TRANSPLANT INTERNATIONAL

Transplant International ISSN 0934-0874

ORIGINAL ARTICLE

Difference in outcomes after antibody-mediated rejection between abo-incompatible and positive cross-match transplantations

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Keywords
ABO-incompatible, antibody-mediated rejection, donor-specific antibodies, kidney transplantation, positive cross-match.

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Conflicts of interest
The other authors have nothing to disclose.

Received: 1 February 2015
Revision requested: 7 April 2015
Accepted: 8 June 2015
Published online: 6 Jul 2015

doi:10.1111/tri.12621

Summary

Graft survival seems to be worse in positive cross-match (HLAi) than in ABO-incompatible (ABOi) transplantation. However, it is not entirely clear why these differences exist. Sixty-nine ABOi, 27 HLAi and 10 combined ABOi+HLAi patients were included in this retrospective study, to determine whether the frequency, severity and the outcome of active antibody-mediated rejection (AMR) were different. Five-year death-censored graft survival was better in ABOi than in HLAi and ABOi+HLAi patients (99%, 69% and 64%, respectively, $P = 0.0002$). Features of AMR were found in 38%, 95% and 100% of ABOi, HLAi and ABOi+HLAi patients that had a biopsy, respectively ($P = 0.0001$ and $P = 0.001$). After active AMR, a declining eGFR and graft loss were observed more frequently in HLAi and HLAi+ABOi than in ABOi patients. The poorer prognosis after AMR in HLAi and ABOi+HLAi transplantations was not explained by a higher severity of histological lesions or by a less aggressive treatment. In conclusion, ABOi transplantation offers better results than HLAi transplantation, partly because AMR occurs less frequently but also because outcome after AMR is distinctly better. HLAi and combined ABOi+HLAi transplantations appear to have the same outcome, suggesting there is no synergistic effect between anti-A/B and anti-HLA antibodies.

Introduction

Antibody-incompatible kidney transplantation has become more common over the last decade. ABO-incompatible (ABOi) transplantation is an established procedure, and is useful for blood type O recipients who have a low chance of finding a compatible donor through paired donation schemes [1]. For highly sensitized patients, positive cross-match (HLAi) transplantation after 'appropriate desensitization' may lead to better patient survival than staying on dialysis [2].

Although both ABOi and HLAi transplantations involve incompatible antibodies, *in vitro* studies have shown that anti-A/B antibodies are more likely to orientate the endothelial cell towards an accommodation phenotype, while high levels of donor-specific HLA antibodies (DSA) are more likely to induce rejection [3–5]. *In vivo*, graft survival seems to be worse in HLAi than in ABOi transplantation [6–9]. However, it is not entirely clear why these differences exist.

It could be explained by the higher incidence of early acute antibody-mediated rejection (AMR) observed in HLAi transplantation (37–50% after HLAi transplantation [10–14], vs. 3.3–33% after ABOi transplantation [6,15–17]). Moreover, HLAi transplantations lead to higher rates of microvascular injury (MVI) (glomerulitis (g) and/or

peritubular capillaritis (ptc)) and transplant glomerulopathy on both 1- and 5-year available protocol biopsies [9]. These data suggest then that the outcome after early AMR could be very different between HLAi and ABOi transplantation.

Following the recent reclassification of AMR [18], we conducted this study to determine whether the frequency, severity and the outcome of AMR were different and could explain the differences in graft survival observed between patients undergoing ABOi, HLAi and combined ABOi+HLAi transplantations.

Patients and methods

Patients

Between January 2005 and November 2012, 69 living-donor ABOi, 27 HLAi and 10 simultaneous ABOi+HLAi kidney transplants were performed at Guy's Hospital. HLAi transplantation was defined as those with both DSA and a positive baseline flow cytometric cross-match. The group of ABOi patients had no DSA detected by Luminex with flow bead assays at the time of transplantation. All the patients included in this retrospective single-centre study were followed until April 2013. Clinical information and laboratory information were extracted from electronic databases and patient medical records.

Desensitization protocol

We have previously published the details of our pretransplant desensitization protocol for ABOi patients [19]. In brief, pretransplant antibody removal using Glycosorb-ABO immunoadsorption (IA) columns was used for patients with baseline titres greater than 64, because these columns have a high capacity for ABO antibody removal and a minimal effect on coagulation [20]. A less specific but more cost-effective alternative, the double-filtration plasmapheresis (DFPP) was used for patients with baseline titres between 16 and 64, as the need for a small number of treatments meant a limited effect on coagulation. Routine pretransplant antibody removal was omitted for those with baseline titres at 8 or lower [19].

In HLAi patients, preoperative antibody removal using DFFP or Therasorb immunoadsorption was systematically performed. Therasorb has minimal effect on coagulation but is more expensive than DFPP. Therasorb was not available during the initial part of our programme, but was used for selected patients with high levels of antibody.

