Renal Replacement Therapy

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Novel double-filtration plasmapheresis preserves fibrinogen while removing immunoglobulin-G antibodies before ABO blood type-incompatible kidney transplantation

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Abstract

Background: Removal of anti-blood group antibodies is important for successful ABO-incompatible kidney transplantation (ABOi-KTx). Double-filtration plasmapheresis (DFPP) using albumin solution removes antibodies effectively. However, fibrinogen is largely removed resulting in hemostatic failure. Herein, we designed an altered combination of plasma membranes in DFPP (novel DFPP, nDFPP) to retain more fibrinogen while removing IgG, and assessed its efficacy and safety compared with conventional DFPP (cDFPP).

Methods: Consecutive ABOi-KTx recipients (from 2015 to 2018) were enrolled. For the first membrane, we used Cascadeflo EC-50W in nDFPP and Plasmaflo OP-08W in cDFPP, and Cascadeflo EC-20W as the second membrane in both modalities. Removal rates (RR) of IgG, IgM and fibrinogen per DFPP session, and adverse events were compared with historical control patients who underwent cDFPP before ABOi-KTx, between 2006 and 2015.

Results: nDFPP and cDFPP groups included 12 and 23 cases, respectively. nDFPP was inferior to cDFPP in RR of IgG and IgM. nDFPP was also inferior to cDFPP in the decline in anti-blood group IgG and IgM antibody titers. However, fibrinogen was more preserved in nDFPP compared with cDFPP, indicating that nDFPP has more selective removal properties (median RR of IgG, IgM, and fibrinogen: 62.1%, 15.7% and 37.6%, respectively, in nDFPP; and 74.5%, 85.0% and 76.6%, respectively, in cDFPP). In the comparison of hemostatic function among the patients who had arteriovenous fistula for hemodialysis, prolonged hemostasis (> 20 min) at the cannulation site was significantly less frequently observed in nDFPP group (1 in 9 cases, 9.1%) than in cDFPP group (all 18 cases, 10%, $p < 0.0001$).

Conclusions: nDFPP preserves fibrinogen while removing anti-blood type IgG antibodies before ABOi-KTx.

Keywords: ABO-incompatible, Antibody removal, Double-filtration plasmapheresis, Kidney transplantation

Introduction

Recent improvements in immunosuppressive regimens have enabled the ability to transplant "antibody-incompatible" kidneys under conditions of ABO blood group-incompatibility or donor-specific antibody-positivity [1]. Pretransplant desensitization protocols for such recipients generally include reagents for suppressing

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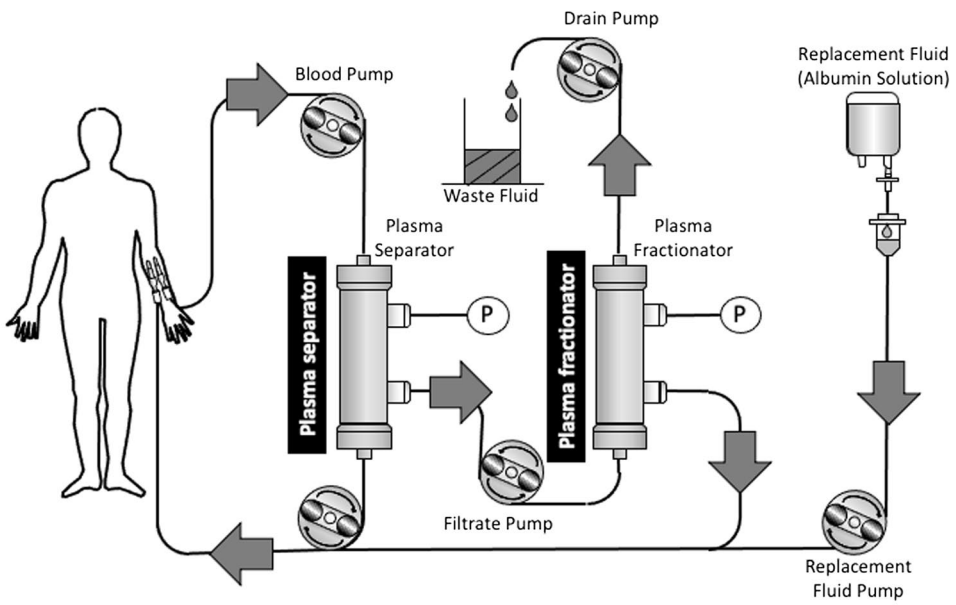
antibody-producing cell populations and apheresis for removal of pre-existing donor-reactive antibodies [2, 3]. There are presently three major apheresis modalities for removing donor-reactive antibodies. Therapeutic plasma exchange (TPE), which replaces recipient plasma with fresh frozen plasma (FFP), is associated with risks such as hyperacute allergic reactions, hypocalcemia (induced by sodium citrate, a preservative in FFP), and microorganism transmission caused by massive infusion of FFP**,** with the incidence widely ranged 3–46.8% [4–6]. The incidence of adverse effects may be influenced by patients' morbidities such as chronic renal failure [6]. Double-filtration plasmapheresis (DFPP) was first introduced by Agishi et al. in the early 1980s [7]. It was designed to remove molecules with sizes between the pore sizes of the first (plasma separator) and second membranes (plasma fractionator). In general, the conventional combination of a plasma separator (Plasmaflo OP-08W) and plasma fractionator (Cascadeflo EC-20W) has been applied in DFPP (i.e., cDFPP). DFPP using albumin (Alb) replacement solution removes high molecular weight molecules such as immunoglobulins (Ig) effectively and reduces the loss of Alb. However, DFPP still results in massive loss of fibrinogen and factor XIII [8], which has high molecular weight. Since these molecule's turnover is relatively long, the effect of their removal of the coagulation factors will be remarkable according to the number of DFPP sessions [8], resulting in increase of international normalized ratio and activated partial thromboplastin time, and potentially hemorrhagic complications [9, 10]. The third modality, immunoadsorption, has unfortunately not yet been approved by the health insurance system in Japan. DFPP is designed to remove particles with sizes that range between the two different pore sizes of plasma membranes. Herein, we describe a new combination of plasma-separating membranes for DFPP (novel DFPP, nDFPP), designed to improve the particle-removal selectivity profile of DFPP, and we assessed the removal patterns of major plasma proteins.

