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Histological and Extended Clinical Outcomes Following ABO-incompatible Renal Transplantation Without Splenectomy or Rituximab

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Abbreviations:

ABGAb	Anti-ABO blood group antibody
AbMR	Antibody mediated rejection
ABOc	ABO compatible renal transplant
ABOi	ABO incompatible renal transplant
ACR	Cellular rejection
CAT	Column agglutination technology
CNI	Calcineurin inhibitor
DSAb	Donor specific anti-HLA antibodies
ESKD	End-stage kidney disease
IF/TA	Interstitial fibrosis and tubular atrophy
IQR	Inter-quartile range
PEx	Plasma exchange
Ritux	Rituximab
SD	Standard deviation
SI	Standard immunosuppression
TGP	Transplant glomerulopathy

Abstract

Background: Excellent short-term results have been reported in ABO-incompatible renal transplant recipients (ABOi) managed solely with antibody removal and conventional immunosuppression. However, long-term clinical outcomes with this regimen and predictive information from protocol biopsies is lacking.

Methods: We compared outcome data in ABOi and ABO compatible (ABOc) recipients receiving this regimen approximately 4 years posttransplant, and histology from biopsies approximately 12 month posttransplant.

Results: Patient and graft survival amongst 54 ABOi recipients were 98.1% and 90.7% respectively at 4 years. Graft function was similar between ABOi (creatinine 140.3 μmol/L) and ABOc recipients (creatinine 140.2 μmol/L) (p=0.99), with no significant change over the study period in either group (Δcreatinine -0.83 vs 6.6 μmol/L) (p=0.59). There was no transplant glomerulopathy (TGP) in biopsies from either group. Interstitial fibrosis and tubular atrophy (IF/TA) was present in 7/25 (28%) ABOi compared with 7/34 (20.6%) ABOc (p=0.52). Progression of IF/TA from implantation was noted in 6/25 (24%) ABOi and 6/34 (17.6%) ABOc respectively. C4d staining without antibody mediated rejection (AbMR) was present in 13/25 (52%) of early posttransplant biopsies from ABOi recipients by immunohistochemistry, but in only 4/25 (16%) at 12 months.

Conclusions: ABOi performed with antibody removal and conventional immunosuppression continues to provide excellent patient and graft survival, and stable renal function over 4 years. Coupled with absent TGP and low rates of progressive IF/TA on earlier biopsies, this suggests that ABOi with conventional immunosuppression and antibody removal, without rituximab or splenectomy, can achieve long-term outcomes comparable to ABO compatible transplantation.

Introduction

Kidney transplantation is the optimal treatment for patients with end-stage kidney disease. With an inadequate supply of cadaveric donor kidneys there has been increasing reliance on living donor kidney transplantation. This confers the additional benefits of reduced cold ischaemic time, earlier graft function and better long-term survival (1). It also enables optimal preparation for marginal recipients with significant comorbidities. Historically, up to 30% of living donors were unable to donate to their intended recipient because of blood group incompatibility (2), with early attempts at crossing the ABO blood group barrier blighted by hyper-acute rejection and premature graft loss (3). A 1987 study reported improved outcomes in ABOi recipients when splenectomy was added to plasmapheresis (4), leading to incorporation of splenectomy in the initial large ABOi programs in Japan (5). Despite splenectomy, plasmapheresis and intense immunosuppression, these ABOi patients experienced increased rates of AbMR and early graft loss, though long-term graft survival for ABOi was comparable to concurrent ABOc kidney transplants (9 year ABOi graft survival 59% versus 57% respectively) (5).

Results from ABOi slowly improved over the next 15 years in association with a clearer understanding of anti-ABO blood group antibody (ABGAb) titres (6-8), and development of diagnostic and therapeutic tools for AbMR (9, 10). However, the improvements also largely paralleled those occurring in ABOc as a consequence of increasingly potent immunosuppression and progress in the prevention and treatment of opportunistic infections (11, 12). Although splenectomy was incorporated in regimens throughout this period of improvement, centres in the USA and Sweden began to replace splenectomy with pretransplant rituximab despite its inability to lower ABGAb prior to extracorporeal antibody removal. A report of successful ABOi transplantation without splenectomy or rituximab in 4

patients (11) was followed by a series of 37 ABOi recipients transplanted with standard immunosuppression and peritransplant antibody removal alone (13) in whom 1 year graft and patient survival was 100% with AbMR in only 2 patients. More recently, successful ABOi has been reported using standard immunosuppression without antibody removal in patients with low titre ABGAb (14, 15).

Publications reporting histological outcomes from ABOi renal transplantation are few, small in size, and come from centres employing peritransplant splenectomy and/or rituximab (16, 17). With concern lingering that omission of splenectomy and rituximab may compromise long-term outcomes, we examined extended clinical outcomes associated with this ABOi protocol in which patients underwent antibody removal and received only conventional immunosuppression. To gain additional insights into long-term outcomes, we reviewed histological findings of 12 month protocol biopsies from a cohort of these patients. To maximise prognostic information, we extended the study group to include both protocol and "for cause" biopsies from 9-18 months posttransplant. This not only increased the sample size, but also allowed us to confirm resolution of any acute pathology (and its treatment), and appreciate the impact of progressive irreversible changes within the microvasculature and interstitium (IF/TA).