Antibody removal was carried out using the HF440 (LINC Medical) machine. Approximately 1–1.5 plasma volumes (around 4–8 l) were processed at each session. Heparin was used for anticoagulation, and replacement was made with human albumin solution after DFFP. For ABOi patients, our target was to reach an anti-A or anti-B antibody titre of 8 or lower. For HLAi patients, our target was to reach a flow cytometry cross-match relative mean fluorescence ratio below 2.3 on T or B cells.

Immunosuppressive regimen

All received a triple maintenance immunosuppression comprising tacrolimus (target trough concentration 10–12 ng/ml), mycophenolate mofetil (1000 mg twice daily) and oral prednisolone (5 mg/day). Tacrolimus and mycophenolate mofetil were started 1 week prior to transplant. In ABOi patients, basiliximab induction was used, and rituximab (375 mg/m^2, 4 weeks prior to transplant) was also given if baseline titres were $\geq$ 1:8 [19]. Alemtuzumab was used for two sensitized patients (presence of third-party HLA antibody, not DSA). In HLAi patients, our pretransplant induction protocol has evolved with time: induction therapy was either basiliximab alone, basiliximab+rituximab, alemtuzumab or antithymocyte globulin. Intravenous immunoglobulin (0.5 g/kg) was given the day before surgery in some patients.

ABO antibody titres

Anti-A and anti-B antibody titres (total immunoglobulin load) were measured by the indirect antiglobulin test using gel cards (DiaMed ID-Card Coombs anti-IgG, catalogue number 004025; Bio-Rad Laboratories, Cressier, Switzerland) in a single laboratory.

DSA identification

In all the patients, antibody detection was performed using a panel flow bead assay on a Luminex platform. All positive assays were next subjected to single antigen flow bead assays, in order to identify DSA. The positivity threshold for the bead MFI was set at 500 after removal of the background. Calculated reaction frequency (cRF) is the percentage of the last 10 000 deceased donors to whom the patient has performed DSA in United Kingdom.

Flow cytometry cross-match

Both baseline and pretransplant flow cytometry cross-match assays were performed prospectively. They were performed on T and B lymphocytes, looking for IgG antibodies. The threshold for performing pretransplant antibody removal was at a relative mean fluorescence ratio between the patient's serum and the negative control serum (pool from AB group normal donors) of 2.3 for T cells and/or B cells. The basic CDC cross-match assay is not routinely performed in our centre.

Histopathological evaluation

'For-cause' biopsies were performed when there was a clinical indication. Protocol biopsies were performed at month 3 when patients agreed, and there was no recent 'for-cause' biopsy. Each biopsy sample was fixed, embedded in paraffin, and stained with haematoxylin and eosin, periodic acid-Schiff, silver methenamine, Masson's trichrome stains. C4d staining was performed on paraffin sections by immunohistochemical (IHC) analysis using a rabbit anti-human C4d polyclonal IgG (AbD Serotec, Kidlington, UK).

All biopsies performed in the first 100 days after transplantation were reviewed and scored according to the updated semi-quantitative Banff classification by R.P who was blinded to the clinical data [21,22].

Based on the Banff 2013 report [18], active AMR was defined when all the three following features were present: (i) histologic evidence of acute tissue injury, including one or more of the following: MVI ($g > 0$ in the absence of recurrent glomerulonephritis and/or ptc>0, and/or thrombotic microangiopathy in the absence of any other cause), arteritis ($v > 0$); (ii) evidence of current/recent antibody interaction with vascular endothelium, including linear C4d staining in peritubular capillaries (C4d > 0 by IHC on paraffin sections) or moderate MVI ([g+ptc] ≥2); (iii) serologic evidence of anti-blood group antibodies or DSA. We differentiated C4d-positive AMR (C4d>0 by IHC) from C4d-negative AMR ([g+ptc] ≥2).

Lesions meeting the criteria for 'active AMR' which resulted in additional therapy were called 'treated AMR' in this report, whereas those not resulting in additional treatment were called 'nontreated' AMR.

Outcome

We calculated the slope of the eGFR (MDRD formula), which was considered as an indicator of ongoing status of transplant renal function. For each patient, the slope of the eGFR versus time curve was calculated by least-square fitting of linear regression using all eGFR values at months 3, 6, 12, 24, 36, 48 and 60 ($y = ax + b$, a = slope, ml/min/1.73 m^2/month). Patients with a slope > 0.1 were considered as having an improving eGFR, patients with a slope between −0.1 and 0.1 a stable eGFR, and patients with a slope<−0.1 a declining eGFR. For the analyses correlating histology with outcome, we stratified the patients into two groups: a group of patients with a declining eGFR and a group of patients with an improving or stable eGFR. We did not obtain any slope in five patients who experienced graft loss and in two who died before month 6. The five patients with an early graft loss (four acute ABMR and 1 renal venous thrombosis) were added to the declining group. Patient and graft survival were also analysed.

