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Prognostic value of C3d-fixing, preformed donor-specific antibodies in crossmatch-positive living kidney transplantation

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ABSTRACT

The occurrence of acute antibody-mediated rejection (ABMR) is higher in flow cytometric crossmatch (FCXM)-positive patients despite desensitization. Accumulating evidence suggests a correlation between the complement-binding ability of donor-specific antibodies (DSAs) and the risk of ABMR. Here, we investigated the correlation between complement C3d-fixing ability of preformed DSA and ABMR risk, the efficacy of a desensitization protocol for patients with C3d-fixing DSA, and the risk of ABMR in 21 DSA- and FCXM-positive patients. We retrospectively analyzed the C3d-fixing ability and mean fluorescence intensity (MFI) of preformed DSA before and after desensitization. Six patients had non-C3d-fixing DSA and 15 had C3d-fixing DSA. The presence of C3d-fixing DSA before desensitization was correlated with the incidence of acute ABMR within 1 year after transplantation ($p = .04$) and chronic ABMR ($p = .03$). Moreover, the MFI of preformed DSA differed between responder and non-responder C3d-fixing DSA after desensitization ($p < .0001$). The C3d-fixing ability of preformed DSA with low MFI disappeared after desensitization. These results indicate that measuring DSA C3d-fixing ability may identify patients with a high risk of ABMR, especially before desensitization.
Clinical trial notation: UMIN Clinical Trials Registry (UMIN-CTR) number: UMIN000033449.

1. Introduction

Kidney transplantation is an important treatment for end-stage renal disease and confers a significant survival advantage over dialysis therapy. However, a serious shortage of organs from deceased donors necessitates living kidney transplantation (LKT) from living donors. Due to donor shortage, flow cytometric crossmatch (FCXM)-positive kidneys from living donors are transplanted despite immunological risks, which increases the survival of recipients compared to that of patients who wait for transplants from deceased donors [1]. Nonetheless, donor-specific antibody (DSA)-positive, FCXM-positive patients have increased risk of antibody-mediated rejection (ABMR) and graft loss following transplantation. Various desensitization protocols have been developed to overcome this problem [2–7]; however, higher rates of ABMR and graft loss are still observed in DSA-positive, FCXM-positive patients [8,9]. For unknown reasons, not all DSAs elicit ABMR [10,11].

Studies have revealed a strong correlation between C1q-binding DSA and risk of ABMR and renal allograft loss [12–16]. C1q is the first molecule in the classic complement activation cascade and determines the cytotoxic potential of antibodies; however, the clinical utility of C1q assays for predicting the risk of ABMR and allograft loss is controversial [17–19]. Another complement-binding antibody detection method known as the C3d-detection assay has recently become commercially available [17,18] and measures the physiological complement activation that occurs when C1q binding triggers the complement cascade. C3d is a downstream effector of the complement pathway, whereas C1q is the first component. Moreover, while C1q is only involved in the classical complement pathway, C3d is generated in all three pathways [20]. In fact, several studies have demonstrated that C3d-fixing de novo (dn)DSAs better predict allograft rejection risk than C1q-binding dnDSA and C4d immunolabeling [17,18]. While these reports describe the effects of complement-fixing antibodies in the context of dnDSA, there is little information on the effect of preformed C3d-fixing antibody in pre-

Abbreviations: ABMR, antibody-mediated rejection; dnDSA, de novo donor-specific anti-human leukocyte antigen antibody; DSA, donor-specific anti-human leukocyte antigen antibody; FCXM, flow cytometric crossmatch; LKT, living kidney transplantation; MFI, mean fluorescence intensity

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transplant serum [21,22].

We recently reported that in patients undergoing appropriate desensitization prior to transplantation, intermediate-term outcomes of graft survival of FCXM-positive and -negative recipients did not differ statistically, and this was because we performed FCXM-positive LKT; however, ABMR-free survival in FCXM-positive recipients was significantly reduced within 90 days of kidney transplantation [23].

2. Objective

We speculated that C3d-fixing ability of preformed DSA in FCXM-positive recipients is a significant predictor of ABMR. To test this hypothesis, we investigated C3d-fixing ability of preformed DSA in FCXM-positive patients with C3d-fixing DSA before and after desensitization to assess the efficacy of this process in preventing acute ABMR. Our results show for the first time that complement-fixing ability before transplantation is an effective biomarker for acute ABMR in highly sensitized patients.