Subjects and methods

General procedure for DFPP

The general procedure for DFPP is shown in Fig. 1. An arteriovenous fistula (AVF) or double-lumen catheter placed in the internal jugular vein was used as a vascular access. A blood pump was run at 100–120 mL/min and plasma was separated through the first membrane (plasma separator) at 30% of the speed of the blood



	Plasma separator	Pore sizes (mm)	Sieving coefficient (IgG : Fib)	Plasma fractionator	Pore sizes (mm)	Sieving coefficient (Albumin : IgG)
cDFPP	Plasmaflo® OP-08W	0.30	0.88 : 0.85	Cascadeflo® EC-20W	0.01	0.62 : 0.15
nDFPP	Cascadeflo® EC-50W	0.03	0.80 : 0.30			

Fig. 1 Protocol for DFPP. As the plasma separator (primary membrane), Plasmaflo OP-08W was used in cDFPP and Cascadeflo EC-50W was used in nDFPP. Large molecules were removed from plasma using a secondary plasma fractionator (secondary membrane), Cascadeflo EC-20W, in both DFPP modalities. Albumin solution was used as replacement fluid and was infused at the same rate as the drain pump

pump. Plasmaflo® OP-08W plasma separators (Asahikasei Medical, Japan) were used as the first membrane in cDFPP, while Cascadeflo® EC-50W fractionators (Asahikasei Medical, Japan) were used as the first membrane in nDFPP. Sieving coefficients for the first membrane for IgG and fibrinogen were 0.88 and 0.85, respectively, with OP-08W membranes (cDFPP), and 0.80 and 0.30, respectively, with EC-50W membranes (nDFPP). Large molecules that did not pass through pores of these sizes were removed using a second membrane (plasma fractionator) with an ethylene vinyl alcohol copolymer membrane (Cascadeflo EC-20W®, Asahikasei medical, Japan). The Alb solution was infused at the same rate as the drain pump. The patients were treated with 30 mg/h of nafamostat mesylate during the session. There were no patients treated with daily oral anticoagulant.

Patient selection and study design

From 2015 to 2018, consecutive ABO-incompatible living-donor kidney transplant recipients were enrolled. Patients who did not undergo apheresis were excluded. This study was approved by the Hokkaido University Hospital institutional review board (No. 014-0266), and written informed consent was obtained from each patient. The study protocol adhered to the statutes of the Declaration of Helsinki. This trial was registered at the UMIN Clinical Trials Registry as UMIN000017605 [https://upload.umin.ac.jp/cgi-open-bin/ctr/ctr_view.cgi?recptno=R000020404].

The general desensitization protocol used for ABO-incompatible kidney transplantation is shown in Fig. 2. Patients were treated with rituximab (200 mg) 2 weeks before transplantation, followed by 1–4 sessions of apheresis (initiated with nDFPP or cDFPP, followed by cDFPP, and lastly TPE on the day before transplantation) and intravenous immunoglobulin (100 mg/kg body weight for 3 consecutive days beginning the day before kidney transplantation). Recipients were administered basiliximab as induction therapy and triple immunosuppressants (tacrolimus, mycophenolate mofetil, and methylprednisolone) as maintenance therapy.

The purpose of the present study was to assess the particle removal profile of nDFPP compared with cDFPP. To assess the removal properties of major proteins under the same condition, patients underwent nDFPP as the first session in their desensitization therapy regimen. They then underwent cDFPP and finally TPE to achieve acceptable antibody titers and to replenish lost coagulation factors including fibrinogen before transplantation. No patients in either group were required to forgo KTx because of inadequately high antibody titers (>1:16), or required exogenous infusion of fibrinogen. We collected blood samples immediately before and after initial

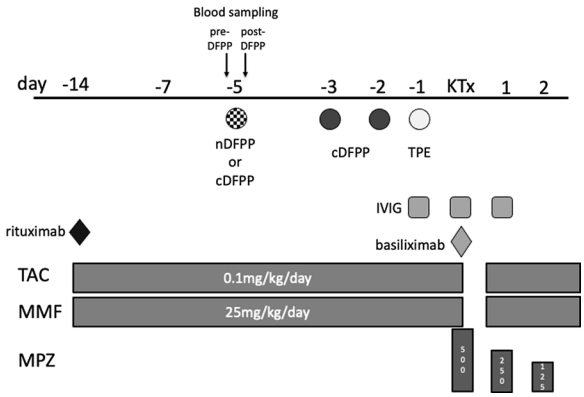


Fig. 2 Desensitization protocol for ABO-incompatible kidney transplantation. Patients were treated with rituximab (200 mg) 2 weeks before transplantation, followed by one to four sessions of apheresis (initiated with nDFPP or cDFPP, followed by cDFPP, and lastly TPE on the day before transplantation) and intravenous immunoglobulin (100 mg/kg body weight for 3 consecutive days from the day before transplantation). The total number of sessions of plasmapheresis was determined according to the titers of anti-blood group antibodies to achieve acceptable titers before transplantation. Recipients were administered basiliximab as induction therapy and maintenance therapy with triple immunosuppressants (tacrolimus, mycophenolate mofetil, and methylprednisolone). Blood sampling for analysis was performed immediately before and after the first session of cDFPP or nDFPP to avoid the cumulative effect of serial sessions of DFPP. cDFPP: conventional double-filtration plasmapheresis, IVIG: intravenous immunoglobulin, KTx: kidney transplantation, nDFPP: novel double-filtration plasmapheresis, MMF: mycophenolate mofetil, MPZ: methylprednisolone, TAC: tacrolimus, TPE: therapeutic plasma exchange

session of cDFPP or nDFPP of each patient (Fig. 2). We analyzed data pertaining to the first session of serial DFPP in each patient even if the patient underwent multiple sessions of DFPP (to avoid the cumulative effects of serial DFPP sessions).

As a historical control, another group of patients who underwent cDFPP as the first session before ABO-incompatible kidney transplantation between 2003 and 2013 was identified. Their clinical data were extracted for analyses. For the setting of the sample size in the current study, we assumed RR of IgG with nDFPP is the same as that with cDFPP and fibrinogen with nDFPP is more preserved than that with cDFPP. With two-sided significance level of 5% and sample size ratio of 2:1 (cDFPP: nDFPP), sample sizes of 22 and 11 are required to achieve the power of 80% using two-sample t-test if the effect size of at least 1.1 was assumed. With dropout rate of 5–10%, we set the target sample size as 23 and 12, respectively.