Statistical analysis

Comparisons of characteristics were performed using conventional statistics. Mc Nemar chi-squared test or Fisher test for qualitative variables, and Wilcoxon rank test or Student's *t*-test for quantitative variables were used when appropriate. Allograft loss, death and infection were analysed with the use of the Kaplan–Meier method, and group differences were assessed by the log-rank test. Analyses were performed with JMP 10.0 (2012; SAS Institute Inc, Cary, NC, USA).

Results

Baseline characteristics

Sixty-nine living-donor ABOi, 27 HLAi and 10 simultaneous ABOi+HLAi kidney transplants patients were followed up for a mean of 1008 ± 667, 867 ± 779 and 998 ± 772 days, respectively. Baseline characteristics are depicted in Table 1. More females were present in the HLAi group. The number of previous transplants, time on dialysis and calculated reaction frequency (cRF) were higher in HLAi and ABOi+HLAi than in ABOi patients. Induction therapy with antithymocyte globulin or alentuzumab was more frequently used in HLAi and ABOi+HLAi patients. Rituximab and intravenous polyclonal immunoglobulins were mainly used as induction treatment in ABOi and HLAi patients, respectively. Preoperative antibody removal was required in only 77% (53/69) of ABOi patients to achieve pretransplant ABO antibody titre ≤ 1/8.

ABO titres were not significantly different between the ABOi and the ABOi+HLAi groups, both at baseline (before antibody removal) and just before transplantation (pretransplant). Baseline and pretransplant class I and II DSA MFI and cross-match relative mean fluorescence ratio were also similar between the HLAi and the ABOi+HLAi groups (Table 2).

Patient and graft survival

Five-year patient survival was similar between ABOi, HLAi and ABOi+HLAi patients (93%, 88% and 100%, respectively, $P = 0.7$). eGFR at 3 months, 1 and 3 years post-transplant were similar in the ABOi, HLAi and ABOi+HLAi groups (data not shown). One-year death-censored graft survival was 99% in ABOi, 80% in HLAi and 80% in ABOi+HLAi patients. Five-year death-censored graft survival was still better in ABOi than in HLAi and ABOi+HLAi patients (99%, 69% and 64%, respectively, ABOi versus HLAi: $P = 0.0002$, ABOi versus ABOi+HLAi: $P = 0.0002$) (Fig. 1a). It was similar between the 16 ABOi patients transplanted without antibody removal and the 53 ABOi

Table 1. Patient characteristics.

(Mean, SD)	ABOi $n = 69$	P ↔	HLAi $n = 27$	P ↔	ABOi+HLAi $n = 10$	P versus ABOi
Age (year)	46.4 ± 16.4	0.2	44.3 ± 10.1	0.3	40.4 ± 12.8	0.1
Sex (F/M)	29/40	0.02	19/8	0.3	5/5	0.6
Kidney disease						
Glomerular	19	0.4	12	0.4	2	0.9
Diabetes	10		1		0	
Tubulo-interstitial	6		2		1	
Vascular	8		3		1	
Obstructive	10		5		2	
APCKD	6		1		1	
Congenital	6		0		2	
Unknown	4		3		1	
Number of previous transplant						
0	59 (86%)	0.0001	12 (44%)	0.7	4 (40%)	0.001
≥1	10 (14%)		15 (56%)		6 (60%)	
Time on dialysis (months)	20.7 ± 23.6	0.0001	87.7 ± 68.5	0.4	69.6 ± 58.1	0.001
cRF	6.7 ± 18.6	0.0001	89.2 ± 14.2	0.5	88.5 ± 13.1	0.0001
Paired scheme	9 (14%)	0.7	4 (17%)	0.2	1 (10%)	0.8
Donor age (year)	45.9 ± 10.3	0.03	39.6 ± 14.8	0.7	41.2 ± 10.9	0.2
Donor sex (F/M)	40/29	0.07	10/17	0.5	5/5	0.6
Induction treatment						
Alentuzumab/ATG	2/0 (3%)	0.0001	7/4 (42%)	0.4	4/0 (40%)	0.0001
Basiliximab	67 (97%)		15 (58%)		6 (60%)	
Rituximab	58 (84%)	0.0001	4 (15%)	0.1	4 (40%)	0.002
IVIG	10 (14%)	0.0001	22 (84%)	0.001	3 (30%)	0.4
Pre-op antibody removal	53 (77%)	0.006	27 (100%)		10 (100%)	0.1
DFPP/IA/both	30/20/3		26/1/0		3/3/4	
Number of sessions	4.2 ± 2.4	0.4	3.8 ± 1.8	0.3	8.6 ± 14.3	0.1

APCKD, autosomic polycystic kidney disease; ATG, antithymocyte globulins; cRF, calculated reaction frequency; DFPP, double-filtration plasmapheresis; IA, immunoadsorbtion; IVIG, intravenous immunoglobulin.

patients with antibody removal (100% and 98%, $P = 0.6$). Graft losses were mainly due to AMR in all groups.

