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ORIGINAL ARTICLE

ABO-incompatible kidney transplant recipients have a higher bleeding risk after antigen-specific immunoadsorption

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Summary

Pretransplant removal of antiblood group ABO antibodies is the cornerstone of all current ABO-incompatible (ABOi) transplantation programmes. In our protocol, plasmapheresis (PP) is performed with a plasmafilter followed by immunoadsorption (IA) of anti-ABO antibodies. The bleeding complications of this technique are not known. We analysed the data of all 65 consecutive ABOi kidney transplantations between March 2006 and October 2013 and compared these with matched 130 ABO-compatible (ABOc) kidney transplantations. Cases differed from controls in the pre-operative regimen, which included IA-PP and rituximab, tacrolimus, mycophenolate mofetil, prednisone and immunoglobulines. Data on platelet count, blood loss and red blood cell (EC) transfusions during 48 h post-operatively were collected. ABOi patients received EC transfusions more frequently than controls (29% vs. 12%, $P = 0.005$). Intra-operative blood loss was higher (544 vs. 355 ml, $P < 0.005$) and they experienced more major bleeding (≥3 EC within 24 h, 15% vs. 2%, $P < 0.0005$). Platelet count decreased by 28% after the pre-operative IA. In a multivariate model, only the number of pre-operative IAs was associated with the number of ECs given (OR per IA 1.9, $P < 0.05$). ABOi kidney transplant recipients have a high postoperative bleeding risk, correlating with the number of pre-operative IA sessions performed.

Introduction

The cornerstone of ABO incompatible (ABOi) and HLA antibody desensitization protocols is reduction of pre-existing concentrations of antibodies against ABO blood group or HLA antigens within the graft recipient prior to transplantation. In particular, ABO-incompatible kidney transplantation is now accepted as a suitable alternative transplantation programme with excellent outcomes for patient and graft survival. This has resulted in increasing numbers of patients receiving an ABOi kidney graft worldwide and a better use of the potential of living kidney donors [1–4]. Plasmapheresis (PP) is an essential procedure within all current desensitization protocols and may consist of plasma separation by either a centrifugation technique or by plasmafiltration using a large pore haemofilter. During PP, the plasma is exchanged for either fresh frozen plasma (FFP) or a human albumin solution. In the case of ABO desensitization, the need for plasma exchange can be circumvented by the use of immunoadsorption (IA). In this particular procedure, the separated plasma is led over an anti-ABO adsorbing column before returning to the circulation of the patient. In patients undergoing PP, an increased risk for haemorrhage has been reported, which can in part be attributed to the removal of coagulation factors when plasma is exchanged for albumin solution instead of FFP. In addition, the anticoagulants used during PP treatment can cause bleeding complications and PP with the

centrifugation technique, leads to substantial loss of platelets of up to 50% [5]. In PP using the filtration technique, an occasional case of platelet depletion was described [6], but any association with bleeding tendency has not been reported. Also in ABOi kidney transplantation, there are indications that the desensitization procedure may be associated with a higher incidence of bleeding complications postoperatively [7–9]. However, there has not been a systematic analysis of bleeding complications associated with the ABOi desensitization procedure in a large group of patients compared with ABO-compatible (ABOc) controls. In our protocol, PP is performed with a plasmafilter followed by IA of anti-ABO antibodies using the Glycorex device. To study the possible bleeding complications in more detail, we analysed intra-operative blood loss and the need for red blood cell (EC) transfusions in 65 consecutive ABOi kidney transplant recipients, compared this with 130 matched ABOc controls and studied the causative mechanisms.

Material and methods

Patients

We analysed all consecutive recipients of a blood group ABOi kidney transplant between March 2006 and October 2013 in the Erasmus Medical Center in Rotterdam, the Netherlands ($n = 65$). Controls were recipients of an ABOc kidney transplant matched for age of donor and recipient in this period ($n = 130$). Cases differed from controls in the pre-operative regimen as has been described in detail before [10]. In short, the regimen included rituximab 375 mg/m^2 4 weeks before transplantation; tacrolimus 0.1 mg/kg BID, mycophenolate mofetil 1000 mg BID; prednisone 20 mg once daily starting 2 weeks before transplantation and immunoglobulines 0.5 mg/kg 1 day pre-operatively. Furthermore, antigen-specific IA was performed pre-operatively in all but three ABOi recipients. PP was performed with a plasmaFlux PSu filter (Fresenius Medical Care, Bad Homburg, Germany) followed by adsorption of anti-ABO antibodies with the Glycosorb® device, coated with synthetically derived blood group A or blood group B antigen (Glycorex Transplantation, Lund, Sweden). In 4 h, 1.5 l plasma/h was led over the column, using unfractionated heparin infusion 1000 U/h to prevent clotting. The first 30 patients additionally received also *postoperative* IA as per protocol, performed on days 3, 6 and 9 (or days 2, 5 and 8 depending on the day of surgery). ABOi patients and ABOc received the same immunosuppressive regimen after transplantation: tacrolimus 0.1 mg/kg BID, mycophenolate mofetil 1000 mg BID, prednisone 50 mg BID for 3 days and 20 mg once daily thereafter. All kidney transplant recipients in our centre receive unfractionated heparin 12 000 IU/24 h from 4 h after renal artery anastomosis until postoperative day 5.

