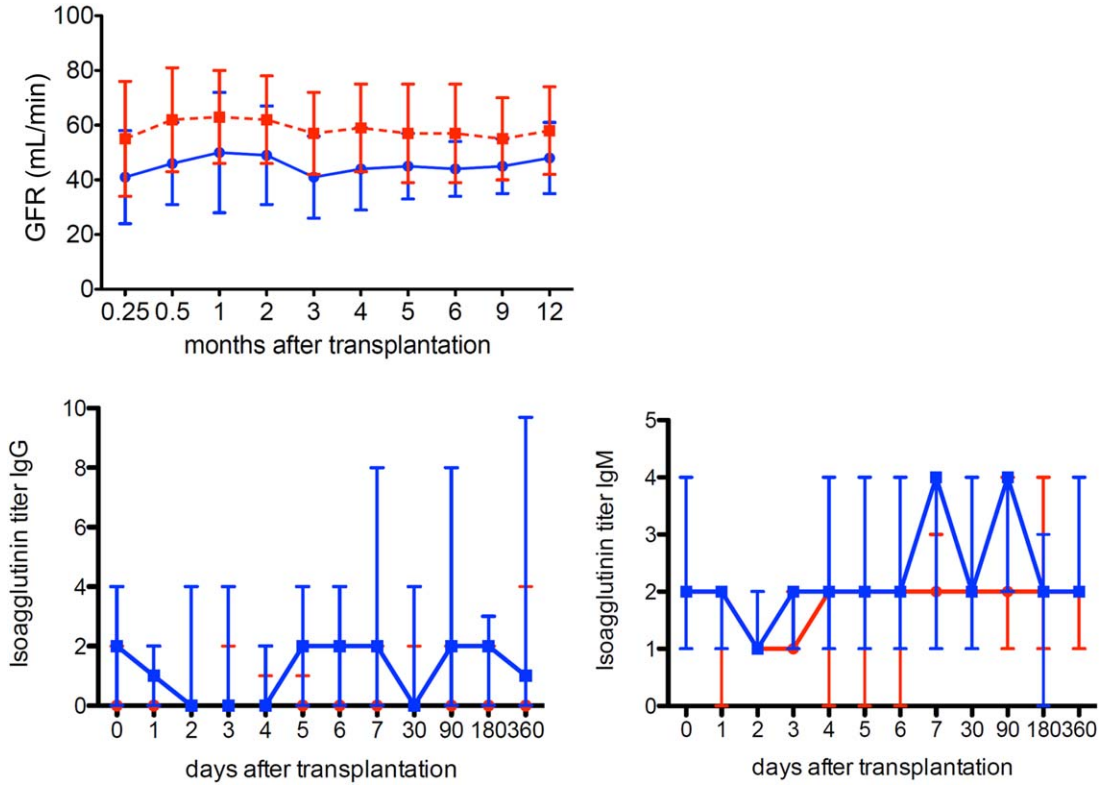

Fig. 1. Posttransplant GFR, isoagglutinin titers IgG, and isoagglutinin titers IgM in the first 12 months after ABO-mismatched kidney transplantation in secretors (■) and nonsecretors (●).

TABLE 2. Protocol biopsies*

Rejection	Secretors (n = 50)	Nonsecretors (n = 11)	p values†
Humoral	9 (23)	4 (36)	0.018
Vascular	6 (15)	1 (9)	0.73
Interstitial	17 (43)	6 (55)	0.32
All	32 (80)	11 (100)	0.25

* Data are presented as number of patients (%).
† Model accounting for secretor status, recipient sex, blood group, initial titer, and plasma transfusion on the risk of rejection.