Treated antibody-mediated rejection

The 1-year incidence of treated AMR was 19% (13/69), 41% (11/27) and 60% (6/10) in ABOi, HLAi and ABOi+HLAi groups, respectively (ABOi versus HLAi: $P = 0.03$, ABOi versus ABOi+HLAi: $P = 0.004$, HLAi versus ABOi+HLAi: $P = 0.3$) (Table 3). It was similar between the 16 ABOi patients transplanted without antibody removal and the 53 ABOi patients with antibody removal (19% and 19%, $P = 0.9$). Retrospective analysis of the biopsies associated with these clinical episodes revealed that all fulfilled the new Banff 2013 criteria for AMR: all were diagnosed in the first 100 days post-transplantation. In ABOi and ABOi+HLAi patients, 85% and 100% of these treated AMR were C4d+ versus only 55% in HLAi patients. Treatment for AMR was different in these three groups: ABOi patients with AMR received steroids more frequently than HLAi and ABOi+HLAi patients (69%, 9% and 33%, respectively). On the other hand, they were less frequently treated with double-filtration plasmapheresis than HLAi and ABOi+HLAi patients (15%, 73% and 67%, respectively).

One-year borderline and T-cell mediated rejection were similar between ABOi, HLAi and ABOi+HLAi patients.

New cases of active antibody-mediated rejection revealed on reanalysis of biopsies according to Banff 13 criteria

We next reviewed all the biopsies performed in these 106 patients during the first 100 days post-transplantation, and we found that 72% (50/69) of ABOi, 78% (21/27) of HLAi and 80% (8/10) of ABOi+HLAi patients had at least one biopsy ($P = 0.8$). Mean time between transplantation and the biopsy was 59 ± 38 days. Among them, 67% (53) were 'for-cause' and 33% (26) protocol biopsies (no difference was observed between the three groups, data not shown).

The re-scoring of the biopsies using the Banff 2013 classification found 17 more patients with criteria for active AMR (13 'for-cause' and four protocol biopsies), all of whom had not been diagnosed or treated for AMR

Table 2. Antibodies characteristics.

	ABOi $n = 69$	*P* ↔	HLAi $n = 27$	*P* ↔	ABOi+HLAi $n = 10$	*P* versus ABOi
Blood group						
Donor blood group incompatibility (A versus B)	45/24				5/5	0.4
A1 versus A2	38/6				5/0	0.4
Recipient blood group A/B versus O	27/42				2/8	0.2
Anti-ABO titres						
Baseline (median, min–max)	64 (0–1024)				64 (2–512)	0.6
Pretransplant (median, min–max)	8 (0–16)				8 (0–32)	0.8
Donor-specific HLA antibodies (DSA) by Luminex (mean, SD)						
A-B-Cw-DR-DQ mismatches	5.5 ± 2.4	0.6	5.3 ± 2.2	0.9	5.3 ± 2.1	0.7
Class I DSA						
Number of patient with class I DSA (%)			23 (85%)	0.7	9 (90%)	
Number of class I DSA/patient			1.74 ± 1.19	0.6	1.50 ± 0.84	
Baseline class I MFI			11380 ± 9317	0.9	9652 ± 5421	
Pretransplant class I MFI			5201 ± 5226	0.9	5258 ± 5633	
Class II DSA						
Number of patient with Class II DSA (%)			17 (63%)	0.3	4 (40%)	
Number of class II DSA/patient			1.00 ± 0.96	0.4	0.80 ± 1.31	
Baseline class II MFI			7185 ± 8791	0.4	9880 ± 11255	
Pretransplant class II MFI			4631 ± 5983	0.6	2586 ± 1975	
Flow cytometry cross-match RMF ratio (mean, SD)						
Baseline T cell	1.03 ± 0.14	0.0001	3.14 ± 2.89	1.0	2.89 ± 2.4	0.0001
Baseline B cell	1.40 ± 0.91	0.0001	5.72 ± 3.88	0.3	5.60 ± 5.67	0.0001
Pretransplant T cell	1.01 ± 0.15	0.0001	1.51 ± 0.65	0.5	1.53 ± 0.38	0.0001
Pretransplant B cell	1.21 ± 0.60	0.0001	2.97 ± 1.39	0.06	2.15 ± 1.06	0.002

DSA, donor-specific HLA antibody; RMF, relative mean fluorescence.

originally. Therefore, within this cohort, there were a total of 47 active AMR (i.e. AMR on biopsy irrespective of clinical features and treatment), which were found in 38% (19/50), 95% (20/21) and 100% (8/8) of ABOi, HLAi and ABOi+HLAi biopsies, respectively (ABOi versus ABOi+HLAi: $P = 0.0001$, ABOi versus ABOi+HLAi: $P = 0.001$) (Table 3). In ABOi and ABOi+HLAi patients, 89% and 100% of these active AMR were C4d+ versus only 55% in HLAi patients.

We next compared Banff scores between all ABOi, HLAi and ABOi+HLAi biopsies exhibiting active AMR. Vascular thrombi, glomerulitis (g), peritubular capillaritis (ptc), arteritis (v) and interstitial inflammation (i) scores were similar between the three groups (Table 3). Tubulitis (t) was more severe in ABOi than in HLAi and ABOi+HLAi biopsies. C4d score tended to be lower in HLAi biopsies. Chronic scores were similar between the three groups.