Since a formula to determine the optimal concentration and volume of Alb replacement fluid (Rf) for nDFPP has not been established, the formula established for cDFPP was applied for nDFPP. Briefly, in a previous study, the

formula for the volume of albumin Rf was determined based on volume-RR curves of IgG obtained from data on patients who underwent cDFPP [11]. Regression analysis between the volume of Alb Rf per kg body weight (Rf/BW) and RR-IgG was performed. Another formula for the Alb concentration of Rf was established to maintain plasma volume within the range of pre-DFPP plasma volume ± 10%, calculated according to post- to pre-DFPP hematocrit ratios to avoid hypotensive events. Consequently, the volume and concentration of Alb Rf (Alb_{Rf}) were determined according to the following two formulae using the targeted RR-IgG as follows [11]:

$$\begin{aligned} \text{Rf (ml)} &= \text{body weight}\,(\text{kg}) \times e^{X}, \quad \text{where} \\ X &= (\text{RR} - \text{IgG}(\%) + 2.427) \div 22.928 \\ \text{Alb}_{\text{Rf}} &= (2.982 - 2.36 \times \text{RR} - \text{IgG}) \times \text{Alb}_{\text{pre}} \\ &\quad + (2.36 \times \text{RR} - \text{IgG} - 0.236) \times \text{TP}_{\text{pre}} \end{aligned}$$

To compare the two methods of apheresis under the same conditions, we set the targeted RR-IgG as 70% in the nDFPP group and selected the sessions where targeted RR-IgG was 70% as the cDFPP group. As the primary endpoint, RR-IgG, RR-IgM, and RR-fibrinogen were determined under each DFPP modality and compared statistically. RR was corrected for hematocrit (Hct) using a formula (C corresponds concentration in the sera),

$$\text{RR} = \left[1 - \left\{\text{Hct}_{\text{pre}}\left(1 - \text{Hct}_{\text{post}}/100\right)\text{C}_{\text{post}}\right\} / \left\{\text{Hct}_{\text{post}}\left(1 - \text{Hct}_{\text{pre}}/100\right)\text{C}_{\text{pre}}\right\}\right] \times 100$$

The drops in titer of anti-blood group IgG and IgM antibodies were also compared between the groups. For calculations, fibrinogen concentrations below the measurement limit (< 50 mg/dL) were assumed to be 50 mg/dL. Next, the selectivity of removal of major proteins such as IgG, IgM, and fibrinogen was evaluated. As the secondary endpoint, the frequency of adverse events during and immediately after a single session of DFPP was evaluated and compared between the groups. We measured the time of manual compression until hemostasis of the AVF was achieved. Prolongation of hemostasis (over 20 min) of the cannulation sites of AVF in patients who had an AVF for hemodialysis at the time of nDFPP session was also analyzed.

Statistical analysis

Patient characteristics between the cDFPP and nDFPP groups were analyzed using a chi-squared test (for differences in proportions) and Wilcoxon rank-sum test (for differences in median values). Statistical analyses were performed using JMP pro12 (SAS Institute Inc., Cary, NC, USA).

Results

Removal rates of IgG, IgM, and fibrinogen in the cDFPP and nDFPP groups

Twelve ABO-incompatible living kidney transplant recipients who underwent nDFPP were enrolled in the study. Furthermore, 23 historical control patients who underwent cDFPP and met the inclusion criteria were identified and analyzed. The demographic data of patients are shown in Table 1. Age at transplantation was significantly higher in the nDFPP group. Body weight, a factor in determining the dose of Alb Rf, was comparable between the groups. Other determinants of the dose of Alb Rf, pre-DFPP total protein (TP) and Alb were also comparable between the groups. Pre-DFPP IgG was comparable between the groups. In contrast, pre-DFPP fibrinogen was significantly higher in the nDFPP group than in the cDFPP group (median: 381 mg/dL in nDFPP and 224 mg/dL in cDFPP, $p = 0.001$). Pre-DFPP anti-blood group IgM antibody titer was significantly lower in the nDFPP group than in the cDFPP group (median: 1:12 in nDFPP and 1:32 in cDFPP, $p = 0.001$). The median amount of albumin was comparable between cDFPP (median 169 g, range 100–313 g) and nDFPP (median 169 g, range 125–200 g, $p = 0.4861$, data not shown). The concentrations of albumin solution were also statistically similar in both group, 14.7% (10.0–17.1%) in cDFPP and 13.8% (13.0–14.9%) in nDFPP ($p = 0.2308$, data not shown).

We compared RR-IgG and RR-IgM between the nDFPP and cDFPP groups. nDFPP was inferior to cDFPP in terms of the RR of both total IgG and IgM (Fig. 3A) and in the drop of anti-blood group IgG and IgM antibody titers (Fig. 3B). Similarly, nDFPP decreased anti-blood group IgM and IgG antibody titers by 1 titer step each (0–2 steps in IgM and 1–2 steps in IgG). Both were significantly lower than in cDFPP (IgM: median 2 steps, range 1–3 steps, and IgG: median 2 steps, range 1–3 steps). We next analyzed the RR of IgG and fibrinogen to determine the selectivity of removal of major serum proteins. The RR-IgG and RR-fibrinogen were comparable in the cDFPP group (median: RR-IgG, 70.7% and RR-fibrinogen, 76.6%, $p = 0.6121$, Fig. 3C), indicating that cDFPP has almost no selectivity in removing serum proteins. In contrast, in the nDFPP group, RR-fibrinogen was significantly lower than RR-IgG (median: RR-fibrinogen, 33.2% and RR-IgG, %, $p < 0.00001$, Fig. 3C), indicating that nDFPP is more selective for removing IgG while preserving fibrinogen. These results indicate that nDFPP is inferior to cDFPP in terms of the RR-IgG and RR-IgM, using