In general, renal tissue of secretors is able to secrete soluble A/B blood group antigens.[9,10] Therefore, one might hypothesize that in ABO-mismatched kidney transplantation soluble A and B blood group antigens secreted by the graft might bind circulating anti-A and/or -B of the recipient. This may reduce posttransplant hemagglutinin titers, prevent AMR, and lastly optimize renal function (in vivo adsorption). Pathophysiologically, AMR is thought to involve donor-specific antigens that can react with DSA, leading to in situ antigen-antibody interaction, complement activation, and donor tissue injury.[17] In particular, HLA Class I and II antibodies are known to be most pathogenic[18] and consequently we excluded these patients from our analysis to avoid interference. However, also ABO antibodies are known to cause AMR and high posttransplant ABO antibody titers are associated with increased graft loss.[19] ABO blood group antigens are more properly defined as histo-blood groups, because they are expressed throughout the body,[20] including embryonic kidney cells.[21] In the adult kidney, A/B antigens are detected on the vascular endothelium, convoluted distal tubules, and collecting tubules.[22] In consequence, naturally occurring antibodies against ABO antigens (hemagglutinins), which are a mixture of IgM- and IgG-type antibodies, are a key mediator of AMR after ABO-mismatched living donor kidney transplantation.[17] To reduce AMR and permit tolerance of ABO-mismatched kidney transplants, ABO-mismatched living donor kidney transplantation is performed after an intensive preparative regimen including the removal of hemagglutinins by therapeutic plasma exchange or IA and immunosuppressive therapy to reduce circulating ABO antibody titers to less than 8 to 16, depending on the protocol of the individual transplant center.[23] Surprisingly, the majority of patients maintain low-level hemagglutinin titers in the posttransplant period.[5] Only rarely posttransplant hemagglutinin titers increase after transplantation, which in turn correlates with increased graft loss.[5,19] Overall, the exact mechanisms responsible for this immunologic accommodation across the ABO blood group barrier remain unknown,[24] but possibly immunomodulation due to the pretransplant hemagglutinin removal and

immunosuppressive regimen plays a role. In this study, posttransplant hemagglutinin titers also stayed low level, but they were not significantly influenced by the donor secretor status. In this respect, difficulties in the determination of anti-A and anti-B titers and the lack of standardization could have influenced our results. The quantification of anti-A and/or anti-B is carried out most frequently by hemagglutination and hemolysis techniques, based on the crosslinking of test RBC with hemagglutinins in the serum of interest. Results are prone to a broad variability. First of all because of technical issues, there is a considerable interexaminer variability related to the determination by visual observation of agglutinated RBCs in tubes.[25] However, our results were measured in only one accredited certified laboratory according to standard operating procedure manuals. Finally, as hemagglutinins are predominantly of the IgM class, the determination of IgG requires additional steps and is more challenging.[26]

Besides hemagglutinin titers we sought specific histologic changes related to the secretor status of the graft to support our hypothesis. In fact, previous studies showed that subclinical rejection detected by protocol biopsies can be observed early after transplantation without overt clinical renal dysfunction and predicts graft function.[27] In our study a significant impact was shown on humoral rejection, but not on vascular and interstitial rejection in the posttransplant protocol biopsies. This might strengthen our hypothesis that in ABO-mismatched kidney transplantation soluble A and B blood group antigens secreted by the graft bind circulating anti-A and/or anti-B of the recipient and therefore may optimize renal function by preventing AMR. However, renal function can be affected by multiple factors, such as blood pressure of the recipient, age of the donor and medication, which might have influenced our results considering the small cohort size and retrospective design of the study. To date, only in 1978 the ABH-secretor status of the recipient instead of the donor was evaluated in patients with cadaver ABO-matched kidney transplantation.[28] Recently, also the role of the ABH-secretor status in the setting of hematopoietic stem cell transplantation has been analyzed by our group.[29] Both published studies showed no effect on clinical outcome.

Several other limitations of the study merit consideration. First, as mentioned, the relatively small number of patients and the retrospective design of the study might have predisposed to selection bias and residual confounding. Moreover, due to the low frequency of some blood group combinations, such as donor with AB and recipient with O blood group, which are not represented in this cohort, we can comment less on their outcome. Technical issues in the measurement of hemagglutinins—as mentioned—is also critical and might have caused inaccurate results. Finally, we cannot comment on patients with DSA and posttransplant IA since such patients were excluded from our study.

In conclusion, our data indicate that the donor ABH-secretor status may have an influence on early posttransplant renal function in patients undergoing ABO-mismatched living donor kidney transplantation in the first year. However, this could not be supported by hemagglutinin titers. In consequence, these results need to be confirmed in larger prospective studies with long-term follow-up and more sensitive techniques for the measurement of hemagglutinins.

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CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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