Outcome of all patients with active AMR

We next analysed both eGFR evolution and graft survival in patients with active AMR to determine whether they had the same outcome in ABOi, HLAi and ABOi+HLAi transplantation.

Patients were classified into two groups: an improving/stable group and a declining group (Fig. 1b). After active AMR, a declining eGFR was observed in only 32% of ABOi patients. On the other hand, after active AMR, a declining eGFR was observed in 60% and 88% of patients in the HLAi and HLAi+ABOi groups, respectively (ABOi versus HLAi: $P = 0.07$, and ABOi versus ABOi+HLAi: $P = 0.01$) (Fig. 1c).

Moreover, after active AMR, 5-year death-censored graft survival was 95% in ABOi patients, versus 63% in HLAi and 56% in ABOi+HLAi groups (ABOi versus HLAi: $P = 0.05$, and ABOi versus ABOi+HLAi: $P = 0.05$) (Fig. 1d).

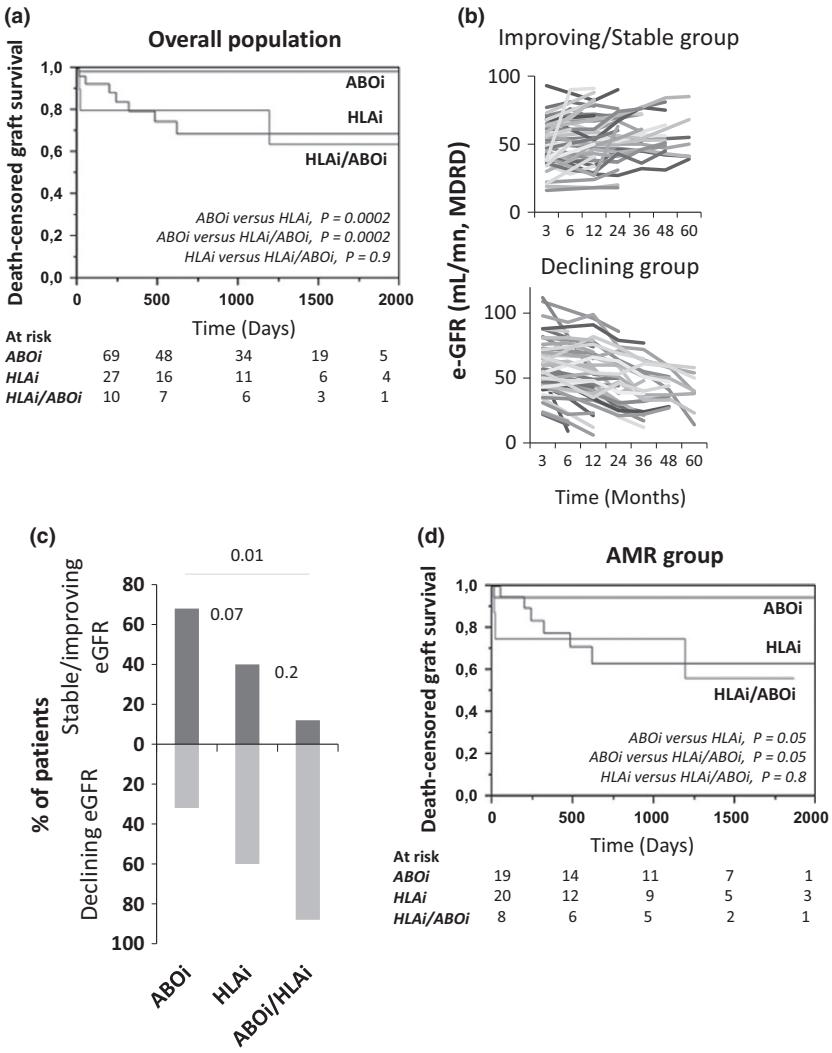

Figure 1 (a) Death-censored graft survival in ABO-incompatible (ABOi, *n* = 69), positive cross-match (HLAi, *n* = 27) and combined (ABOi+HLAi, *n* = 10) transplantations. (b) Slope of the eGFR. For each patient, the slope of the eGFR versus time curve was calculated by least-square fitting of linear regression using all eGFR values. This value was then used to stratify patients in two groups: patients with improving/stable eGFR and patients with a declining eGFR. (c) Percentage of patients with a stable/improving, or a declining eGFR after active antibody-mediated rejection (AMR) in ABO-incompatible (ABOi, *n* = 20), positive cross-match (HLAi, *n* = 19) and combined (ABOi+HLAi, *n* = 8) transplantations. Active AMR was diagnosed using Banff 2013 criteria irrespective of clinical features and treatment. (d) Death-censored graft survival after active AMR in ABO-incompatible (ABOi, *n* = 19), positive cross-match (HLAi, *n* = 20) and combined (ABOi+HLAi, *n* = 8) transplantations. Active AMR was diagnosed using Banff 2013 criteria irrespective of clinical features and treatment.