Table 1 Patient characteristics

			cDFPP ($n=23$)	nDFPP ($n=12$)	*p* value
Original Disease		IgAN	5	4	.676[#]
		CGN	6	0	
		ADPKD	5	4	
		DM	2	1	
		Unknown or Others	5	3	
Duration of pre-transplant dialysis (years)			0.5 (0.0–14.1)	1.2 (0.0–14.2)	.856[$]
Sex (male:female)			15:8	3:7	.469[#]
Age at transplant (years)			51 (14–68)	61 (26–67)	.022[$]
Height (cm)			161.5 (150.1–180.0)	160.0 (148.0–179.5)	.790[$]
Weight (kg)			59.15 (37.7–78.0)	57.53 (43.75–73.90)	.348[$]
Body mass index			21.2 (16.0–27.7)	23.3 (16.7–26.7)	.099[$]
Pre-DFPP data	Hematocrit (%)		34.2 (23.3–47.8)	31.3 (23.3–36.9)	.215[$]
	Total protein (mg/dL)		6.4 (4.9–7.1)	6.2 (5.5–6.6)	.278[$]
	Albumin (mg/dL)		3.6 (3.0–4.3)	3.5 (3.3–3.8)	.517[$]
	Total IgG (mg/dL)		905 (351–1884)	1011 (585–1463)	.948[$]
	Platelet count (/μL)		17.8 (11.4–37.3)	18.6 (11.6–28.4)	.824[$]
	PT-INR		0.98 (0.82–1.20)	1.02 (0.94–1.30)	.179[$]
	APTT (s)		28.1 (23.7–39.6)	29.3 (26.0–42.6)	.840[$]
	Total IgM (mg/dL)		83 (21–220)	54 (21–162)	.122[$]
	Fibrinogen (mg/dL)		224 (129–304)	381 (222–471)	.001[$]
	Anti-ABO IgG antibody titer		1:16 (1:4–1:512)	1:32 (1:8–1:1024)	.493[$]
	Anti-ABO IgM antibody titer		1:32 (1:8–1:128)	1:12 (1:4–1:16)	.001[$]

ADPKD Autosomal-dominant polycystic kidney disease, *APTT* activated partial thromboplastin time, *CGN* chronic glomerulonephritis, *DM* diabetes mellitus, *IgAN* IgA nephropathy, *PT-INR* international normalized ratio of prothrombin time. #: chi-squared test, $: Wilcoxon rank-sum test

the same concentration and volume of Alb Rf. However, nDFPP has improved selectivity at protein removal resulting in higher preservation of fibrinogen per session compared with cDFPP. IgM was barely removed in the nDFPP group (median: RR-IgM, 15.8%) while IgM removal was comparable to that of IgG in the cDFPP group (median: RR-IgM, 84.7%, $p<0.0001$, Fig. 3C).

In additional study to measure the lost fibrinogen in the wasted fluid in limited cases the data available ($n=8$), the median concentration and total amount of fibrinogen were 123 mg/dL (50–234 mg/dL) and 1555 mg (680–2668 mg), but not zero, respectively.

nDFPP is superior to cDFPP in preservation of total protein during treatment

When the changes in pre- and post-DFPP Alb values were compared for each DFPP session, Alb was significantly increased in both the cDFPP (median: 3.6 mg/dL and 5.3 mg/dL, $p=0.0039$, Fig. 4A) and nDFPP groups (median: 3.6 mg/dL and 5.0 mg/dL, $p=0.0010$, Fig. 4A), and there were no significant differences in the ratio of post- to pre-DFPP Alb (median: 141.8% in cDFPP and 134.3% in nDFPP, $p=0.6137$, Fig. 4B). When the changes in pre- and post-DFPP TP values were compared for each DFPP session, TP was significantly decreased in the cDFPP group (median: 6.4 mg/dL and 5.8 mg/dL, $p=0.0005$, Fig. 4C). In contrast, median TP was similar between pre- and post-DFPP in the nDFPP group (6.2 mg/dL and 6.3 mg/dL, $p=0.5313$, Fig. 4C). When the post- to pre-DFPP ratio for TP was compared between the groups, the ratio was significantly higher in the nDFPP group than in the cDFPP group (103.2% vs. 88.1%, $p=0.0015$, Fig. 4D). These results indicate that nDFPP can preserve higher amounts of serum protein than cDFPP under the same dose of Alb Rf. The data also suggest that nDFPP can preserve large molecules such as fibrinogen and IgM, while changes in Alb were comparable, resulting in higher serum TP after nDFPP, than after cDFPP.

nDFPP has lower incidence of hemostatic prolongation compared with cDFPP

The frequency of adverse events during and immediately after each DFPP session in the two groups is shown in Table 2. When the frequency of adverse events was evaluated, no occurrence of hypotension was observed during or immediately after any sessions of DFPP. Regarding hemostasis-related adverse events, 18 patients in the