3. Material and methods

3.1. Study population

This retrospective study included 420 consecutive patients who received LKT between 2012 and 2016 at the Department of Urology, Tokyo Women's Medical University Hospital. Of these subjects, 21 were FCXM-positive with preformed DSA and were subjected to the same desensitization protocol before transplantation. We analyzed the C3d-fixing ability of DSA in pre-operative serum before and after desensitization in these patients. C3d-positive cases (C3d+) were compared with C3d-negative cases (C3d−) in terms of incidence of acute and chronic ABMR following LKT. Among C3d + cases, those demonstrating a loss of DSA C3d-fixing ability after desensitization were defined as responders (R-C3d+), whereas those retaining this activity were defined as non-responders (NR-C3d+). We also compared R-C3d + and NR-C3d + patients with acute and chronic ABMR patients. Moreover, we investigated MFI of preformed DSA before and after desensitization and compared the results with the C3d-fixing ability. The endpoint of follow-up was 1 year after transplantation. The study was conducted in accordance with the 2008 Declaration of Istanbul and 2013 Declaration of Helsinki.

3.2. Immunosuppression

Standard immunosuppression for kidney transplantation involved a calcineurin inhibitor (tacrolimus), mycophenolate mofetil, prednisone, and basiliximab. Desensitization for FCXM-positive patients with preformed DSA consisted of high-dose immunoglobulin (Ig) (2–4 g/kg), rituximab (200–500 mg), and plasma exchange or double filtration plasmapheresis before transplantation. A steroid bolus, high-dose intravenous Ig, plasma exchange, or a combination of rituximab and bortezomib were administered when clinical or subclinical acute ABMR was observed.

3.3. Immunological monitoring

All patients underwent FCXM evaluation and single-antigen screening prior to transplantation. Briefly, FCXM was measured by flow cytometry on a FACSCalibur instrument (Becton Dickinson, San Jose, CA, USA). FCXM was routinely performed at the first visit and 1 day before the surgery to verify the status and confirm the positive-to-negative conversion by FCXM irrespective of DSA intensity with the Luminex (Austin, TX, USA) single-antigen beads assay. Analysis using pronase was conducted after rituximab administration to avoid the false-positive reaction of lymphocytes absorbed with rituximab. The presence of DSA was determined by comparing human leukocyte antigen (HLA)-A, -B, -DR, and -DQ antibody specificity at the serological level with donor HLA type. Circulating preformed DSA was detected with a single-antigen flow bead assay (Lifecodes Immucor, Norcross, GA, USA), and mean fluorescence intensity (MFI) of preformed DSA was measured with the LX200 for Luminex platform. FCXM-positive and preformed DSA-positive patients were also analyzed for C3d-fixing ability with the single-antigen bead assay according to the manufacturer's protocol and using the manufacturer-specific software. Beads were defined as positive when two or more of the three adjusted values were above the cutoff level. We defined positive DSA as an MFI > 1000 and positive FCXM as a shift greater than over 10 and/or ratio over 2.0 compared to the median MFI of a negative control.

3.4. Diagnosis of rejection

ABMR was diagnosed from graft biopsies that were routinely performed (at 3 and 12 months following transplantation) and from clinical indications (graft function decline and/or proteinuria) whenever rejection was suspected. ABMR was diagnosed based on any of the following microvascular injuries: peritubular capillaritis (ptc > 0), glomerulitis (g > 0), thrombosis, or transplant glomerulopathy (cg > 0). Rejection types were defined according to the Banff classification (2009 and 2013). Rejection was diagnosed in a blinded manner by the same two pathologists.

3.5. Statistical analysis

Data are presented as mean ± standard deviation or as count and percentage if categorical. $p < .05$ was considered statistically significant. Event-free rates were estimated with the Kaplan-Meier method and compared between groups with the log-rank test. The global score chi-square statistic was used to measure the goodness of fit of models. Patients who did not experience ABMR were censored at the end of follow-up. All cases were observed for 1 year after transplantation and were treated if rejection occurred.

4. Results

4.1. Characteristics and outcomes of the study population according to C3d status

A total of 21 FCXM-positive and preformed DSA-positive patients were enrolled. Baseline demographic and transplant characteristics determined based on the presence of C3d-fixing DSA are summarized in Table 1. Nine (42.9%) of the 21 patients received transplants from ABO-incompatible donors and 10 (47.6%) underwent re-transplantation. The maximum MFI was 7877. Eleven (52.3%) of the 21 patients had acute ABMR and required treatment, and 11 (52.3%) were chronic cases. The average estimated glomerular filtration rates after 3 months and 1 year were 45.9 and 42.5 ml/min/1.73 m^2, respectively. Patient and graft survival rates were 100% at 1 year after transplantation.