In the HLAi group, the percentages of patients with a declining eGFR and graft survival were not statistically different between patients with C4d-positive and C4d-negative AMR (declining eGFR: 72% vs. 44%: $P = 0.2$; 5-year death-censored graft survival: 51% vs. 76%: $P = 0.5$, respectively).

Importantly, we were not able to detect statistically significant differences in outcomes between treated and nontreated AMR in the ABOi group, in which a declining eGFR was observed in 38% (5/13) of treated AMR and 17% (1/6) of untreated AMR ($P = 0.3$), nor in the HLAi group, in which a declining eGFR was seen in 64% (7/11) of treated AMR and 56% (5/9) of untreated AMR ($P = 0.7$). Five-year death-censored graft survival was also similar between treated and untreated ABMR in the ABOi (92% vs. 100%, $P = 0.5$, respectively) and HLAi groups (59% vs. 69%, $P = 0.4$, respectively). These analyses were not performed in ABOi+HLAi patients because the number of patients was too low.

Table 3. Rejection.

	ABOi $n = 69$	*P* ↔	HLAi $n = 27$	*P* ↔	ABOi+HLAi $n = 10$	*P* versus ABOi
1-year treated AMR	19% (13)	0.03	41% (11)	0.3	60% (6)	0.004
C4-positive AMR	85% (11)	0.1	55% (6)	0.05	100% (6)	0.3
C4d-negative AMR	15% (2)		45% (5)		0% (0)	
Treatment for AMR						
Steroids	69% (9)	0.005	9% (1)	0.4	33% (2)	0.05
ATG	15% (2)		0% (0)		0% (0)	
IVIG	0% (0)		18% (2)		0% (0)	
DFPP	15% (2)		73% (8)		67% (4)	
1-year borderline rejection	3% (2)	0.3	7% (2)	0.3	0% (0)	0.6
1-year T-cell mediated rejection	20% (14)	0.3	30% (8)	0.6	20% (2)	0.9
Grade I	9% (6)	0.8	15% (4)	0.9	10% (1)	0.9
Grade II	10% (7)		11% (3)		10% (1)	
Grade III	1% (1)		4% (1)		0% (0)	
Patients with a biopsy	ABOi $n = 50$	*P* ↔	HLAi $n = 21$	*P* ↔	ABOi+HLAi $n = 8$	*P* versus ABOi
Active AMR	38% (19)	0.0001	95% (20)	0.4	100% (8)	0.001
C4d-postive active AMR	89% (17)	0.02	55% (11)	0.02	100% (8)	0.3
C4d-negative active AMR	11% (2)		45% (9)		0% (0)	
Active AMR	ABOi $n = 19$	*P* ↔	HLAi $n = 20$	*P* ↔	ABOi+HLAi $n = 8$	*P* versus ABOi
Vascular thrombi	26% (5)	0.6	20% (4)	0.1	50% (4)	0.2
Banff scores						
g (mean ± SD)	1.05 ± 0.62	0.2	1.45 ± 0.94	0.9	1.37 ± 0.52	0.2
ptc (mean ± SD)	0.95 ± 0.91	0.3	1.15 ± 0.67	0.9	1.12 ± 0.83	0.6
v (mean ± SD)	0.28 ± 0.75	0.3	0.20 ± 0.52	0.9	0.00 ± 0.00	0.2
t (mean ± SD)	1.05 ± 1.13	0.04	0.35 ± 0.59	0.8	0.25 ± 0.46	0.08
i (mean ± SD)	0.84 ± 0.89	0.1	0.45 ± 0.76	0.6	0.25 ± 0.46	0.1
C4d (mean ± SD)	2.21 ± 0.98	0.07	1.40 ± 1.35	0.1	2.25 ± 0.89	0.9
cg (mean ± SD)	0.00 ± 0.00	0.09	0.15 ± 0.37	0.3	0.00 ± 0.00	–
cv (mean ± SD)	1.17 ± 0.79	0.2	0.85 ± 0.75	0.9	0.88 ± 0.99	0.5
ct (mean ± SD)	0.95 ± 0.23	0.7	1.00 ± 0.56	0.3	0.75 ± 0.46	0.2
ci (mean ± SD)	0.89 ± 0.32	0.9	0.90 ± 0.64	0.6	0.75 ± 0.46	0.4

AMR, antibody-mediated rejection; ATG, antithymocyte globulins; DFPP, double-filtration plasmapheresis; IVIG, intravenous immunoglobulin.

Infections and cancer

At the end of the follow-up, the incidence of infections and cancers (including post-transplant lymphoproliferative disorders) was low and similar in the three groups (Table 4).

Discussion

Our study shows that *in vivo:* (i) long-term graft survival is better after ABOi than HLAi or combined ABOi/HLAi transplantation; (ii) by Banff 2013 criteria, an active AMR phenotype occurs in 38% of ABOi biopsies, whereas it is observed in almost all the HLAi or combined ABOi+HLAi group biopsies; and (iii) outcome after active AMR is much better in ABOi than in HLAi or combined ABOi+HLAi transplantations.