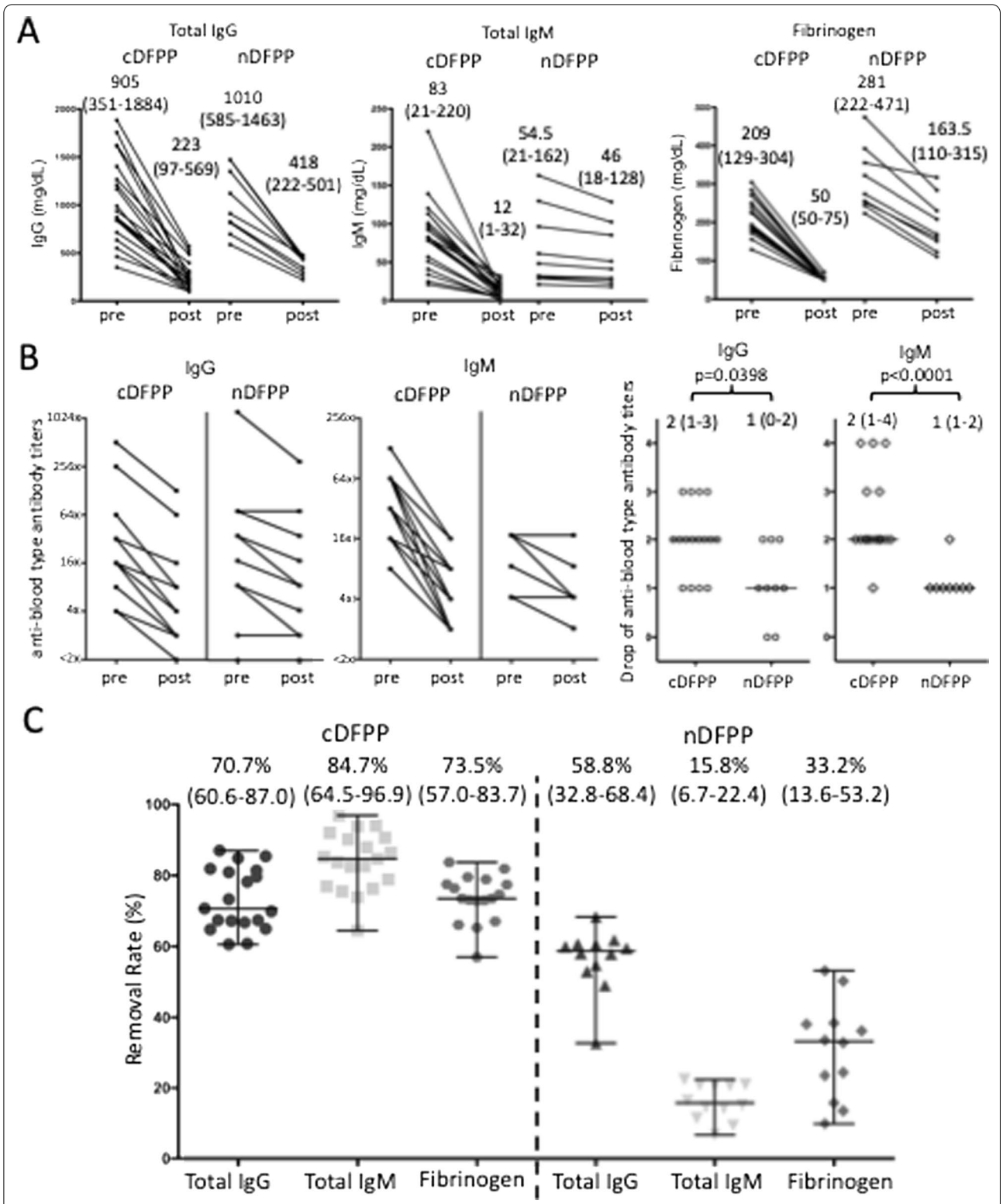


Fig. 3 nDFPP is inferior to cDFPP in removing IgG and IgM but can preserve more fibrinogen than cDFPP. **A** Changes in IgG, IgM, and fibrinogen in the first session in each group. **B** The changes of anti-blood group antibody titers pre- and post-initial DFPP (left two panels) and the drop in anti-blood group antibody titers (right panel) in each group. The drops in anti-blood group antibody titers in each first DFPP session were compared between the cDFPP and nDFPP groups. **C** Removal rates of IgG, IgM, and fibrinogen in the cDFPP and nDFPP groups demonstrating the improved removal selectivity of nDFPP compared with cDFPP. Blood sampling for analysis was performed immediately before and after the first session of cDFPP or nDFPP