Table 1 shows baseline and immunological characteristics of the study population according to C3d-fixing ability of DSA in the 21 renal transplant recipients, which included six C3d − and 15 C3d + patients. There was no difference in ABO incompatibility between the two groups (C3d+, 46.7% vs. C3d−, 33.3%). No differences in estimated glomerular filtration rate were noted at 3 months and 1 year post transplantation. The presence of C3d-fixing DSA was significantly associated with re-transplantation (C3d+, 66.7% vs. C3d−, 0%; $p = .01$). Further, the C3d-fixing ability of DSA was correlated with higher MFI of DSA before desensitization (C3d−, 3869 vs. C3d+, 9480; $p = .02$) (Fig. 1A).

Fig. 3 shows acute ABMR-free survival vs. C3d-fixing ability of DSA before desensitization. Patients with C3d-fixing DSA had a lower rate of acute ABMR-free survival for 1 year than those with non-C3d-fixing DSA (C3d+, 68.8% vs. C3d−, 16.7%; $p = .04$). Moreover, at 1 year

Table 1
Characteristics of the study population before desensitization[a].

	total(n = 21)	C3d + (n = 15)	C3d-(n = 6)	p
Recipients data				
Sex (male), no	8(38.1%)	7(46.7%)	1(16.7%)	0.18
Age, yr	52.8 ± 14.9	51.1 ± 15.8	56.8 ± 11.8	0.44
Re-transplant, no	10(47.6%)	10(66.7%)	0(0%)	0.002
Diabetes, no	4(19.0%)	2(13.3%)	2(33.3%)	0.31
HD duration, m	57.1 ± 58.8	65.7 ± 65.6	35.7 ± 33.2	0.3
Donors data				
Sex(male), no	16(76.2%)	12(80%)	4(66.7%)	0.52
Age, yr	58.3 ± 8.5	58.2 ± 8.6	58.5 ± 8.3	0.94
Related (parents, brothers), no	6(28.6%)	5(33.3%)	1(16.7%)	0.43
Immunological data				
MFI shift of FCXM	51.9 ± 44.3	63.4 ± 47.6	23.4 ± 10.8	0.04
ABO incompatibility, no	9(42.9%)	7(46.7%)	2(33.3%)	0.57
HLA mismatch (A.B.DQ.DR), no	4.1 ± 1.3	3.8 ± 1.2	4.8 ± 1.5	0.11
DSA number, no	1.6 ± 1.0	1.5 ± 0.7	1.8 ± 1.5	0.53
Max DSA MFI before desensitization	7877 ± 4567	9480 ± 1179	3869 ± 1864	0.02
Class 1 DSA positive (max MFI)	8(38.1%)	6(40%)	2(33.3%)	0.79
Acute ABMR, no	12(57.1%)	11(73.3%)	1(16.7%)	0.04
C4d + at biopsy	14(66.6%)	11(73.3%)	3(50%)	0.31
TMR, no	5(23.8%)	4(26.7%)	1(16.7%)	0.64
Chronic ABMR (I yr), no	11(52.3%)	10(66.7%)	1(16.7%)	0.03
3 m eGFR, mL/min/1.73m^2	45.9 ± 12.3	43.8 ± 3.2	51.1 ± 5.0	0.23
12 m eGFR, mL/min/1.73m^2	42.5 ± 12.6	41.7 ± 13.3	44.2 ± 11.0	0.69

Baseline and immunological characteristics of the study population according to C3d-fixing ability of DSA.
ABMR, antibody-mediated rejection; C3d+, positive for C3d-fixing ability; C3d−, negative for C3d-fixing ability; DSA, donor-specific antibody, eGFR, estimated glomerular filtration rate; FCXM, flow cytometric crossmatch; MFI, mean fluorescence intensity; TMR, T cell-mediated rejection.

post transplantation, the incidence of chronic ABMR was higher in patients with C3d + DSA than in those with C3d − DSA (C3d+, 66.7% vs. C3d−, 16.7%; p = .03).

4.2. C3d-fixing ability of DSA after desensitization

Of the 15 C3d-fixing DSA cases, nine were responders and six were non-responders. Table 2 shows the characteristics of these subjects in terms of C3d-fixing ability of preformed DSA. *Re*-transplantation was more frequent among non-responders (NR-C3d+, 100% vs. R-C3d+, 44.4%; p = .01). Donor age was lower for the latter group (NR-C3d+, 52.7 years vs. R-C3d+, 61.9 years; p = .04). Although there was no difference in MFI of preformed DSA before desensitization between the two groups, a difference was observed after desensitization (R-C3d+, 2898 vs. NR-C3d+, 13,103; p < .0001). C3d + DSA with low MFI after desensitization (especially < 5000) changed to R-C3d + and lost C3d-fixing ability (Fig. 1B) (see Fig. 2).

Fig. 3 shows acute ABMR-free survival according to the persistence of C3d-fixing ability after desensitization. No differences in survival (83.3% vs. 66.7%; p = .92) or incidence of chronic ABMR 1 year after transplantation (50% vs. 77.8%; p = .26) were observed between non-responders and responders.