As expected, baseline characteristics were different between the three groups. HLAi and ABOi+HLAi recipients were more likely to exhibit risk factors associated with HLA sensitization (female, retransplantation) and then have spent more time on dialysis. On the other hand, ABOi transplantation involved mainly blood type O recipients who had a restricted access to transplantation through paired donation schemes [1].

Recent studies have shown that MVI is a more reliable indicator of early injury from DSA than C4d deposition in peritubular capillaries [23–25], which has resulted in the recognition of C4d-negative AMR in the recent Banff classification [18]. We defined treated AMR as the concomitant presence of histological signs of AMR [18], and graft dysfunction that led to additional therapies. All treated AMR occurred in the first 3 months. The incidence was lower in

Table 4. Causes of graft loss, infections and cancers.

% (*n*)	ABOi *n* = 69	*P* ↔	HLAi *n* = 27	*P* ↔	ABOi+HLAi *n* = 10	*P* versus ABOi
Causes of graft loss	1 AMR		5 AMR 1 renal venous thrombosis 1 FSGS recurrence		3 AMR	
Viral infections						
CMV disease	10% (7)	0.1	0% (0)		0% (0)	0.3
BK virus Nephropathy	9% (6)	0.1	0% (0)	0.1	10% (1)	0.7
VZV infection	7% (5)	0.4	17% (4)	0.3	0% (0)	0.4
Bacterial infections						
Bacteremia	1% (1)	0.07	11% (3)	0.9	10% (1)	0.2
Pneumonia	17% (12)	0.8	15% (4)	0.4	30% (3)	0.4
Wound infection	3% (2)	0.4	0% (0)		0% (0)	1
Fungal infections						
Pneumocystis pneumonia	6% (4)	0.3	0% (0)	0.2	10% (1)	0.5
Candida infections	3% (2)	0.4	0% (0)	0.2	10% (1)	0.3
Cryptosporidium diarrhoea	0% (0)	0.1	4% (1)	0.6	0% (0)	
Cancer	3% (2)	0.4	0% (0)	0.3	10% (1)	0.3
PTLD	1.5% (1)		0% (0)		0% (0)	
Skin	1.5% (1)		0% (0)		10% (1)	

AMR, antibody-mediated rejection; FSGS, focal segmental glomerulosclerosis.

ABOi (19%) than in HLAi transplantation (41%). Nevertheless, these figures are similar to those reported in the literature using the previous AMR Banff definitions [6,10–17].

However, on reanalysis of all biopsies in these patients using the Banff 2013 criteria, we also found other patients displaying active AMR who did not receive antirejection therapy. In these patients, MVI lesions were sometimes missed because of the low interoperator reproducibility of the semi-quantitative analysis [26,27]. In others, mild MVI was considered a benign lesion [28] or often ignored, as C4d-negative AMR was not recognized in previous Banff classifications [29]. Due to patient refusal or for logistical reasons, 28% of our patients did not have a biopsy. The lack of systematic and serial protocol biopsies may have resulted in overestimating the percentage of patients with AMR in this single-centre study. However, in protocol biopsy studies, glomerulitis, peritubular capillaritis and MVI have been observed in 73%, 60% and 80% of HLAi patients, respectively [24,30,31]. Here, active AMR (and then MVI) was found to be a constant feature in biopsies of patients transplanted with preformed DSA (95%). It included C4d-negative active AMR in 50% of cases, as previously reported [24]. In ABOi protocol biopsy studies, glomerulitis and peritubular capillaritis have previously been observed in 7% and 10–30% of patients, respectively [11,31]. Here, around one-third of ABOi biopsies exhibited criteria for active AMR. No *de novo* HLA-DSA were identified during the follow-up period in these ABOi patients (data not shown). Therefore, this high rate of AMR in ABOi patients is unexplained; however, it is within the range of the previous studies (3.3–33%). As peritubular capillary C4d deposition is observed in 75–94% of ABOi biopsies [11,31], it was not surprising to observe that 90% of AMR were C4d-positive and that C4d-negative AMR was a rare diagnosis after ABOi transplantation. In addition to a low rate of protocol biopsy, another limitation of this study is the relatively small groups; nevertheless, few studies involving large numbers of antibody-incompatible patients have been published. Furthermore, histological appearances are clearly not definitive. The diagnosis of AMR remains difficult, and individual clinicians may vary in their interpretation of findings, which partly explains why some patients with AMR were not treated.