Data collection

The following data were collected from the medical files: sex and age of donor and recipient, ABO blood group, blood urea nitrogen (BUN), dialysis duration prior to transplantation, platelet count before IA, pre-operatively and during 2 weeks postoperatively; coagulation tests, including the activated partial thromboplastin time (aPTT) and prothrombin time-based International Normalized Ratio (PT-INR); transfusion with EC within 48 h after surgery and during two postoperative weeks. Major bleeding was defined as ≥3 EC within 24 h. Intra-operative blood loss documented in the anaesthesia files was also recorded.

Statistics

For analysis, the data obtained within the total ABOi and ABOc patient groups were compared for the first 48 h after surgery. After this period, the ABOi group was divided into two groups of patients, one that had received postoperative IA and one group that had not. Data were analysed using GRAPHPAD PRISM version 6.0 (GraphPad Software, Inc., La Jolla, CA, USA). Mann–Whitney *U*-test, Wilcoxon's signed rank test, paired t-test and unpaired *t*-test were used to determine differences between groups. Fisher's exact test was performed on discrete variables. Multivariate binary regression analysis was performed on variables known for influencing the risk of bleeding, for example blood group O [11,12]. For this analysis, IBM SPSS (IBM Corporation, Armonk, New York, USA) Statistics 21 was used. The statistical significance level was determined as $P \leq 0.05$.

Results

Baseline characteristics

Age and sex of donor and recipients did not differ between groups. Recipients did not differ in age (54 vs. 53 years in controls, $P > 0.1$) with the majority of recipients being male (68% vs. 67% in controls). More ABOi recipients were on dialysis than controls [72% vs. 55%, $P < 0.05$ or months dialysis 14.1 (1.99) vs. 10.9 (1.41), $P = 0.02$] and their BUN was significantly higher than of ABOc recipients, 25 vs. 21 μmol/l, $P = 0.003$ (Table 1). ABOi patients had higher peak panel-reactive antibodies [peak PRA 10.9 (2.4) vs. 8.5 (1.5), $P = 0.04$], but the number of HLA mismatches on A, B and DR loci did not differ, except for locus A [total mismatches 3.92 (0.20) vs. 3.56 (0.15), $P = 0.18$ and mismatches on locus A 1.29 (0.08) vs. 1.08 (0.06), $P = 0.04$]. As expected, blood group O was overrepresented in the ABOi programme (65%), as their anti-ABO antibodies against all nontype O donors limit their donor pool. In ABOc controls, the reverse phenomenon was present: 38% of patients were blood group O, which is less than the

Table 1. Baseline characteristics of ABO-incompatible and ABO-compatible kidney transplant recipients.

Variables	ABO incompatible	ABO compatible	*P*
Number of patients	65	130	–
Age recipient (mean, SEM)	53.6 (1.7)	53.5 (1.1)	NS
Male sex recipient (*n*, %)	44 (68)	87 (67)	NS
BUN day-1 (mmol/l, mean, SD)	25 ± 9	21 ± 7	<0.005
Dialysis dependency (*n*, %)	47 (72)	71 (55)	<0.05
Months on dialysis (mean, SEM)	14.1 (1.99)	10.9 (1.41)	<0.05
Recipient blood group (*n*, %)			
	O *n* = 42 (65)	O *n* = 50 (38)	<0.005
	A *n* = 11 (17)	A *n* = 57 (44)	–
	B *n* = 12 (18)	B *n* = 18 (14)	–
	AB *n* = 0	AB *n* = 5 (4)	–
Number of pre-operative immunoadsorption (median, IQR)	4 (2)	–	–
Number postoperative immunoadsorption *n* = 30 (median, IQR)	3 (0.25)	–	–
aPTT (median, IQR)	30 (9.5)	28 (6)	NS
PT-INR (median, IQR)	1.0 (0.1)	1.0 (0.1)	NS
peak PRA %(mean, SEM)	10.9 (2.4)	8.5 (1.5)	<0.05
Total mismatches HLA loci A, B and DR	3.92 (0.20)	3.56 (0.15)	NS
MM on A	1.29 (0.08)	1.08 (0.06)	<0.05
MM on B	1.39 (0.08)	1.39 (0.06)	NS
MM on DR	1.25 (0.08)	1.09 (0.06)	NS

SEM, standard error of the mean; BUN, blood urea nitrogen; SD, standard deviation; IQR, interquartile range; aPTT, activated partial thromboplastin time.

reported prevalence (47%) of blood group O in blood donors in the Netherlands. A median of 4 IAs was performed pre-operatively, with a range of 0 to 10 sessions depending on the original anti-ABO titre and its decline during treatment with IA (Table 1).