Materials and Methods

Transplant and Immunosuppressive Protocol

Patient selection, immunological assessment, immunosuppressive protocol and antimicrobial prophylaxis were as previously described (13). Briefly, the study included unsensitised ABOi and ABOc recipients receiving identical tacrolimus based immunosuppression except that

ABOi recipients underwent peritransplant antibody removal and commenced mycophenolate mofetil 10-14 days pretransplant. All patients had negative crossmatches by CDC and flow, with donor specific anti-HLA antibodies (DSAb) <2000 MFI as measured by Luminex multi-antigen beads.

Plasmapheresis was prescribed according to baseline ABGAb titre (see below). Tacrolimus was commenced 2-3 days pretransplant but omitted on the morning of transplant, with basiliximab induction and methylprednisolone 250-500 mg intravenously at time of transplant. Oral prednisolone was administered from the first postoperative day, initially 25mg daily for 4 weeks, weaned to 5mg daily by week 8-12 (20). Biopsies were performed at implantation, with protocol biopsies scheduled at 3 and 12 months post-transplant. All episodes of rejection were biopsy proven, with repeat biopsies following treatment. If a patient underwent a "for cause" biopsy in proximity to a scheduled protocol biopsy, the protocol biopsy was not performed. Hence, rather than restricting the current analysis to 12 month protocol biopsies, the collection and analysis period was extended to between 9-18 months. This enabled inclusion of biopsies performed after treatment of rejection or any other acute pathology.

Titering methods

Assessment of baseline ABGAb titre was performed as previously described (13). Briefly, 3 methods were used: the "classic" nonenhanced tube technique (National Immunohematology Continuing Education – Australian Consensus Forum (NICE) method (18)), and 2 column agglutination technologies (CAT): Diamed Gel card (utilising gel beads) (DiaMed GmbH, Cressier, Switzerland) and BioVue card (utilising glass beads) (Ortho Clinical Diagnostics, Raritan, United States).

The tube method was conducted as follows: ABGAb titres were assayed using a direct agglutination test at room temperature with normal saline solution – to measure IgM titres, and an indirect anti-globulin test at 37ºC with CSL Coombs Reagent – monovalent anti-IgG (anti-human globulin; AHG), using the NICE method. A master dilution panel using platelet-poor ethylenediamine tetraacetic acid (EDTA) plasma aliquots derived from a patient venous blood EDTA sample was prepared without using low ionic strength solution or other enhancers. This was subjected to doubling dilutions, with a 5% bovine albumin solution as the diluent. Agglutination was scored using the Marsh 0-12 scoring system and the titre was recorded as the reciprocal of the highest doubling dilution at which a reaction was noted by IAT (19).

Initial testing for each patient included titering with commercially available group A_1, A_2 or B "test" cells (CSL, Parkville, Australia) and renal donor erythrocytes. If the blood group of the renal donor was typed as non-A_1, then donor erythrocytes were used exclusively. If titres using donor and reagent erythrocytes differed by more than 2 dilutions, or if the score difference was ≥10, then donor red cells were used for all further measurements; otherwise reagent red cells were used.

When the program began, patients had serial titres performed using all 3 methods. However, after August 2007, workload and the desire for maximal consistency and reproducibility of results led to peritransplant titre monitoring using only CAT with the Ortho Clinical Diagnostics AutoVue Innova semi-automated method ("ortho" method).

Plasmapheresis

ABOi patients received peritransplant plasmapheresis to reduce ABGAb titres as previously described (13). Briefly, preoperative plasmapheresis treatments were scheduled according to

the baseline ABGAb IgG isoagglutinin level, aiming for titres of ≤1:32 (tube method) or ≤1:8 (ortho method). Postoperative plasmapheresis was scheduled according to the baseline titre, and adjusted according to clinical need. Exchanges were generally conducted against albumin with fresh frozen plasma (group AB) used for exchanges performed in proximity to surgery or other interventions (eg biopsies), or when serum fibrinogen <2 g/L. Treatment volumes were generally 1x blood volume on each occasion. The median number of exchanges performed is shown in Table 1.

Analysis

Transplant biopsies were initially assessed by at least 1 of 3 experienced renal pathologists, and again at a departmental meeting involving all renal pathologists and transplant physicians. Biopsies with any ambiguity were evaluated on at least 1 further occasion to reach consensus. In assessing the biopsy, no distinction was made between protocol and "for cause" biopsies, and all were assessed according to revised BANFF 97 criteria (20). The primary outcome measure was the presence of TGP (tg ≥1). Secondary outcome measures were interstitial fibrosis (ci ≥1), tubular atrophy (ct ≥1), and peri-tubular capillary and/or glomerular C4d deposition (scored according to the Banff 90/07 criteria).