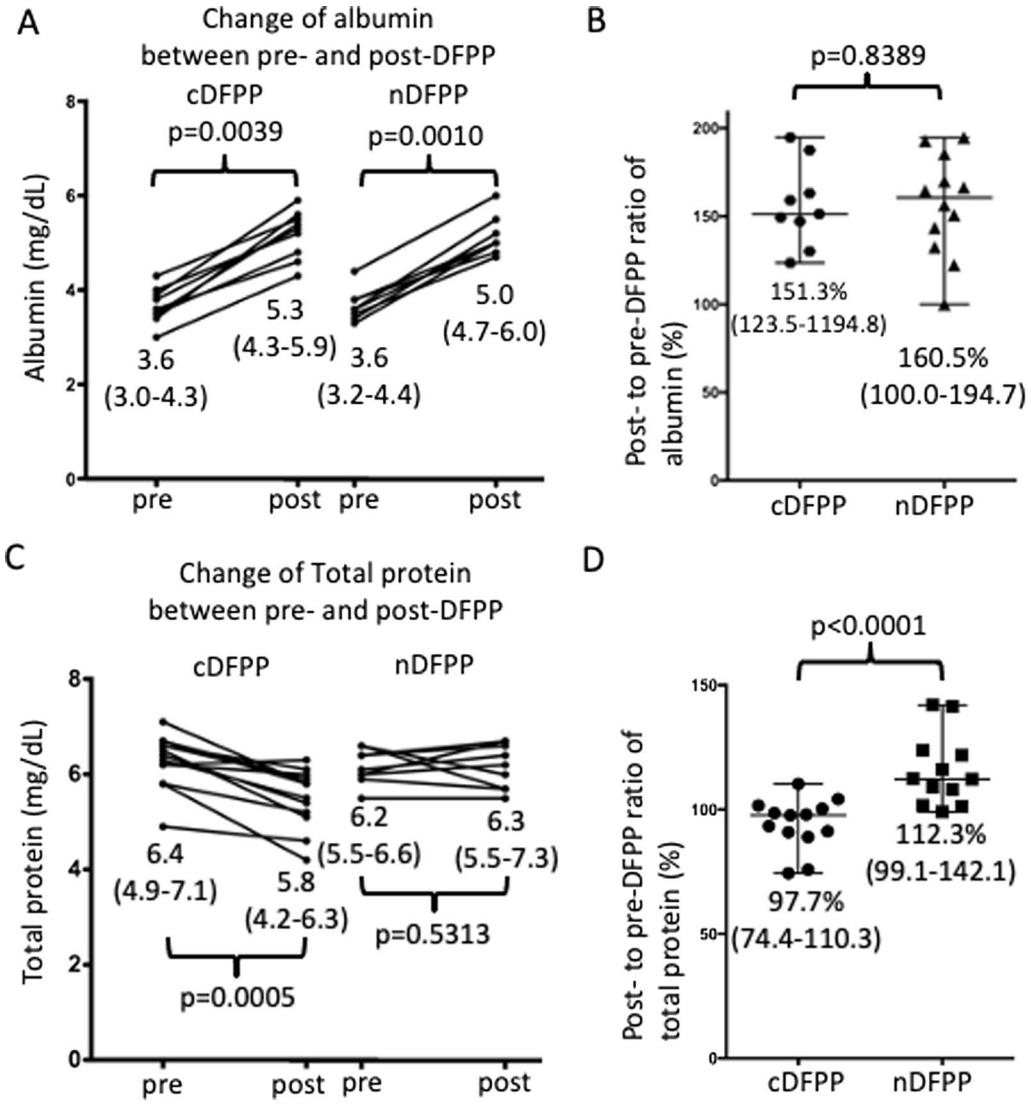


Fig. 4 nDFPP is superior to cDFPP in preserving total serum protein when using the same concentration and volume of albumin replacement fluid. Change before and after initial DFPP session (**A**) and post- to pre-DFPP ratio (**B**) of serum albumin and those of total protein (**C** and **D**) in each DFPP group. Blood sampling for analysis was performed immediately before and after the first session of cDFPP or nDFPP

cDFPP group and nine in the nDFPP group had AVF. Among these patients, prolonged hemostasis (over 20 min) of cannulation sites of AVF occurred in all 18 cases with an AVF (100%) in the cDFPP group, while there was only 1/9 case in the nDFPP, group (11.1%), in which it was significantly lower ($p < 0.0001$). These results suggest that nDFPP is better at preserving fibrinogen compared with cDFPP, resulting in improved hemostasis. Moreover, it may be safer to perform nDFPP, than cDFPP during the perioperative period (i.e., pre- and post-KTx).

Table 2 Adverse events with each DFPP modality

	cDFPP ($n = 23$)	nDFPP ($n = 12$)	*p* value
Hypotension	0/23	0/12	n.s.[#]
Nausea and /or vomit	0/23	1/12 (8.3%)	n.s.[#]
Hemostasis prolongation of AVF after DFPP session	18/18 (100%)[$]	1/9 (11.1%)[$]	< 0.0001[#]

[#] : chi-squared test, $: Hemostasis prolongation was defined as hemostasis of the AVF over 20 min in patients who had an AVF for hemodialysis, and the incidence was calculated in those who had an AVF for hemodialysis at the time of the DFPP session

AVF Arteriovenous fistula, *cDFPP* conventional double-filtration plasmapheresis, *nDFPP* novel DFPP

Discussion

DFPP is effective in various diseases where autoreactive or alloreactive IgG is responsible for development and progression of the diseases to achieve complete remission, reduce the treatment period and reduce the total amount of steroid [12–14], as well as in the treatment of antibody-mediated rejection after kidney transplantation [10]. However, as mentioned above, massive loss of coagulation factors especially fibrinogen resulting in bleeding risk perioperative period is still unsolved. To overcome the problem, Gong et al. also reported the effect of our similarly modified combination of membranes of DFPP in ANCA-associated vasculitides to remove anti-ANCA IgG more selectively while preserving other major serum proteins [15]. Our report was the first study to investigate the effect of nDFPP in ABO-incompatible kidney transplant recipients compared to conventional DFPP in terms of IgG clearance and preservation of fibrinogen.

Based on our results, the RR of IgG in nDFPP were lower than those of cDFPP using the same volume of Rf, suggesting that the optimal volume of Rf for nDFPP may be larger than that calculated according to the formula for cDFPP. In contrast, nDFPP demonstrated improved preservation of large molecules such as fibrinogen and IgM after one session of nDFPP, resulting in improved preservation of total serum protein and colloid osmotic pressure and improved hemostasis. Serum proteins, notably Alb, have critical roles in maintaining colloid osmotic pressure, and extreme reductions in their levels can result in hypotensive events. Our results suggest that nDFPP is advantageous for preventing hypotensive events as it was superior at preserving both TP and serum Alb compared with cDFPP. TP was increased following one session of nDFPP while it was significantly decreased following one session of cDFPP (Fig. 4C and 4D). The concentration of Alb may be lower than that calculated according to the formula we established previously for cDFPP [11]. These results indicate that the concentration of albumin solution can be reduced to achieve the same colloid osmotic pressure resulting in reduced consumption of albumin. Additional studies are necessary to establish the optimal dose of Alb Rf specific for nDFPP to achieve more effective removal of IgG and to minimize consumption of Alb solution.

nDFPP showed low RR in IgM antibody titers in our study. nDFPP may not be suitable for such patients who has high anti-blood type IgM antibody titer. Such patients with high IgM isoagglutinin titer may need combination therapy with TPE and/or cDFPP. Hanaoka et al. reported the effectiveness of selective plasma exchange in desensitization therapy for ABO-incompatible kidney transplantation. They showed acceptable reduction of isoagglutinin IgG by multiple sessions of selective plasma exchange; however, very low reduction of IgM indicating other apheresis therapies such as DFPP or TPE is necessary to reduce isoagglutinin IgM titers to the acceptable range [16]. On the contrary, such patients with low IgM isoagglutinins can avoid unnecessary sessions of DFPP. Actually, our patients had lower anti-blood type IgM antibody titers (median, 1:16, 1:4–1:128) compared to that of IgG titers (1:32, 1:2–1:1028). In other words, more than 60% of our patients don't need to eliminate anti-blood type IgM antibodies in our desensitization protocol.

There are recently major modalities available such as TPE, DFPP, immunoadsorption (IA), as well as selective PE. These apheresis treatments have each advantages and disadvantages as summarized in Table 3. It is important to know which apheresis technique is effective and safe for the ABO-i transplant recipient; however, there are few randomized controlled studies comparing apheresis modalities. In addition, it has not been determined whether the elimination of complements has additional benefits on prevention and/or treatment of AMR in ABO-i kidney transplant recipients. In the systematic review involving 83 studies and 4810 ABOi KTx recipients, a total of 3041 (63.2%) patients underwent apheresis (plasma exchange and plasmapheresis) and 1294 (26.9%) received immunoadsorption as part of pre-transplant desensitization therapy [17]; however, they didn't discuss the isoagglutinin IgM titer presumably because of lacking in reliable data in association with incidence of antibody-mediated rejection. Thus, in the clinical setting, the selection of apheresis modalities is discussed mainly based on their influence on the coagulation system and their eliminating efficacy of IgG and IgM.