4.3. Immunological parameters according to acute ABMR

Table 3 shows the relationship between acute ABMR and immunological parameters. Acute ABMR was significantly likely to occur in recipients with re-transplantation (ABMR+, 66.7% vs. ABMR-, 22.5%; p = .04), high MFI shift of FCXM (ABMR+, 73.1 ± 48.4 vs. ABMR-, 23.7 ± 10.5; p = .004), high MFI of DSA before desensitization (ABMR+, 10,925 ± 4323 vs. ABMR-, 3812 ± 2859; p = .0003), and positive C3d-fixing ability (ABMR+, 91.7% vs. ABMR-, 44.4%; p = .02). For FCXM positive preformed DSA positive cases, high MFI shift of FCXM, high MFI of DSA, and C3d-fixing ability were remarkable pre-transplant predictors of ABMR.

5. Discussion

We retrospectively analyzed C3d-fixing ability of preformed DSA and the effect of desensitization in 21 FCXM-positive LKT patients. Our findings showed that not only the MFI of performed DSA and the MFI shift of FCXM, but also C3d-fixing ability of preformed DSA before desensitization increased the risk of acute ABMR. Correlations were observed before desensitization between C3d-fixing ability and the state of re-transplantation, the MFI of preformed DSA, and the MFI shift of FCXM; moreover, there was a significant correlation between C3d response and the MFI of preformed DSA before and after desensitization. These results suggest that preoperative measurement—especially before desensitization—of DSA C3d-fixing ability in FCXM-positive transplant recipients may be useful for predicting ABMR, and there may be strong relationship between desensitization and C3d-fixing ability.

FCXM-positive recipients with preformed DSA reportedly have a higher risk for ABMR and early graft loss [24]. Our results demonstrate that short-term outcomes in FCXM-positive LKT recipients can be predicted based on complement-fixing ability. Multiple desensitization protocols are currently available; ours consists of plasmapheresis, high-dose intravenous Ig, and rituximab for 1 month prior to transplantation. We found no significant differences in patient and graft survival rates and graft function between FCXM-positive and -negative groups at the intermediate-term follow-up after desensitization, although ABMR-free survival rate differed significantly between the groups [23].

Regarding patient characteristics, re-transplantation is a significant risk factor for complement-fixing ability and is known to be a high-risk factor for FCXM positivity, and our results reinforced this. Previous transplantation strongly sensitized recipients, leading to the production of complement-fixing DSAs upon subsequent transplantation. Moreover, nine ABO-incompatible recipients were included in this study. ABO-incompatible LKT is an acceptable treatment for end-stage renal disease, and 3-month biopsies suggest comparable graft pathology between ABO-compatible and -incompatible patients after desensitization with rituximab and by plasmapheresis [25,26]. As all 21 patients received rituximab, plasmapheresis, and intravenous Ig in our study, ABO incompatibility had no effect on ABMR risk. However, ABO incompatibility affected C4d staining at biopsy, explaining why C3d staining was better than C4d staining in predicting ABMR.

Complement-fixing DSAs are reportedly associated with ABMR and graft loss [12]. The development of solid-phase C1q-binding assays offered a new approach for assessing HLA antibody risk by distinguishing potentially harmful complement-fixing antibodies from those that do not fix complement and are thus less likely to induce ABMR [14,17,27]. C1q-binding assay reportedly detects differences in DSA function, possibly due to the composition of complement-binding IgG isotypes [28,29]. Conversely, the C3d assay allows for direct detection of C3 fragments generated by the complement activation process. C1q measures the potential of antibodies to initiate the classic pathway, whereas the downstream C3d is an indicator of complete complement activation [17,18] and may better reflect physiological complement activation and complement-mediated injury in allografts [30].

The presence of complement-fixing dn DSA at the time of ABMR has been investigated in some studies [17,18]. However, to date, there are no reports of C3d-fixing ability of preformed DSA in FCXM-positive patients and its effects following desensitization. FCXM is used to determine whether the preformed recipient serum will attack donor lymphocytes by measuring antibody–antigen binding activity.