C4d staining was performed on all renal allograft biopsies by immunoperoxidase, using a Leica Bond-III immunostainer with Leica Detection System DS9800 on paraffin processed sections. Antigen retrieval was by Leica Microsystems; "Epitope Retrieval Solution 2" code AR9640, with C4d antibody (Quidel Cat#213) used at 1:300 dilution.

All episodes of AbMR and acute cellular rejection (ACR) were recorded, including those subsequent to the "9-18 month biopsy" on which assessment of IF/TA and TGP was made.

Information on patient and graft survival, graft function, and proteinuria was obtained. Outcome measures of ABOi and ABOc cohorts were compared using the Chi^2 or 2 tailed un-paired student's *t* test, and reported as significant if $P<0.05$.

Results

Patient Population and Outcomes

To maximise the number of "12 month" biopsies from the 54 patients receiving standard immunosuppression and undergoing peritransplant antibody removal (Figure 1: SI + PEx), the selection period for the "index biopsy" was extended to between 9-18 months posttransplant. This ensured histopathological assessment was performed at a time sufficiently distant from any acute pathology and its treatment, and includes "quiescent" biopsies performed near the 12 month period which may have led clinicians to forgo a 12 month protocol biopsy. While only 25 of the 54 ABOi recipients had suitable biopsies for assessment, the demographic and clinical characteristics of these 25 patients were similar to those of the 29 who did not have appropriate biopsy material. Of the 54 ABOi recipients, 15 with stable function did not have a protocol biopsy (11 had returned to their parent unit, 3 refused to have a biopsy, and 1 had a biopsy outside of the designated period of 9-18 months posttransplant), while 14 had undergone transplantation less than 12 months prior to the time of analysis. This left biopsies from 25 ABOi recipients receiving this regimen to analyse. ABGAb titres in these recipients are shown in Figure 2 and Figure 3.

As the study group comprised unsensitised ABOi recipients, the comparator group chosen consisted of concurrent, sequential unsensitised recipients of living donor ABOc kidneys (n=95), of whom 48 had a graft biopsy from between 9-18 months post-transplant. Thirteen patients were excluded as they were in a clinical trial comparing cyclosporine/mycophenolate

mofetil to cyclosporine/everolimus, while interpretation of 1 biopsy was limited by a cortical scar, leaving biopsies from 34 ABOc recipients available for analysis. The remaining 47 patients did not have an available biopsy (27 had returned to their parent unit, 5 refused, 2 had biopsies outside the 9–18 month window, and 13 were censored at the date of analysis). As with the ABOi cohort, included biopsies were predominantly 12 month protocol biopsies, with allowance made for any "quiescent" for-cause biopsies performed near the 12 month period, resulting in the decision to forgo a 12 month protocol biopsy. Both groups received the same tacrolimus based regimen, with 5mg maintenance prednisolone.

Outcome Measures

Within a total of 90 ABOi kidney transplants performed at the Royal Melbourne Hospital from December 2005 to January 2012, there were 54 patients without concomitant DSAb who were transplanted with conventional immunosuppression and antibody removal without rituximab. Amongst the 54 patients meeting study criteria, patient survival was 98.1% and graft survival was 90.7%. Within this group, 25 patients had biopsies from the appropriate period (Figure 1), amongst whom patient survival was 100% and graft survival was 96% with a median follow up of 1340 days (IQR 1117-1635). Mean serum creatinine was 140.3 μmol/L (SD 39.4), eGFR 48.1 mL/min (SD 13.9) and urine protein/creatinine ratio 51.7 mg/mmol (SD 78.7) (Table 2). Mean tacrolimus levels at the end of the study period were 3.3 ng/mL (median 3.7, range 1.2–6.4). These results were similar to those from eligible patients who did not undergo a protocol biopsy. Notably, there was little change in serum creatinine from nadir (1 month posttransplant) to the time of analysis, with a mean fall in creatinine of 0.83 μmol/L (Δ creatinine -0.83 μmol/L, SD 41.3). One of the 25 ABOi grafts was lost at 68 months from progressive damage following steroid resistant ACR 15 months posttransplant. This patient had a normal protocol biopsy at 12 months but then experienced rejection within weeks of mycophenolate mofetil being replaced by azathioprine to permit pregnancy.

Amongst the 34 eligible ABOc recipients with evaluable biopsies, patient survival was 97.1% at a median of 1684 days posttransplant (IQR 1577–1946) (1 death from multiple myeloma 1532 days posttransplant), and graft survival was 91.2% (2 additional grafts lost at 46 and 53 months to chronic allograft nephropathy). Amongst the 31 surviving ABOc grafts, mean serum creatinine was 140.2 μmol/L (SD 62.47), eGFR 50.8 mL/min (SD 17.4) and urine protein/creatinine ratio 48.0 mg/mmol (SD 78.4) (Table 2). Immunosuppression was managed identically to that of ABOi recipients with final mean tacrolimus levels of 3.5 ng/mL (median 3.5, range 0.3–7.5). The Δ creatinine was +6.6 μmol/L (SD 55.0). ACR was diagnosed in 4 ABOi patients and 4 ABOc patients. AbMR occurred in 2 ABOi patients but no ABOc patients.