Agishi et al. demonstrated a comparative study between DFPP and IA, Biosynsorb, which employed N-Acetyl-D-Galactosamine and D-Galactosamine as ligands for A and B antigens, respectively. In the study, they demonstrated that DFPP removed both IgG and IgM ABO antibodies more effectively than IA [18].

It is also important that DFPP should be avoided in the peri-operative phase because DFPP eliminates coagulation factors together with antibodies [19]. Accordingly, American Society for Apheresis (ASFA) recommends using either TPE or IA for desensitization protocols in ABO-i kidney transplantation [20]. Although IA, especially Glycosorb-IA, is most antigen-specific, unfortunately, IA hasn't been approved in Japan, yet. Selective PE is a newly developed apheresis technique which was approved in 2015 in Japan and is advantageous to preserve fibrinogen. However, selective PE is apparently inferior to TPE and DFPP in terms of elimination of IgM antibody.

Table 3 Advantages and disadvantages of various methods of antibody removal (modified from reference [23])

	Supplement fluid	Advantages	Disadvantages
TPE	FFP or crystalloid with albumin solution	Simple	Nonselective elimination of other plasma components
		Readily available	Application of FFP is necessary with risk of subsequent infections or anaphylactic reactions
		Simultaneous elimination of ABO Abs and HLA Abs, immune-complexes, and unknown circulating factors	
		Relatively low costs	
DFPP	Crystalloid with albumin solution	Selective (only elimination of high molecular weight plasma fractions)	Elimination of other plasma components
		Simultaneous elimination of ABO Abs and HLA Abs, immune-complexes, and unknown circulating factors	Low biocompatibility of the cascade filter Evaflux 2A (Kuraray, Kurashiki, Japan)
		No application of FFP needed	
Glycosorb-IA (antigen-specific)	Unnecessary	Most selective	No elimination of HLA Abs
		Simple to use technique	In blood type O recipients and AB donors, AB two different columns are needed
		Elimination of both IgG and IgM antibodies for ABO antigens	Short shelf life
		No replacement fluid is needed	High costs
Immunosorba-IA (nonantigen-specific)	Unnecessary	Selective but less than Glycosorb	Elimination of all Ig fractions
		Simultaneous elimination of ABO Abs and HLA Abs	Different affinity for various Ig-subtypes (insufficient removal of IgG3 and IgM)
		No replacement fluid is needed	Rarely anaphylactic reactions triggered by staphylococcus-protein A
			High costs
Ig Therasorb-IA (nonantigen-specific)	Unnecessary	Selective but less than Glycosorb	Removal of all Ig-fractions
		Simultaneous elimination of ABO Abs and HLA Abs	High costs, but less costs with double-columns system
		Elimination of all Ig-fractions	
		36 months shelf live	
		High removal capacity	
		No replacement fluid is needed	
Selective PE	Crystalloid with albumin solution	Simple	Almost no Elimination of IgM-fraction
		Readily available	Elimination of IgG-fractions less than TPE and DFPP
		Elimination of IgG-fractions	
		Fibrinogen mostly preserved	
		No application of FFP needed	

ABO Abs ABO antibodies, *DFPP* double-filtration plasmapheresis, *FFP* fresh frozen plasma, *HLA Abs* human leukocyte antigen antibodies, *IA* immunoadsorption, *Ig* immunoglobulin, *TPE* therapeutic plasma exchange

The present study showed that more fibrinogen was removed than expected in nDFPP, in which the first membrane has as low sieving coefficient (0.30). Indeed, the wasted fluid that was separated by the second membrane (see Fig. 1) in nDFPP contained substantial amounts of fibrinogen, implying that fibrinogen can pass through the pores of the first membrane but not those of the second membrane, leading to loss of fibrinogen. Because fibrinogen has an elastic chain-like structure [21], it may be difficult to control the filtration of fibrinogen compared with other molecules. Otherwise, fibrinogen may be also trapped by membrane fouling as reported by Ohkubo et al. in the selective plasma exchange using Evacure® EC-4A [22], leading to the loss of fibrinogen more than expected.

When the costs were compared between the modalities, for cDFPP, the membranes utilized in the two DFPPs, Plasmaflo OP-08W for cDFPP costs 25,600 yen

corresponding to 239US$, and Cascadeflo EC-20W for nDFPP costs 26,600 yen corresponding to 248US$, respectively. Plasmaflo OP-08W for both DFPP costs 25,600 yen, which corresponds to 239US$. Thus, the costs were very similar between the two modalities.

The major limitation of this study was that it was not a prospective randomized study but designed to evaluate the molecular removal properties of a single session of DFPP, not to evaluate clinical outcomes such as the incidence of antibody-mediated rejection rate, graft function and graft survival. It remains necessary to determine if nDFPP alone can achieve desirable reduction in IgG antibody titers while preserving fibrinogen without supplementation of FFP to recover coagulation activity, and achieve an acceptable clinical outcome such as through evaluation of the incidence of antibody-mediated rejection caused by anti-blood group antibodies.

In conclusion, our altered combination of plasma-separating membranes showed higher selectivity of removal of molecules resulting in elimination of IgG antibodies and improved preservation of fibrinogen. This modality is also applicable for diseases where IgG subclass autoantibodies are associated with disease development and progression (i.e., demyelinating disease and collagen diseases) because of its higher selectivity to remove IgG antibodies, while preserving fibrinogen compared with cDFPP.

Abbreviations

ABOi KTx: ABO-incompatible kidney transplantation; Alb: Albumin; ASFA: American society for apheresis; AVF: Arteriovenous fistula; DFPP: Double-filtration plasmapheresis; FFP: Fresh frozen plasma; IA: Immunoadsorption; IgG: Immunoglobulin-G; IVIG: Intravenous immunoglobulin; MMF: Mycophenolate mofetil; MPZ: Methylprednisolone; Rf: Replacement fluid; RR: Removal rate; TAC : Tacrolimus; TP: Total protein; TPE: Therapeutic plasma exchange.

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Authors' contributions

DI, TM, KH, MO, YC, HS, TH, HH, YT, NI, SM, NS, DI, KH, HS, TH, HH, YT, NI and NS participated in study design and writing of the manuscript; SM participated in study design; DI, TM, KH, HS, TH, HH, YT, NI, YC and MO participated in research; KH, MO and NS provided advice on the study. All authors contributed substantially to interpretation of the results and writing of the manuscript. All authors have read and approved the final manuscript.