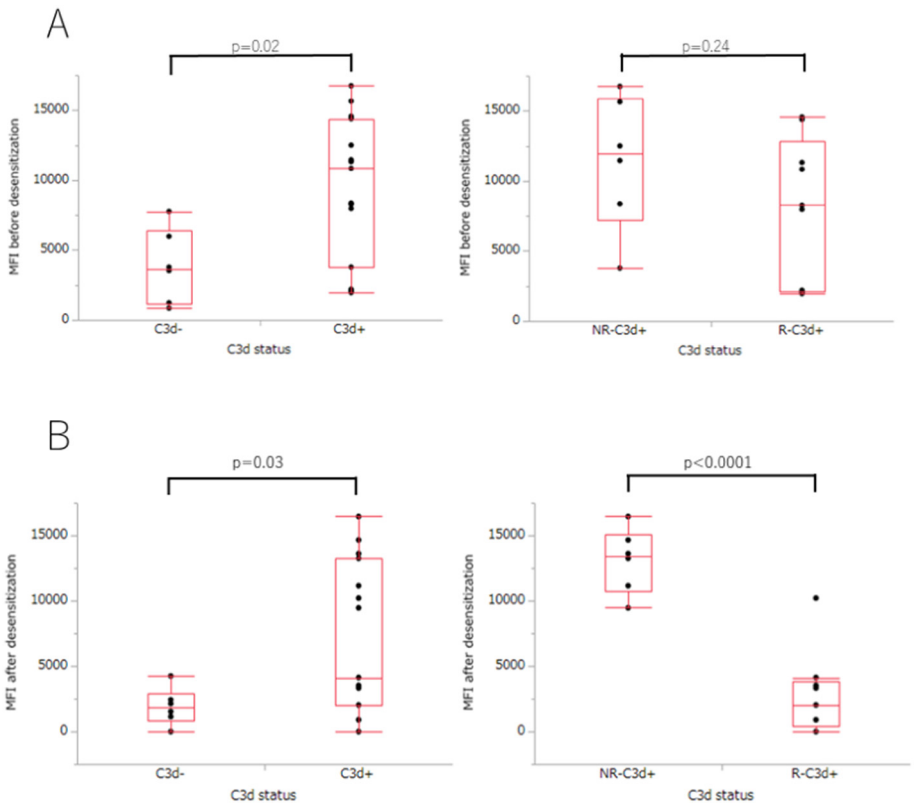

Fig. 1. MFI of DSA according to C3d status. (A) MFI of DSA in the Luminex assay according to C3d-fixing status before desensitization. The plot on the left depicts non-C3d-fixing (C3d−; $n = 6$) and C3d-fixing (C3d+; $n = 15$) DSA groups, respectively ($p = .02$); the plot on the right shows non-responders (NR-C3d+) vs. responders (C3d+) ($p = .24$). (B) MFI of DSA after desensitization. The right plot shows that MFI of DSA was higher for NR-C3d + than for R-C3d + ($p < .0001$).

Preformed serum contains both antibodies and complement; detection of additional complement fixing by DSA is critical for predicting ABMR. We found that FCXM and C3d assays before transplantation were useful in preventing and predicting ABMR, respectively. In addition to MFI of preformed DSA, C3d-fixing ability was also one of the effective examinations.

Desensitization is thought to influence the MFI of DSA by lowering antibody titer [7]. Here, not only DSA titer but also C3d-fixing ability decreased after desensitization. However, we did not observe a trend towards an improved ABMR-free rate in the response to C3d fixing by DSA after desensitization. As DSA production is a central event leading to graft damage and acute ABMR, a strategy for removing or

Table 2
Characteristics of the study population after desensitization[a].

	C3d + (n = 15)	NR-C3d + (n = 6)	R-C3d + (*n* = 9)	p
Recipients data				
Sex (male), no	7(46.7%)	3(50%)	4(44.4%)	0.83
Age, yr	51.1 ± 16.1	47.8 ± 15.3	53.3 ± 16.7	0.53
Re-transplant, no	10(66.7%)	6(100%)	4(44.4%)	0.01
Diabetes, no	2(13.3%)	2(33.3%)	0(0%)	0.13
HD duration, m	65.7 ± 68.1	64.3 ± 28.4	66.6 ± 83.8	0.95
Donors data				
Sex (male), no	12(80%)	5(83.3%)	7(77.8%)	0.79
Age, yr	58.2 ± 7.4	52.7 ± 7.2	61.9 ± 7.6	0.04
Related (parents, brothers), no	5(33.3%)	3(50%)	2(22.2%)	0.26
Immunological data				
MFI shift of FCXM	63.4 ± 47.6	57.2 ± 29.0	67.5 ± 58.2	0.35
ABO incompatibility, no	7(46.7%)	3(50%)	4(44.4%)	0.83
HLA mismatch(A.B.DQ.DR), no	3.8 ± 1.2	3.3 ± 1.4	4.1 ± 1.1	0.23
DSA number, no	1.5 ± 0.8	1.5 ± 0.3	1.6 ± 0.3	0.89
Max DSA MFI before desensitization	9480 ± 4983	11,424 ± 4795	8183 ± 5097	0.24
Max DSA MFI after desensitization	6980 ± 5878	13,103 ± 2490	2898 ± 3119	< 0.0001
Class 1 DSA positive (max MFI)	6(40%)	2(33.3%)	4(44.4%)	0.69
Acute ABMR, no	11(73.3%)	5(83.3%)	6(66.7%)	0.92
C4d + at biopsy				
TMR, no	4(26.7%)	1(16.7%)	3(33.3%)	0.51
Chronic ABMR (I yr), no	10(66.7%)	3(50%)	7(77.8%)	0.26
3 m eGFR, mL/min/1.73m^2	43.8 ± 13.4	41.1 ± 5.5	45.5 ± 4.5	0.55
12 m eGFR, mL/min/1.73m^2	41.7 ± 13.9	42.8 ± 6.2	40.8 ± 5.2	0.81