There was no TGP on light microscopic analysis of biopsies from ABOi or ABOc recipients. Amongst ABOi recipients, 7/25 (28%) had IF/TA on the index biopsy versus 7/34 (20.6%) ABOc patients (P=0.52) (Table 2; Figure 2). In 1 of these 7 ABOi recipients, IF/TA was present at implantation, with no progression over the study period (Table 3). In 3 ABOi recipients, minor IF/TA was noted on the implantation biopsy (ci, ct) but was insignificant on the subsequent index biopsy. Two patients in the ABOi cohort, and 1 in the ABOc cohort developed ACR subsequent to the index biopsy.

Immunohistochemistry revealed peri-tubular capillary C4d deposition without AbMR in 13/25 (52%) of biopsies from ABOi recipients prior to the index biopsy (ie in biopsies that were “for cause” and/or 3 month protocol biopsies). However, only 4/25 (16%) had C4d staining in the subsequent biopsy 9-18 months posttransplant. No biopsies from the ABOc group had C4d staining.

Discussion

We have previously reported patient and graft survival of 100% at a median of 26 months in 37 ABOi kidney transplant recipients receiving a regimen employing conventional immunosuppression in conjunction with perioperative ABGAb removal (13). Over time, the cohort of ABOi patients (without DSAb) receiving this regimen increased to 54, with 1 death & 4 graft losses at a median of 48 months posttransplant (4 year graft survival 91%). This is consistent with the good ABOi outcomes reported by others in the modern era (5, 21, 22); the notable exceptions being some centres employing T cell depletion from which higher rates of AbMR and TGP and have been reported with associated reduction in graft survival (23, 24).

Historically, the excess graft loss associated with ABOi occurred within the first year, with long-term outcomes comparable to ABOc recipients (5). The extended clinical outcomes reported here and accompanying histological results provide further reassurance for those undertaking ABOi transplantation without splenectomy or rituximab, with no significant differences in patient and graft survival, renal function, or light microscopic findings on biopsies at around the 1 year mark between ABOi and ABOc recipients. Notably, there was no TGP in the ABOi group to suggest chronic antibody mediated damage, even in the 2 ABOi patients who had experienced early acute AbMR. While a number of patients receiving this regimen did not have suitable biopsy material, the clinical characteristics and outcomes of these patients were similar to those who had undergone protocol biopsy. The absence of subclinical disease in those biopsied is also reassuring.

The revised BANFF 97 criteria for acute AbMR include glomerulitis, peri-tubular capillaritis, acute tubular injury and/or positive C4d staining in patients with donor specific alloantibody (ABGAb or DSAb) (20). While antibody induced damage is not always complement

mediated (23), involvement of the complement pathway is characteristic of allograft injury from either blood group incompatibility or DSAb. Complement binding characteristically deposits a "footprint" denoted by staining for the complement split product C4d (9, 25, 26). Indeed, the advent of C4d facilitated identification of the histological changes signifying acute and chronic humoral rejection (9, 25), though it is now apparent that C4d is neither completely specific nor sensitive for antibody mediated injury (23, 27, 28). A unique feature of ABOi transplantation is the presence of C4d staining with stable function and otherwise normal histology. This apparent activation of proximal complement without rejection or graft dysfunction is commonly referred to as "accommodation", with peritubular capillary C4d detectable in around 80% of histologically "normal" ABOi transplant biopsies interrogated using immunofluorescence (29, 30). Detection of C4d by immunohistochemistry is generally associated with reduced sensitivity (23, 31-34). A recent study reported C4d detection by immunohistochemistry in approximately 40% of quiescent biopsies from ABOi recipients at 3 months (35). This series was, however, notable for an uncommonly high rate of rejection (AbMR in 46%, ACR in 24%). In our current series C4d staining (without AbMR) was present in biopsies from 13/25 patients (52%), and became less frequent as time post-transplant increased, persisting in only 4/25 of "12 month" biopsies. The significance of this apparent change in complement binding over time is uncertain, but raises new questions with regards to the evolution of graft acceptance/"accommodation" in the presence of ABGAb. Additional questions regarding the immunobiology of ABOi graft acceptance have been raised by the absence of C4d staining in a series of ABOi recipients with low baseline titre ABGAb who underwent ABOi without antibody removal (15).