Antigen-specific immunoadsorption substantially lowers platelet count but does not result in changes of coagulation tests

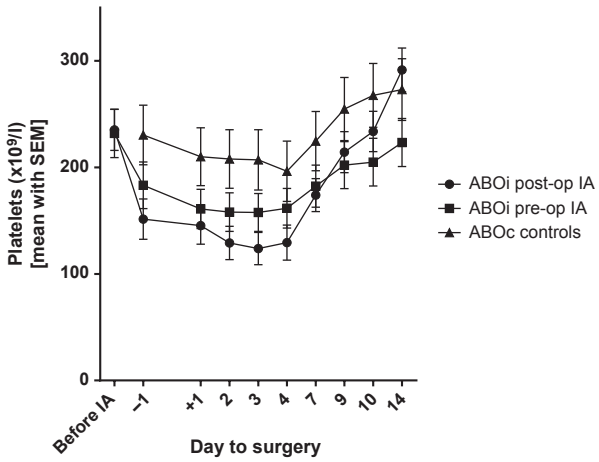
The platelet count before the start of the IA was 233 × 10^9/l on average, comparable to a mean of 230 × 10^9/l in ABOc controls 1 day pre-operatively ($P > 0.1$). However, after the pre-operative IAs, platelet count decreased with 28%, leading to 169 × 10^9/l platelets on average 1 day pre-operatively in ABOi patients ($P < 0.0001$, Fig. 1). This decline in platelet count led to a pre-operative platelet count of <100 × 10^9/l in 14 patients (22% of ABOi recipients), which was different from the control group, where only one patient (1%) had a platelet count <100 × 10^9/l ($P < 0.0001$). One week after transplantation, the platelet count was comparable to pre-operative levels (mean 178 × 10^9/l day 7) and returned to pre-IA levels after 2 weeks in the patients receiving only pre-operative IA ($n = 35$, mean 223 × 10^9/l day 14).

The first 30 patients received additional *postoperative* IA and their platelet count remained nonsignificantly lower within the first week compared with the group with only pre-operative IA ($n = 21$), but their platelet count was restored to significantly higher levels 2 weeks after transplantation (postoperative IA 291 vs. only pre-operative IA 223 × 10^9/l; $P < 0.05$). The platelet count on day 14 in the group of patients with postoperative IA exceeded even the numbers recorded before the desensitization procedure started (291 vs. 235 × 10^9/l; $P < 0.05$). Platelet count in the total ABOi group remained significantly lower than in ABOc controls from the day prior to transplantation to day 10 (all $P < 0.0005$).

Both the aPTT and the PT-INR did not differ between groups (aPTT median 30 s vs. 28 s in controls, $P > 0.1$, laboratory normal range 22–32 s; PT-INR median 1.0 IU both groups, $P > 0.1$, Table 1).

The number of preoperative IAs is strongly associated with the need for red blood cell transfusion 48 h postoperatively

The ABOi patients needed EC transfusion more frequently than the ABOc controls within 48 h postoperatively: 29% of ABOi patients received EC transfusion vs. 12% of controls ($P < 0.005$, Fig. 2a-1). In the group of patients that received EC transfusion, more ECs per patient were given in the ABOi patients than in controls (median 2 EC vs. 1 EC, $P < 0.001$, Fig. 2a-2). The following variables were tested for correlation with the dichotomous dependent EC transfusion within 48 h postoperatively: sex of recipient,



ABOi post-op PP	ABO-incompatible kidney transplantation with pre- and post-operative plasmapheresis
ABOi pre-op PP	ABO-incompatible kidney transplantation with only pre-operative plasmapheresis
ABOc controls	ABO-compatible controls

Figure 1 Platelet count in ABO-incompatible patients and their ABO-compatible controls. Platelet count before start of the immunoadsorption (IA) (mean 233 × 10^9/l) was comparable to controls (230 × 10^9/l) 1 day pre-operatively ($P > 0.1$). Platelet count fell by 28% 1 day pre-operatively in ABO-incompatible patients (169 × 10^9/l, $P < 0.0001$). Platelets in ABO-incompatible patients remained lower up to day 10 compared with controls (all $P \leq 0.001$). In the group of ABOi patients with postoperative IA (first 30 patients), platelet count was higher after 2 weeks than without postoperative IA (292 vs. 223 × 10^9/l; $P = 0.02$). Platelet count after postoperative IA was even higher at day 14 than before IA (292 vs. 235 × 10^9/l; $P = 0.02$).