One of the main findings of this study is that the difference in graft survival between ABOi and HLAi patients is only partly explained by the low frequency of AMR seen after ABOi transplants. Instead, there appears to be a real difference in the prognosis of AMR occurring after ABOi compared to HLAi. We found that stabilization or improvement in eGFR was common after AMR in ABOi transplantation leading to very little graft loss, while decline in eGFR and graft loss occurred frequently after AMR in HLAi patients. This finding should not lead us to consider AMR as a benign condition in ABOi transplantation because it can sometimes lead to an excess graft loss [8,11,13]. However, our data suggest that the majority of AMR in ABOi transplantation is followed by subsequent development of a stable state consistent with 'accommodation' [32,33]. In HLAi patients, accommodation has been

reported very rarely [34]. On the contrary, DSA has been associated with a high risk of development of chronic AMR [24,30,35]. Moreover, MVI and transplant glomerulopathy are more frequently observed in HLAi than in ABOi patients in both 1- and 5-year protocol biopsies [9,36].

One explanation for this unexpected result might be a difference in the induction therapy used. Rituximab was used more frequently in ABOi patients. B-cell depletion protocols have been reported to reduce *de novo* DSA incidence and chronic AMR [37,38], but whether rituximab has particular advantage is unknown. The large ongoing randomized ReMIND trial (Clinical-Trials.gov number NCT01095172) may answer this question.

A second potential explanation might be a difference in the histological severity of AMR lesions in ABOi versus HLAi patients, but our data do not support this. Indeed, tubulitis was more severe during AMR after ABOi transplantation, consistent with previous reports [11], but it does not seem to be associated with poor outcome during AMR. Although future studies using transcriptome analyses (or the 'molecular microscope') could help to further differentiate AMR in ABOi from that in HLAi transplants [39], this still appears impossible using the latest Banff criteria.

A third potential explanation might be the fact that AMR was treated differently in ABOi versus HLAi patients; however, the similar outcomes observed between treated and untreated patients in both ABOi and HLAi groups suggest additional mechanisms are at play. In ABOi transplantation, the concomitant presence of tubulitis directed the therapy more towards the use of antithymocyte globulin, because their primary mechanism of action is T-cell depletion [40]. Antithymocyte globulins have been also shown to contain antibodies against a number of B-cell antigens [41]; however, they have been shown ineffective to reverse some B cells containing or C4d+ rejection [42,43]. For this reason, antibody removal and intravenous polyclonal immunoglobulins, regarded as the gold-standard treatment for ABMR [43,44] was more frequently used in HLAi recipients. Rituximab induction may reduce the magnitude of HLA antibody rebound [45] and be effective in the treatment of rejection with significant B-cell infiltrate [42]. However, the others reports of its use as a treatment for AMR have been purely descriptive and included a variety of other treatments [46]. Therefore, definitive evidences for use of rituximab for treating AMR are still lacking.

A review of the literature of accommodation indicates two factors that may be relevant in trying to interpret our findings [3]. First, the characteristics of the antibody and the interactions with donor endothelium have a role in mediating spontaneous accommodation, and there is some *in vitro* evidence to suggest that cytoprotective effects of anti-blood group antibodies are less dependent on antibody levels than anti-HLA antibodies [3,4]. Secondly, accommodation is only ever seen when antidonor T-cell responses are completely controlled, so the inherent differences in sensitization and then in antidonor T cells priming between ABOi and HLAi patients might be very relevant [47].

Previous reports have suggested good short-term outcomes in small numbers of ABOi+HLAi patients, although these reports included no Luminex analyses to identify DSA [48,49]. The 10 patients reported here had a much longer follow-up (998 $\pm$ 772 days). They displayed the same baseline characteristics, had a similar incidence of biopsy proven active AMR and similar graft survival as HLAi patients. These data suggest that the presence of anti-A/B antibodies fail to influence the outcome in HLAi patients.

Finally, the rate of viral infection, including cytomegalovirus and BK virus infections, has been suggested as being higher after ABOi transplantation [50,51]. However, the incidences of infections and cancers were low and similar between the three groups.

In conclusion, ABOi transplantation offers better results than HLAi transplantation, partly because the incidence of ABMR is lower, but also because there are distinct differences in outcomes following ABMR between the two groups.

Authorship

LC, RP, AD and NM: contributed to the design of the study. LC, AD and NM: participated in the writing of the manuscript. LC, RP, MM, OS, ZA and NK: participated in the performance of the research. LC, MM, AD and NM: participated in data analysis. LC, RP, MM, ZA, OS, NK, AD and NM: were involved in critical revision of the manuscript.

Funding

There is no study sponsor. Only one author of this manuscript has conflict of interests to disclose. LC has research contracts with Novartis, Roche and Astellas companies.

Acknowledgements

This research was supported by the National Institute for Health Research (NIHR) Biomedical Research Centre based at Guy's and St Thomas' NHS Foundation Trust and King's College London. The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health. The authors acknowledge the support of the Medical Research Council (MRC) Centre for Transplantation, King's College London, UK – MRC grant no. MR/J006742/1. We also acknowledge Grainne

Walsh (Evelina London Children's Hospital), Amanda Sawdon (King's College Hospital), Dr Michelle Webb (East Kent Hospitals University NHS Foundation Trust), Dr Keith Graetz (Queen Alexandra Hospital, Portsmouth) and Dr Edward Kingdon (Royal Sussex County Hospital, Brighton) for their valued assistance.

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