ABMR, antibody-mediated rejection; DSA, donor-specific antibody; FCXM, flow cytometric crossmatch; MFI, mean fluorescence intensity; NR-C3d+, non-responder-C3d after desensitization; R-C3d+, responder-C3d after desensitization.

[a] Baseline and immunological characteristics of the study population according to C3d-fixing response after desensitization.

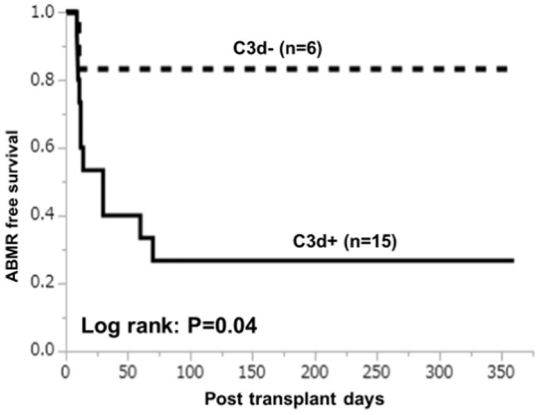

Fig. 2. ABMR-free survival according to C3d status. ABMR-free rate according to C3d-fixing ability of DSA at the time of crossmatching. Kaplan-Meier curves for ABMR-free rate are shown for patients with C3d-fixing DSA (C3d +) and non-C3d-fixing DSA (C3d −). The results of the log-rank test are shown (p = .04).

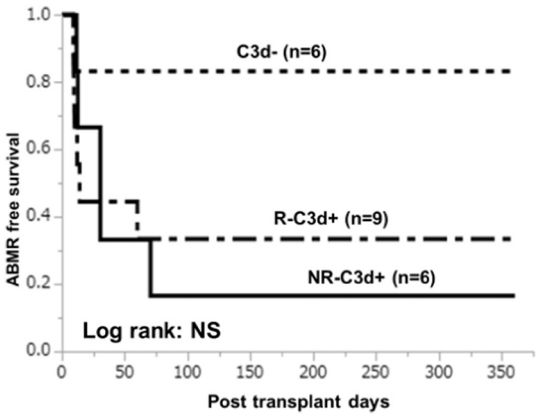

Fig. 3. ABMR-free survival according to C3d after desensitization. ABMR-free rate according to C3d status and response to C3d-binding DSA after desensitization. Kaplan-Meier curves for ABMR-free rates are shown for non-responders with C3d-fixing DSA (NR-C3d +) after desensitization and before transplantation, responders with C3d-fixing DSA (R-C3d +), and patients with non-C3d-fixing DSA (C3d −). The results of the log-rank test are shown (p = .92).

inactivating preformed DSA is needed to minimize this risk. The correlation between the efficacy of desensitization before transplantation and C3d-fixing ability of preformed DSA is unknown. Nevertheless, C3d-fixing ability was suggested to be linked to the MFI of DSA, which is a powerful predictor of C3d positivity [31]. It is possible that the negative C3d assay result post desensitization is simply due to a lower titer of antibodies, which may not be able to fix complement onto the beads. Our results suggest that these antibodies are of the same subtype as those that are present prior to desensitization and can still fix complement in vivo, but not onto beads. Indeed, we showed that an effective way to determine the response to desensitization and predict ABMR is to measure the C3d-fixing ability of preformed DSA before desensitization.

The important application of the C3d-fixing assay is to identify patients who would benefit from treatment with eculizumab [32,33]. This monoclonal antibody is capable of blocking the complement cascade but cannot be administered to every patient diagnosed with ABMR or that requires desensitization owing to its high cost and adverse effects. Physicians can recommend desensitization with eculizumab in C3d-positive cases to not only treat but also prevent ABMR; at the same time, treatment of patients who are C3d-negative and are not likely to benefit from the therapy can be avoided. In future studies, we would focus on changing the desensitization agents according to the C3d status.