age of recipient, BUN, dialysis (and duration in months) requirement prior to transplantation, platelet count before IA, platelet count 1 day pre-operatively both linear and dichotomous <100 × 10^9/l, delta platelet count (decrease from IA to 1 day pre-operatively), aPTT, PT-INR, ABO blood group, the number of pre-operative IAs and intra-operative blood loss. Only the number of pre-operative IAs and blood group O correlated with the need for EC transfusion (univariate analysis: OR 1.9, OR 7.1, all $P < 0.05$). In multivariate regression analysis, only pre-operative IA predicted postoperative EC transfusion within 48 h: the risk of transfusion increased independently with every pre-operative IA session (OR 1.9, $P = 0.011$, Table 2). Only two of the 18 patients with <4 pre-operative IAs received EC transfusion within the first 48 h, while 16 of the 44 patients with four or more IAs received EC transfusion ($P < 0.05$), irrespective of platelet count (platelet >100 and IA ≥4: 38% vs. platelet <100 and IA ≥4: 40% of patients receiving EC in this time period, $P > 0.9$, Fig. 3a). The number of pre-operative IA sessions did influence pre-operative platelet count: the 21 patients with <4 pre-operative IAs had a higher platelet count than the 44 patients with four or more IA sessions (mean 209 × 10^9/l vs. 143 × 10^9/l, $P = 0.001$, Fig. 3b).

ABOi blood group O recipients received EC transfusion more frequently than nongroup O recipients during 48 h postoperatively (40% vs. 9%, $P = 0.008$). Blood group O recipients underwent more pre-operative IA sessions (median 5 vs. 2, $P < 0.0001$). The strong association between blood group O and EC transfusion disappeared after correction in multivariate analysis for the number of pre-operative IAs. Of note is a nonsignificantly higher blood transfusion rate in ABOc blood group O recipients: nine of 41 blood group O vs. seven of 73 nonblood group O patients, 18% vs. 9%, $P = 0.17$. Interestingly, of these patients, only blood group O patients received more than one EC transfusion: five blood group O vs. none nonblood group O patients ($P = 0.007$).

Major bleeding is more frequent in the ABOi patient group, especially in women

Ten ABOi recipients experienced major bleeding within 48 h postoperatively compared with only one ABOc patient (15% vs. 1%, $P < 0.0001$ Fig. 2b). Three of these ABOi patients underwent relaparotomy, in four patients, the anaesthesia report described massive blood loss and difficult haemostasis with intra-operative blood loss ranging from 1200 to 2800 ml. One patient had hypotension and acute kidney injury for which haemofiltration was initiated. In the other two patients, the major bleeding was contributed to 'oozing'. Women were overrepresented in major bleeding: 70% of major bleeders were female, while only 35% of ABOi recipients were female (Fisher's exact test $P = 0.02$).

Blood transfusion within 2 weeks postoperatively

A substantial part of ABOi recipients needed EC transfusion after the direct postoperative period, from 48 h up to day 14: 40% of ABOi patients received EC transfusion vs. 15% of controls in this period (Fig. 2c, $P = 0.0001$).

Intra-operative blood loss

Of five ABOi and nine ABOc patients, data on intra-operative blood loss were missing, all in patients without EC transfusion the day of surgery. ABOi patients lost more blood intra-operatively than controls (543 ± 65 vs. 355 ± 34 ml, $P < 0.005$, Fig. 4).

Haemoglobin level

We studied whether the decision for blood transfusion was made at comparable haemoglobin levels. The haemoglobin