The hallmark of chronic antibody mediated damage is TGP, with mesangial expansion and interposition, progressing to the characteristic double contour of the glomerular basement

membrane seen on light microscopy (36). Although TGP is often preceded by episodes of AbMR, transplantation in the presence of DSAb may lead to TGP and premature graft loss even without overt episodes of AbMR (37, 38). The incidence of AbMR and TGP in our ABOc cohort is lower than historically reported rates of approximately 7% (39-41), reflecting the ability of contemporary pre-transplant solid phase assays to detect pre-existing DSAb and avoid donors bearing the HLA antigens targeted by these DSAb (42). In a previous study comparing ABOi recipients, ABOc recipients with DSAb and a positive crossmatch, and a group of unsensitised ABOc recipients, AbMR occurred in 46% of ABOi recipients with 13% developing TGP (16). In a subsequent study of 48 ABOi recipients undergoing peri-transplant splenectomy, AbMR was noted in 27% of patients and AbMR was the strongest predictor of TGP noted in 15% of the cohort (17). It was gratifying that TGP was absent in biopsies from the current ABOi cohort amidst the concerns that a simplified ABOi regimen (omitting rituximab and splenectomy) may predispose to chronic AbMR, TGP, proteinuria and premature graft loss. This was true even in 12 month biopsies from the 2 patients who had experienced early AbMR, confirming that acute antibody mediated damage may resolve without TGP in some cases. We cannot rule out that some patients in this series have early TGP, undetectable by light microscopy - the earliest changes visible only by electron microscopy (43). However, proteinuria remained absent even after 4 years and this uncertainty applies equally to both ABOi and ABOc.

Beyond detecting TGP, protocol transplant biopsies may reveal subclinical rejection, drug toxicity, and enable quantification of IF/TA. The extent of IF/TA and its progression may help predict the long-term fate of the transplant, reflecting accumulation of donor related injury, perioperative ischaemia, viral infection, allo-immune injury and drug toxicity. In the current study IF/TA was relatively mild in both ABOi and ABOc (all patients had ci $\leq$1 and

ct ≤1). Availability of implantation biopsies revealed significant donor related scarring in 1 patient's serial biopsies, and overall low rates of progressive IF/TA in both the ABOi and ABOc recipients. The apparent "regression" of IF/TA sometimes observed in serial biopsies (as in 3 cases of the current series) is attributable to obtaining cores from different parts of the graft and the ever-present risk of sampling areas not representative of the general state of the allograft. Some have suggested this may occur in up to 25% of protocol biopsies (44). The value of protocol biopsies can be diminished by inadequate core size (45), sampling variability (44), and inter-observer variation (45, 46). The absence of subclinical rejection on the 12 month protocol biopsies of this cohort is consistent with reduced rates of subclinical rejection observed with contemporary immunosuppression (46), while the minimal progressive IF/TA in both cohorts may reflect a variety of factors. These include: transplantation from living donors, low rates of rejection, and relatively low exposure to calcineurin inhibitor (CNI). While the vascular damage once considered pathognomonic of CNI toxicity is now recognised as nonspecific (47), a correlation is recognised between CNI levels and graft damage (46), with a significant decline in IF/TA reported by Cosio in association with a 15% reduction in tacrolimus exposure (48). The tacrolimus levels to which ABOi and ABOc recipients in the present study were exposed (13) are approximately 30% lower than the "Low Tacrolimus" group of Cosio (48), and may at least partially explain the low rates of progressive IFTA. It is notable that these low levels (median 3.7 and 3.5 ng/ml in ABOi and ABOc respectively) were not associated with any increase in rejection.

The use of B cell depleting induction therapies, such as splenectomy and/or rituximab for sensitised or ABOi kidney transplant recipients remains widespread despite conflicting reports regarding its efficacy, and the lack of a biological rationale for their inclusion. In support of its use, recent studies have suggested lower rates of chronic AbMR and de novo

DSAb formation in ABOi patients receiving induction therapy with splenectomy or rituximab compared to ABOc patients receiving conventional immunosuppression (49). A rapid benefit in reduction of humoral immunity with rituximab seems unlikely given the absence of CD20 on plasma cells, and, a number of studies have found no benefit for either splenectomy or rituximab in suppressing AbMR or de novo DSAb formation (50, 51). The low rate of acute AbMR, lack of TGP and chronic AbMR as well as good graft survival in the ABOi patients reported in the current study is reassuring and argues strongly against the need for these treatments in unsensitised ABOi patients.

This study is notable for the uniformity of immunosuppression, clinical management, and histopathological assessment, with implantation biopsies enabling distinction between preexisting donor related damage and progressive posttransplant IF/TA. Although only around 50% of the cohort had appropriate biopsies for inclusion, the clinical characteristics and outcomes of the biopsied transplants were similar to the broader group. The excellent initial results, current histological analysis, and the stability of renal function over a period of 4 years provide reason for optimism with respect to the longevity of ABOi allografts transplanted with this protocol. The absence of TGP despite omission of splenectomy and rituximab is particularly reassuring, while the apparent decline in C4d staining over time raises questions regarding the biological mechanisms controlling graft acceptance in the presence of ABGAb. Furthermore, while historic series of ABOi transplantation report excess AbMR and early graft loss, long-term outcomes have been comparable to contemporaneous ABOc. Although reporting of long-term outcomes with this regimen are awaited, these interim results with a relatively simple and safe regimen represent a further increment in the quest to improve transplant options for the ESKD population.