Our study had several limitations. Firstly, the small sample size limited the power of the study to determine the significance of outcomes due to the rarity of FCXM-positive cases. Although we propose that there is a significant difference only between C3d + and C3d − patients before desensitization, we need more cases to prove a significant difference between R-C3d + and NR-C3d + after desensitization due to power analysis. Another shortcoming was the short duration of observation as our aim was to focus on preformed DSA; long observation periods obfuscate preformed and dn DSAs which are typically generated few months after transplantation. Finally, as FCXM assays and definitions vary across centers, our results must be interpreted with caution.

In conclusion, this study showed that the C3d-fixing ability and the MFI of preformed DSA in FCXM-positive patients before desensitization are strong predictors of acute ABMR. Additionally, MFI and C3d-fixing ability of preformed DSA are remarkably related to each other in the aspect of desensitization. These findings provide a basis for future clinical trials aimed at evaluating the efficacy of desensitization in this subgroup of high-risk patients.

Declaration of Competing Interest

None.

Acknowledgments

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Table 3
Correlation between ABMR and each parameter before desensitization.

	total(n = 21)	ABMR + (n = 12)	ABMR-(n = 9)	p
Re-transplant, no	10(47.6%)	8(66.7%)	2(22.2%)	0.04
MFI shift of FCXM	51.9 ± 44.3	73.1 ± 48.4	23.7 ± 10.5	0.004
ABO incompatibility, no	9(42.9%)	4(33.3%)	5(55.6%)	0.31
HLA mismatch(A.B.DQ.DR), no	4.1 ± 1.3	3.9 ± 1.3	4.3 ± 1.4	0.47
DSA number, no	1.6 ± 1.0	1.8 ± 0.9	1.4 ± 0.7	0.37
Max DSA MFI before desensitization	7877 ± 4567	10,925 ± 4323	3812 ± 2859	0.0003
Max DSA MFI after desensitization	5531 ± 5098	7222 ± 5356	3277 ± 5105	0.082
Class 1 DSA positive (max MFI)	8(38.1%)	6(50.0%)	2(22.2%)	0.21
C3d +	15(71.4%)	11(91.7%)	4(44.4%)	0.02
C4d + at biopsy	14(66.7%)	8(66.7%)	6(66.7%)	1

ABMR, antibody-mediated rejection; DSA, donor-specific antibody; C3d + , positive for C3d-fixing ability; C4d + , positive for C4d staining at biopsy; MFI, mean fluorescence intensity; eGFR, estimated glomerular filtration rate.

References

[1] B.J. Orandi, X. Luo, A.B. Massie, B.J. Orandi, X. Luo, A.B. Massie, J.M. Garonzik-Wang, B.E. Lonze, R. Ahmed, K.J. Van Arendonk, M.D. Stegall, S.C. Jordan, J. Oberholzer, T.B. Dunn, Survival benefit with kidney transplants from HLA-incompatible live donors, N. Engl. J. Med. 374 (2016) 940–950, https://doi.org/10.1056/NEJMoa1508380.

[2] R.M. Higgins, D.J. Bevan, B.S. Carey, C.K. Lea, M. Fallon, R. Buhler, R.W. Vaughan, P.J. O'donnell, S.A. Snowden, M. Bewick, B.M. Hendry, Prevention of hyperacute rejection by removal of antibodies to HLA immediately before renal transplantation, Lancet 348 (1996) 1208–1211, https://doi.org/10.1016/S0140-6736(96)03452-6.

[3] D. Glotz, C. Antoine, P. Julia, C. Suberbielle-Boissel, S. Boudjeltia, R. Fraoui, C. Hacen, A. Duboust, J. Bariety, Desensitization and subsequent kidney transplantation of patients using intravenous immunoglobulins (IVIg), Am. J. Transplant. 2 (2002) 758–760, https://doi.org/10.1034/j.1600-6143.2002.20809.x.

[4] S.C. Jordan, D. Tyan, D. Stablein, M. McIntosh, S. Rose, A. Vo, M. Toyoda, C. Davis, R. Shapiro, D. Adey, D. Milliner, Evaluation of intravenous immunoglobulin as an agent to lower allosensitization and improve transplantation in highly sensitized adult patients with end-stage renal disease: report of the NIH IG02 trial, J. Am. Soc. Nephrol. 15 (2004) 3256–3262, https://doi.org/10.1097/01.ASN.0000145878.92906.9F.

[5] J.J. Thielke, P.M. West-Thielke, H.L.U. Herren, T. Bareato, V. Ommert, S.A. Vidanovic, I.G. Campbell-Lee, H.N. Tzvetanov, B. Sankary, E.B. Kaplan, Living donor kidney transplantation across positive crossmatch: the University of Illinois at Chicago experience, Transplantation 87 (2009) 268–273, https://doi.org/10.1097/TP.0b013e3181919a16.