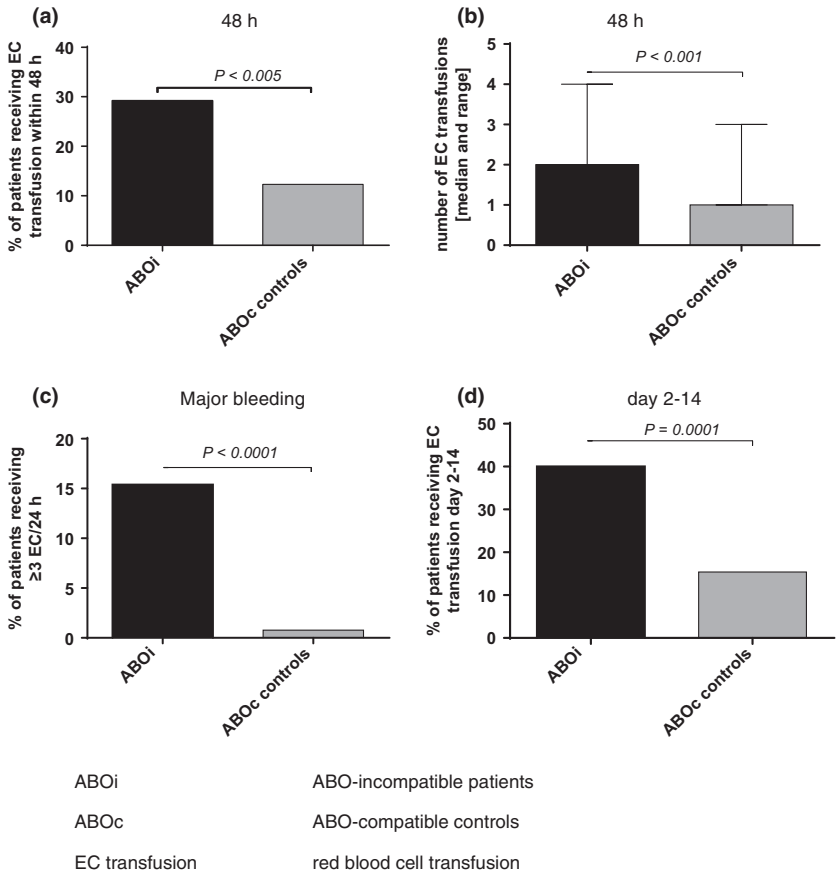


Figure 2 ABO-incompatible patients received red blood cell transfusions more frequently than controls. ABO-incompatible patients received blood transfusions more frequently than ABO-compatible controls (29% vs. 12%, $P = 0.005$) during 48 h postoperatively (a). In this time period, more red blood cell (EC) transfusions were given in the ABO-incompatible patients needing transfusion than in controls (median 2 EC vs. 1 EC, $P < 0.001$) (b). ABO-incompatible recipients experienced more major bleeding (three or more EC within 24 h) in 48 h postoperatively than ABO-compatible controls (15% vs. 1%, $P < 0.0001$) (c). ABO-incompatible patients received blood transfusion more frequently from 48 h after transplantation up to day 14 than ABO-compatible controls (40% vs. 15%, $P = 0.0001$) (d).

Table 2. Multivariate analysis of need for red blood cell transfusion 48 h postoperatively.

	OR*	95% CI†	*P*
Male sex recipient	0.46	0.10–2.11	NS
Number of pre-operative IA‡	1.91	1.16–3.16	0.01
Platelet count 1 day pre-operatively	1.01	1.00–1.02	NS
Blood loss surgery	1.00	1.00–1.00	NS
Blood group O	3.37	0.36–31.41	NS

*Odds ratio.
†Confidence interval.
‡Immunoadsorption.

level in patients receiving EC transfusion within 48 h did not differ between ABOi and ABOc controls: the median haemoglobin level the day of EC transfusion was 4.6 vs. 4.8 mmol/l, $P > 0.1$. Haemoglobin levels did not differ between ABOi patients before start of IA and ABOc controls 1 day pre-operatively (7.3 ± 0.14 vs. 7.5 ± 0.08 mmol/l, respectively, $P > 0.1$). After pre-operative IA, however, the average haemoglobin level was lower compared with ABOc controls 1 day pre-operatively (6.8 ± 0.15 vs. 7.5 ± 0.08 mmol/l, $P < 0.0001$, Fig. 5). The average decrease in haemoglobin concentration from the day before surgery to day +2 postoperatively was comparable between ABOi patients and controls (-0.7 vs. -0.9 mmol/l, $P > 0.05$), but the former received significantly more EC transfusions. This finding is in line with the observation that ABOi patients in general have a higher intra-operative blood loss and have more major bleedings postoperatively.

Discussion

ABOi kidney transplant recipients needed more blood transfusions than their ABOc controls. In this cohort of 65

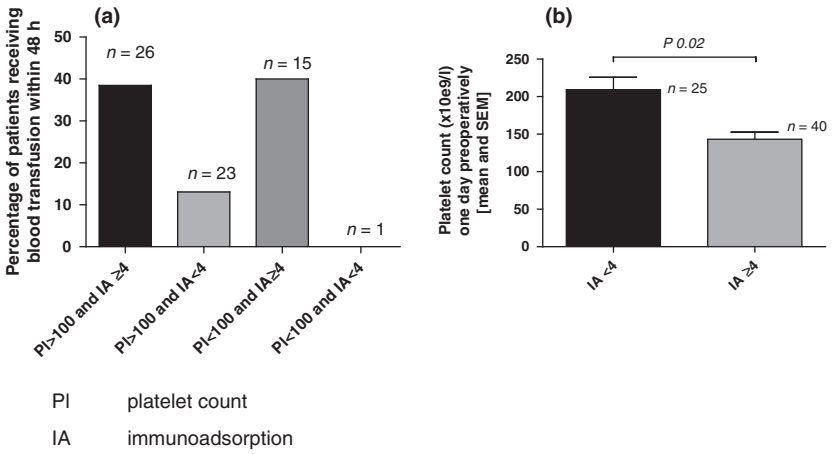


Figure 3 Immunoadsorption correlates with red blood cell transfusion and with platelet count. Only two of the 18 patients with <4 immunoadsorption sessions (IA) received red blood cell (EC) transfusion within 48 h postoperatively, while in the 44 patients with ≥4 IAs, 16 of them received EC transfusion, irrespective of platelet count platelet >100 and IA ≥4: 38% vs. platelet <100 and IA ≥4: 40% of patients receiving EC in this time period, $P > 0.9$) (a). The number of pre-operative IAs did influence pre-operative platelet count: 21 patients with IA<4 had an average platelet count of 209×10^9/l vs. 143×10^9/l, $P = 0.001$, f (b).