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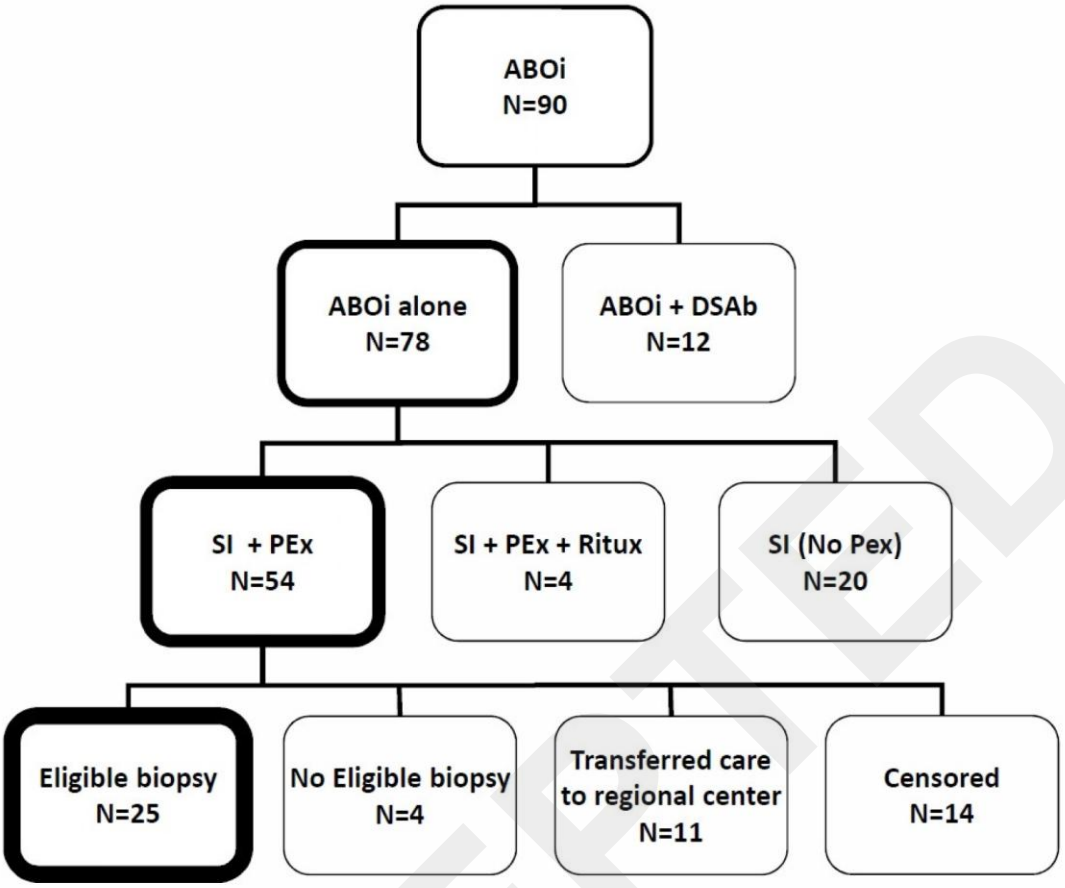
Figure Legends

Figure 1: ABOi at our institution. SI = standard immunosuppression, PEx = plasma exchange, Ritux = rituximab. From 54 patients undergoing plasma exchange and receiving conventional immunosuppression (SI + PEx), 25 had a suitable biopsy for analysis.

Figure 2: Pre-transplant ABGAb for ABOi patients (Tube RT and Tube AHG) and heat map of index biopsy results by BANFF 97 criteria.

Figure 3: ABGAb titres as measured by tube method (RT and AHG), card method (Diamed) and ortho method. Titres are shown at baseline (Base), pre-transplant (PreTx), week 1 post-transplant (W1) and week 2 post-transplant (W2). Each line indicates an individual patient.