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Availability of data and materials

Please contact corresponding author for data requests.

Declarations

Ethics approval and consent to participate

This study was approved by the Hokkaido University Hospital institutional review board (No. 014-0266) and written informed consent was obtained from each patient. The study protocol adhered to the statutes of the Declaration of Helsinki. This trial was registered at the University hospital Medical Information Network Clinical Trials Registry (UMIN-CTR) as UMIN000017605 [https://upload.umin.ac.jp/cgi-open-bin/ctr/ctr_view.cgi?recptno=R000020404].

Consent for publications

Informed consent for publication was obtained from each participant of the study.

Competing interests

Kota Ono is an employee of AbbVie GK. However, being part of the company has not influenced the results and discussion in this paper. The other authors declare that they have no competing interests (both financial and non-financial) related to this manuscript.

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References

1. Higgins RM, Daga S, Mitchell DA. Antibody-incompatible kidney transplantation in 2015 and beyond. Nephrol Dial Transplant. 2015;30(12):1972–8.
2. Takahashi K, Saito K, Takahara S, et al. Excellent long-term outcome of ABO-incompatible living donor kidney transplantation in Japan. Am J Transplant. 2004;4(7):1089–96.
3. Lawrence C, Galliford JW, Willicombe MK, et al. Antibody removal before ABO-incompatible renal transplantation: how much plasma exchange is therapeutic? Transplantation. 2011;92(10):1129–33.
4. Mokrzycki MH, Kaplan AA. Therapeutic plasma exchange: complications and management. Am J Kidney Dis. 1994;23(6):817–27.
5. Basic-Jukic N, Kes P, Glavas-Boras S, Brunetta B, Bubic-Filipi L, Puretic Z. Complications of therapeutic plasma exchange: experience with 4857 treatments. Ther Apher Dial. 2005;9(5):391–5.
6. Eguchi M, Okumura K, Torigoe K, Toyoda M, Uekihara S, Yamanaga S. Chronic renal failure as a possible risk factor for allergic reaction in therapeutic plasma exchange using fresh frozen plasma. Ther Apher Dial. 2019;23(3):261–5.
7. Agishi T, Kaneko I, Hasuo Y, et al. Double filtration plasmapheresis. Trans Am Soc Artif Intern Organs. 1980;26:406–11.
8. Jouve T, Marlu R, Malvezzi P, et al. Reducing fibrinogen and factor xiii using double-filtration plasmapheresis for antibody-mediated rejection: predictive models. Blood Purif. 2018;46(3):239–45.
9. Vatazin AV, Zulkarnaev AB. The impact of therapeutic plasma exchange and double filtration plasmapheresis on hemostasis in renal transplant recipients. Ter Arkh. 2018;90(6):22–7.
10. Marlu R, Malvezzi P, Seyve L, et al. Effect of double-filtration plasmapheresis for antibody-mediated rejection on hemostasis parameters and thrombin generation. Thromb Res. 2018;166:113–21.
11. Iwami D, Miura M, Chiba Y, et al. Optimal settings for double filtration plasmapheresis with targeted removal rate of preexisting antibody in antibody-incompatible kidney transplant. Transplant Proc. 2018;50(10):3478–82.
12. Higashihara T, Kawase M, Kobayashi M, et al. Evaluating the efficacy of double-filtration plasmapheresis in treating five patients with drug-resistant pemphigus. Ther Apher Dial. 2017;21(3):243–7.
13. Li M, Wang Y, Qiu Q, et al. Therapeutic effect of double-filtration plasmapheresis combined with methylprednisolone to treat diffuse proliferative lupus nephritis. J Clin Apher. 2016;31(4):375–80.

14. Tsai MK, Wu MS, Yang CY, et al. B cells and immunoglobulin in ABO-incompatible renal transplant patients receiving rituximab and double filtration plasmapheresis. J Formos Med Assoc. 2015;114(4):353–8.
15. Gong D, Ji D, Xu B, Liu Z. More selective removal of myeloperoxidase-anti-neutrophil cytoplasmic antibody from the circulation of patients with vasculitides using a novel double-filtration plasmapheresis therapy. Ther Apher Dial. 2013;17(1):93–8.
16. Hanaoka A, Naganuma T, Kabata D, et al. Selective plasma exchange in ABO-incompatible kidney transplantation: comparison of substitution with albumin and partial substitution with fresh frozen plasma. Sci Rep. 2020;10(1):1434.
17. Lo P, Sharma A, Craig JC, et al. Preconditioning therapy in ABO-incompatible living kidney transplantation: a systematic review and meta-analysis. Transplantation. 2016;100(4):933–42.
18. Agishi T, Takahashi K, Ota K, Yagisawa T. Comparative evaluation of immunoadsorption and double filtration plasmapheresis for removal of anti-A or -B antibody in ABO-incompatible kidney transplantation. Japanese Biosynsorb Research Group. Transplant Proc. 1992;24(2):557–8.
19. Hanafusa N, Hamasaki Y, Kawarasaki H, et al. The effect of different apheresis modalities on coagulation factor XIII level during antibody removal in ABO-blood type incompatible living related renal transplantation. Transfus Apher Sci. 2013;49(2):254–8.
20. Schwartz J, Padmanabhan A, Aqui N, et al. Guidelines on the use of therapeutic apheresis in clinical practice-evidence-based approach from the writing committee of the American Society for Apheresis: the seventh special issue. J Clin Apher. 2016;31(3):149–62.
21. Hall CE, Slayter HS. The fibrinogen molecule: its size, shape, and mode of polymerization. J Biophys Biochem Cytol. 1959;5(1):11–6.
22. Ohkubo A, Okado T, Miyamoto S, et al. Fibrinogen reduction during selective plasma exchange due to membrane fouling. Ther Apher Dial. 2017;21(3):232–7.
23. Iwami D, Shinohara N. Chapter 8; anti-ABO antibody elimination pre- and post-ABO-incompatible kidney transplantation. In: Wang Y, editor. ABO-incompatible organ transplantation. Springer; 2019. p. 117–29.

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