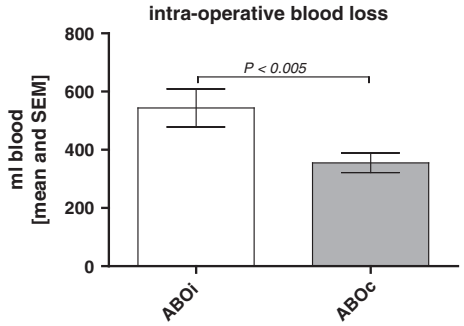


Figure 4 Intra-operative blood loss. ABOi patients lost more blood intra-operatively than controls (536 ± 66 vs. 364 ± 34 ml, mean and SEM, $P < 0.005$).

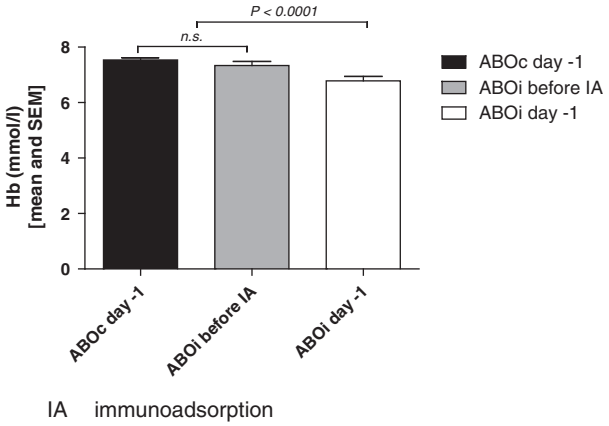


Figure 5 Haemoglobin level. Haemoglobin levels were comparable between ABOc patients 1 day pre-operatively and ABOi patients before immunoadsorption [7.54 (0.08) vs. 7.34 (0.14), $P = 0.12$], but decreased after pre-operative immunoadsorption [6.79 (0.15), $P < 0.0001$].

ABOi patients and 130 ABOc controls, ABOi patients had more blood intra-operative blood loss, received EC transfusions more than twice as frequently as their controls in the first 48 h postoperatively and significantly more major bleeding was noted.

The number of IAs appeared to be strongly associated with the need for EC transfusion. It has been known that PP via the centrifugation technique leads to thrombocytopenia, but little is known about thrombocytopenia as a result of PP using a plasma filter. In this study, we show that IA by plasma filtration also leads to a remarkable decrease in platelet count. However, despite the marked decrease in platelet count in our ABOi cohort, the platelet count did not correlate with EC transfusion in uni- and multivariate regression analysis. Only the number of pre-operative IAs predicted postoperative EC transfusion, irrespective of the platelet count. For instance, the higher transfusion rate in patients receiving four or more IAs was not influenced by platelet count. In other words, although frequent IA decreases platelet count, the blood transfusion rate is largely independent of the platelet count. The singular association between IA and EC transfusion was further strengthened by our analysis of the postoperative period beyond 48 h. Again in the subgroup of patients receiving postoperative IA, we noticed an increased need for blood transfusion as compared to the other ABOi patients, while their platelet count was not significantly lower within this period.

Our findings are supported by anecdotal data in the literature on a higher bleeding tendency in ABOi kidney

transplant recipients treated with PP, performed with and without IA [7–9,13]. The correlation between number of IA/PP and risk of bleeding can be extrapolated from two other studies. ABOi paediatric kidney transplant recipients had more bleeding complications when more PP sessions were applied [14]. Higher anti-ABO titres necessitating an intensified PP regimen led to more bleeding complications compared with low anti-ABO titres in a cohort of 14 Korean ABOi patients [15].

Frequent contact with the plasma filter membrane thus seems to cause a bleeding tendency, which may be explained by coagulation abnormalities or platelet dysfunction rather than thrombocytopenia. The plasma dialyser membrane used in our ABOi patients is a full barrier for platelets. However, platelet count and function are influenced during a haemodialysis session using dialysers made from similar types of synthetic membranes as during PP. Daugirdas and Bernardo review this topic extensively, including studies on polysulfone membranes and its inhibitory effect on platelet count and function [16–18]. Platelet count falls approximately 10% during the first 30 min of dialysis and typically returns to predialysis values thereafter. Both the formation of platelet aggregates and the activation of platelets can lead to this decrease in platelet count [19]. The type of membrane (synthetic vs. cellulose), as well as the sterilization method, influences these phenomena [20]. The increased blood shear stress or the formation of microbubbles might play a role in platelet activation [21]. Activated platelets have a shortened lifespan and prolonged bleeding times can be measured directly after haemodialysis [22,23]. Therefore, thrombocytopenia and platelet dysfunction may follow repeated PP as a consequence of the procedure and materials used, similar to haemodialysis.