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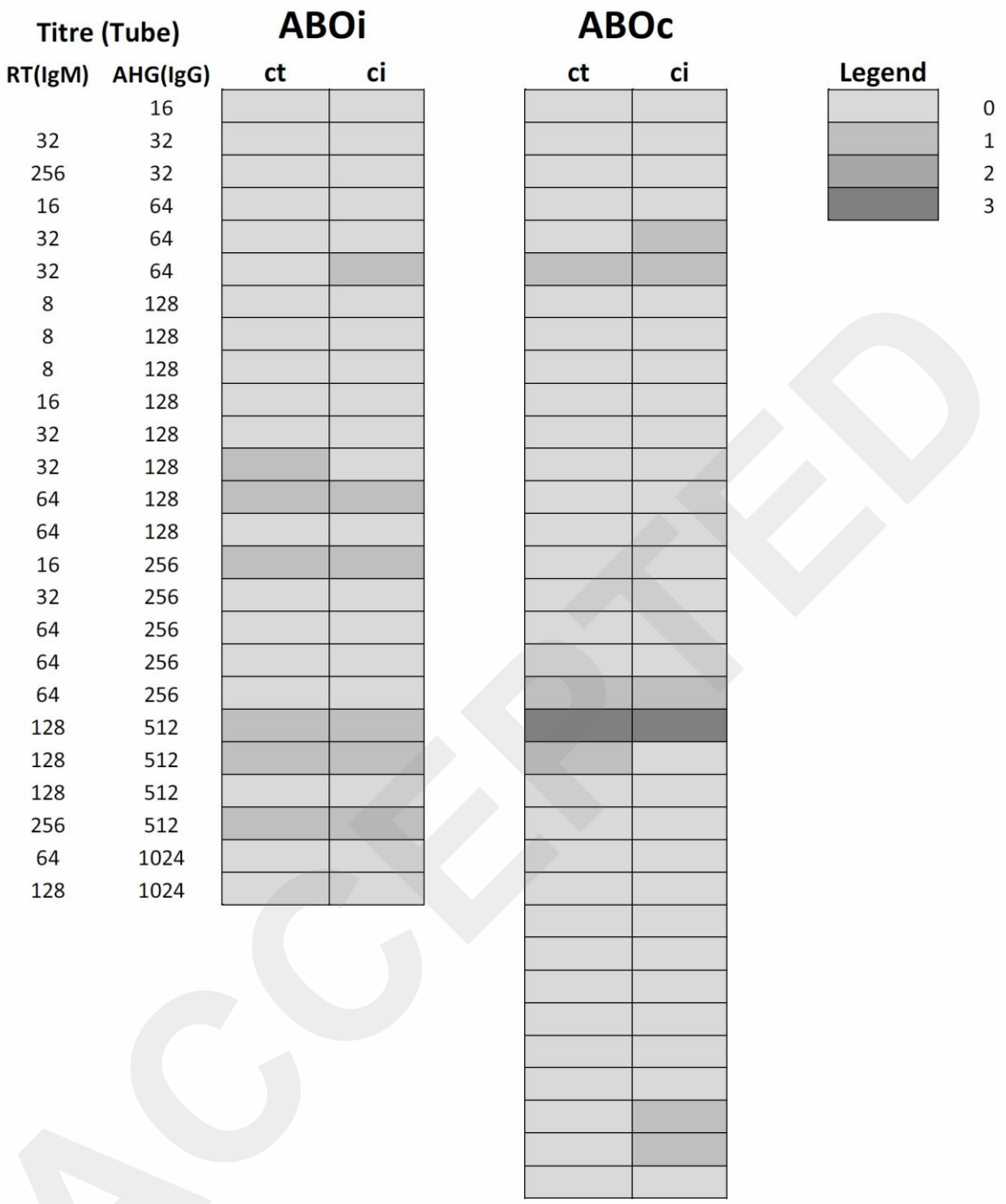

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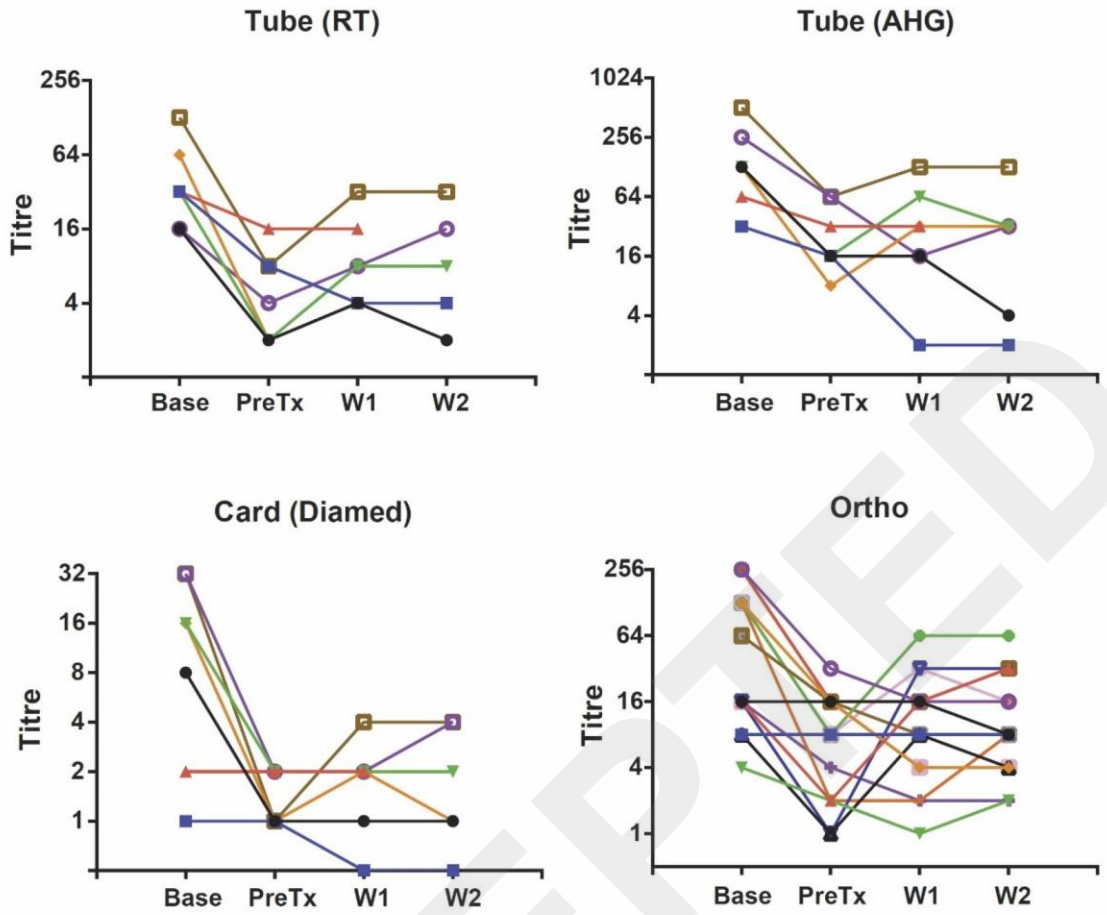