Besides platelet dysfunction induced by the polysulfone membrane, coagulation abnormalities could be an alternative hypothetical explanation for the higher transfusion need. The normal PT-INR and aPTT argue against this possibility. This is further supported by a report on 14 ABOi German patients in whom D-dimer, fibrinogen, plasminogen, thromboelastography and antithrombine-III were found to be similar before and after PP with IA [8].

Notably, the haemoglobin level in ABOi patients after the pre-operative IA sessions was lower than in ABOc controls before surgery, while a similar transfusion policy was used for both groups. Bone marrow depression by mycophenolate mofetil could have attributed to the lower haemoglobin in the ABOi group. This raised the possibility that the EC transfusion rate was increased in the ABOi group postoperatively solely because of a lower haemoglobin level before surgery. However, the higher intra-operative blood loss and the higher rate of major bleeding argue against the lower haemoglobin level pre-operatively as a singular explanation for the higher transfusion rate.

Two remarkable findings need to be discussed. Blood group O recipients needed EC more frequently during 48 h postoperatively. The association between blood group O and EC transfusion could be explained by a higher number of IAs performed in patients with blood group O, as a consequence of higher anti-ABO titres in blood group O recipients [24]. Another explanation for the higher transfusion rate might be a higher bleeding tendency in blood group O subjects *per se*. Blood group O is also overrepresented in, for example, women with heavy menstrual bleeding [11] and children with post-tonsillectomy haemorrhage [12]. The reciprocal also holds true, with a higher incidence of thrombosis in nongroup O individuals [25]. This may be related to the 25% lower levels of von Willebrand Factor in individuals with blood group O vs. non-O [26]. An argument for a higher bleeding tendency *per se* in blood group O patients is the nonsignificant higher blood transfusion rate in blood group O ABOc controls and the finding that only blood group O patients received more than one blood transfusion in controls. Another outcome was the higher risk of major bleeding in women. Studies in anticoagulation therapy and management of cardiovascular disease also reveal a higher risk of bleeding among women [27]. The reason for this bleeding tendency in women is not clear.

Our results reveal a higher transfusion rate and bleeding tendency in patients undergoing IA. We hypothesize that the causative mechanism is platelet dysfunction induced by contact with the plasma filter membrane, combined with blood loss caused by the filtration technique. We have not excluded so far the possibility that the higher blood transfusion rate was caused by the adsorption column and not the plasma filter membrane alone. Fibrinogen levels can indeed be significantly reduced after IA [28]. More bleeding compared with controls, however, was also observed in ABOi patients receiving PP without IA [7,15]. Also the normal coagulation times in our ABOi cohort argue against loss of coagulation factors by adhesion to the column, as hypofibrinogenemia would prolong coagulation tests, which require the production of a fibrinogen clot as an end point, like aPTT and PT-INR.

In conclusion, antigen-specific IA prior to ABOi kidney transplantation exposes patients to a significantly higher bleeding risk than ABOc controls. Blood transfusion leads to HLA sensitisation, especially in patients with baseline panel-reactive antibodies [29,30] such as ABOi kidney transplant recipients [2,7]. Therefore, further investigations are warranted to clarify the precise mechanisms and to implement anticoagulation protocols for this specific patient category.

Authorship

AEW: collected the data, analysed the results and wrote the manuscript. MA: coordinates the ABO-incompatible

kidney transplantation programme, analysed the data and corrected the manuscript. FWL: helped with interpretating the data and correcting the manuscript. JNMI: the principal surgeon performing the ABO-incompatible kidney transplantations and he corrected the manuscript. WW: treated the majority of the patients and corrected the manuscript. MGHB: analysed the data and helped with writing and revising the manuscript.