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Tables

	ABOi n=25	**ABOc** n=34	
Recipient details			
Female recipient	6 (24%)	10 (29.4%)	p = 0.65
Recipient age (years, IQR)	47 (31-59.5)	38 (33-50.8)	p = 0.23
Preexisting diabetes	4 (16%)	3 (8.8%)	p = 0.41
Transplant details			
ABO incompatibility			
A1 -> B	2		
A1 -> O	11		
A2 -> O	3		
AB -> A	2		
B -> A	3		
B -> O	4		
HLA MM	3.44	3.09	p = 0.42
Transplant > 1	1 (4%)	4 (11.8%)	p = 0.30
PRA maximum > 0	4 (16%)	3 (8.8%)	p = 0.41
Preemptive transplant	9 (36%)	4 (8.3%)	p = 0.03
Median titre			
Tube – RT (IgM)	32 (8–256)		
Tube – AHG (IgG)	128 (16–1024)		
Card – Diamed	16 (1-128)		
Ortho (IgG)	64 (4-256)		
Plasmapheresis (number)			
Pre-transplant	4 (1-7)		
Albumin	2 (0-6)		
FFP	1 (0-3)		
Posttransplant*	2 (0-16)		
Albumin	2 (0-5)		
FFP	0 (1-11)		
Donor details			
Female donor	19 (76%)	23 (67.6%)	p = 0.49
Donor age (years, IQR)	54 (47-59)	54 (50-61)	p = 0.54
Related	11 (44%)	25 (73.5%)	p = 0.02

Table 1: Baseline characteristics of ABOi and ABOc transplants included in this study. Data are in n (%) or median (range) unless otherwise indicated. *Posttransplant plasmapheresis includes 1 patient treated for AbMR.

	ABOi n=25	**ABOc** n=34	
Survival			
Patient survival	25 (100%)	33 (97.1%)	p = 0.40
Graft survival	24 (96%)	31 (91.2%)	p = 0.48
Graft function			
1 month creatinine (µmol/L)	139.9 ± 35.1	134.3 ± 43.7	p = 0.61
Final creatinine (µmol/L)	140.3 ± 39.4	140.2 ± 62.5	p = 0.99
Δ Creatinine (µmol/L)	-0.83 ± 41.3	6.6 ± 55.0	p = 0.59
Final eGFR (mL/min)	48.1 ± 13.9	50.8 ± 17.4	p = 0.57
Urine protein/creatinine mg/mmol	51.7 ± 78.7	48.0 ± 78.4	p = 0.87
Tacrolimus levels			
Mean (ng/mL)	3.3 ± 1.3	3.5 ± 1.7	p = 0.37
Median (ng/mL)	3.7 (1.2-6.4)	3.5 (0.3-7.5)	
Episodes of Rejection			
Episodes of ACR	4	4	p = 0.65
Episodes of AbMR	2	0	p = 0.10
Histology			
TGP	0	0	
IF/TA	7 (28%)	7 (20.6%)	p = 0.52
C4d			
< 3 months	13 (52%)	0	
9 - 18 months	4 (16%)	0	

Table 2: Outcome measures. Data are in n (%), median (range) or mean ± SD unless otherwise indicated. Values under *Graft function* and *Tacrolimus levels* include results from functioning grafts at last follow up, except 1 month creatinine which represent results from all grafts at 1 month post transplantation. Δ Creatinine indicates mean change in serum creatinine compared with results at 1 month post transplantation.

	ABOi	ABOc	
Patients with increase in ct score	4/25	4/34	p = 0.65
Patients with increase in ci score	6/25	5/34	p = 0.37
Average Δ ct score	0.16	0.18	p = 0.90
Average Δ ci score	0.2	0.21	p = 0.81

Table 3: Change in IF/TA scores from the implantation biopsy to the index biops