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References

1. Genberg H, Kumlien G, Wennberg L, Tyden G. Long-term results of ABO-incompatible kidney transplantation with antigen-specific immunoadsorption and rituximab. *Transplantation* 2007; **84**(12 Suppl): S44.
2. Montgomery JR, Berger JC, Warren DS, James NT, Montgomery RA, Segev DL. Outcomes of ABO-incompatible kidney transplantation in the United States. *Transplantation* 2012; **93**: 603.
3. Fuchinoue S, Ishii Y, Sawada T, *et al.* The 5-year outcome of ABO-incompatible kidney transplantation with rituximab induction. *Transplantation* 2011; **91**: 853.
4. Roodnat JI, van de Wetering J, Claas FH, Ijzermans J, Weimar W. Persistently low transplantation rate of ABO blood type O and highly sensitised patients despite alternative transplantation programs. *Transpl Int* 2012; **25**: 987.
5. Keller AJ, Chirnside A, Urbaniak SJ. Coagulation abnormalities produced by plasma exchange on the cell separator with special reference to fibrinogen and platelet levels. *Br J Haematol* 1979; **42**: 593.
6. Sanz-Guajardo D. Plasmapheresis in the treatment of glomerulonephritis: indications and complications. *Am J Kidney Dis* 2000; **36**: liv.
7. 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.
8. Renner FC, Czekalinska B, Kemkes-Matthes B, *et al.* Postoperative bleeding after AB0-incompatible living donor kidney transplantation. *Transplant Proc* 2010; **42**: 4164.
9. 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.
10. 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**: 6.
11. Amesse LS, Pfaff-Amesse T, Gunning WT, Duffy N, French JA 2nd. Clinical and laboratory characteristics of adolescents with platelet function disorders and heavy menstrual bleeding. *Exp Hematol Oncol* 2013; **2**: 3.
12. Leonard DS, Fenton JE, Hone S. ABO blood type as a risk factor for secondary post-tonsillectomy haemorrhage. *Int J Pediatr Otorhinolaryngol* 2010; **74**: 729.
13. 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.
14. Schaefer B, Tonshoff B, Schmidt J, *et al.* Bleeding complications in pediatric ABO-incompatible kidney transplantation. *Pediatr Nephrol* 2013; **28**: 327.
15. Chung BH, Lee JY, Kang SH, *et al.* Comparison of clinical outcome between high and low baseline anti-ABO antibody titers in ABO-incompatible kidney transplantation. *Ren Fail* 2011; **33**: 150.
16. Daugirdas JT, Bernardo AA. Hemodialysis effect on platelet count and function and hemodialysis-associated thrombocytopenia. *Kidney Int* 2012; **82**: 147.
17. Andrassy K, Ritz E, Bommer J. Effects of hemodialysis on platelets. *Contrib Nephrol* 1987; **59**: 26.
18. Elshamaa MF, Elghoroury EA, Helmy A. Intradialytic and postdialytic platelet activation, increased platelet phosphatidylserine exposure and ultrastructural changes in platelets in children with chronic uremia. *Blood Coagul Fibrinolysis* 2009; **20**: 230.
19. Bonomini M, Sirolli V, Stuard S, Settefrati N. Interactions between platelets and leukocytes during hemodialysis. *Artif Organs* 1999; **23**: 23.
20. Amato M, Salvadori M, Bergesio F, Messeri A, Filimberti E, Morfini M. Aspects of biocompatibility of two different dialysis membranes: cuprophane and polysulfone. *Int J Artif Organs* 1988; **11**: 175.
21. Barak M, Katz Y. Microbubbles: pathophysiology and clinical implications. *Chest* 2005; **128**: 2918.
22. Sloand JA, Sloand EM. Studies on platelet membrane glycoproteins and platelet function during hemodialysis. *J Am Soc Nephrol* 1997; **8**: 799.
23. Rinder HM, Murphy M, Mitchell JG, Stocks J, Ault KA, Hillman RS. Progressive platelet activation with storage: evidence for shortened survival of activated platelets after transfusion. *Transfusion* 1991; **31**: 409.
24. Toki D, Ishida H, Horita S, Yamaguchi Y, Tanabe K. Blood group O recipients associated with early graft deterioration in living ABO-incompatible kidney transplantation. *Transplantation* 2009; **88**: 1186.
25. Sode BF, Allin KH, Dahl M, Gyntelberg F, Nordestgaard BG. Risk of venous thromboembolism and myocardial infarction

associated with factor V Leiden and prothrombin mutations and blood type. *CMAJ* 2013; **185**: E229.
26. Lazzari MA, Sanchez-Luceros A, Woods AI, Alberto MF, Meschengieser SS. Von Willebrand factor (VWF) as a risk factor for bleeding and thrombosis. *Hematology* 2012; **17** (Suppl 1): S150.
27. Cosma RM, Waeber G, Wasserfallen JB, Nakov K, Aujesky D. Hospitalized women experiencing an episode of excessive oral anticoagulation had a higher bleeding risk than men. *J Womens Health (Larchmt)* 2009; **18**: 321.
28. Fadul JE, Linde T, Sandhagen B, Wikstrom B, Danielson BG. Effects of extracorporeal hemapheresis therapy on blood rheology. *J Clin Apher* 1997; **12**: 183.
29. Yabu JM, Anderson MW, Kim D, *et al.* Sensitization from transfusion in patients awaiting primary kidney transplant. *Nephrol Dial Transplant* 2013; **28**: 2908.
30. Fidler S, Swaminathan R, Lim W, *et al.* Peri-operative third party red blood cell transfusion in renal transplantation and the risk of antibody-mediated rejection and graft loss. *Transpl Immunol* 2013; **29**: 22.