[6] M.K. Jin, J.H. Cho, O. Kwon, K.D. Hong, J.Y. Choi, S.H. Yoon, S.H. Park, Y.L. Kim, C.D. Kim, Successful kidney transplantation after desensitization using plasmapheresis, low-dose intravenous immunoglobulin, and rituximab in highly sensitized patients: a single-center experience, Transplant. Proc. 44 (2012) 200–203, https://doi.org/10.1016/j.transproceed.2011.11.040.

[7] A.A. Vo, M. Lukovsky, M. Toyoda, J. Wang, N.L. Reinsmoen, C.H. Lai, A. Peng, R. Villicana, S.C. Jordan, Rituximab and intravenous immune globulin for desensitization during renal transplantation, N. Engl. J. Med. 359 (2008) 242–251, https://doi.org/10.1056/NEJMoa0707894.

[8] P. West-Thielke, H. Herren, J. Thielke, J. Oberholzer, H. Sankary, V. Raofi, E. Benedetti, B. Kaplan, Results of positive cross-match transplantation in African American renal transplant recipients, Am. J. Transplant. 8 (2008) 348–354, https://doi.org/10.1111/j.1600-6143.2007.02085.x.

[9] A. Haririan, J. Nogueira, D. Kukuruga, E. Schweitzer, J. Hess, C. Gurk-Turner, S. Jacobs, C. Drachenberg, S. Bartlett, M. Cooper, Positive cross-match living donor kidney transplantation: longer-term outcomes, Am. J. Transplant. 9 (2009) 536–542, https://doi.org/10.1111/j.1600-6143.2008.02524.x.

[10] R.W. O'Rourke, R.W. Osorio, C.E. Freise, C.D. Lou, M.R. Garovoy, P. Bacchetti, N.L. Ascher, J.S. Melzer, J.P. Roberts, P.G. Stock, Flow cytometry crossmatching as a predictor of acute rejection in sensitized recipients of cadaveric renal transplants, Clin. Transpl. 14 (2000) 167–173, https://doi.org/10.1034/j.1399-0012.2000.140212.x.

[11] R. Higgins, M. Hathaway, D. Lowe, H. Kashi, L.C. Tan, C. Imray, S. Fletcher, D. Zehnder, K. Chen, N. Krishnan, R. Hamer, Blood levels of donor-specific human leukocyte antigen antibodies after renal transplantation: resolution of rejection in the presence of circulating donor-specific antibody, Transplantation 84 (2007) 876–884, https://doi.org/10.1097/01.tp.0000284729.39137.6e.

[12] A. Loupy, C. Lefaucheur, D. Vernerey, C. Prugger, J.P.D. van Huyen, N. Mooney, C. Suberbielle, V. Frémeaux-Bacchi, A. Méjean, F. Desgrandchamps, D. Anglicheau, Complement-binding anti-HLA antibodies and kidney-allograft survival, N. Engl. J. Med. 369 (2013) 1215–1226, https://doi.org/10.1056/NEJMoa1302506.

[13] C. Lefaucheur, D. Viglietti, C. Bentlejewski, J.P.D. van Huyen, D. Vernerey, O. Aubert, J. Verine, X. Jouven, C. Legendre, D. Glotz, A. Loupy, IgG donor-specific anti-human HLA antibody subclasses and kidney allograft antibody-mediated injury, J. Am. Soc. Nephrol. 27 (2016) 293–304, https://doi.org/10.1681/ASN.2014111120.

[14] J. Bamoulid, A. Roodenburg, O. Staeck, K. Wu, B. Rudolph, S. Brakemeier, F. Halleck, L. Lehner, C. Schönemann, N. Lachmann, K. Budde, Clinical outcome of patients with de novo C1q-binding donor-specific HLA antibodies after renal transplantation, Transplantation 101 (2017) 2165–2174, https://doi.org/10.1097/TP.0000000000001487.

[15] C. Wiebe, A.J. Gareau, D. Pochinco, I.W. Gibson, J. Ho, P.E. Birk, T. Blydt-Hansen, M. Karpinski, A. Goldberg, L. Storsley, D.N. Rush, Evaluation of C1q status and titer of de novo donor-specific antibodies as predictors of allograft survival, Am. J. Transplant. 17 (2017) 703–711, https://doi.org/10.1111/ajt.14015.

[16] D.S. Ramon, Y. Huang, L. Zhao, T. Rendulic, J.M. Park, R.S. Sung, M. Samaniego, Use of complement binding assays to assess the efficacy of antibody mediated rejection therapy and prediction of graft survival in kidney transplantation, Hum. Immunol. 78 (2017) 57–63, https://doi.org/10.1016/j.humimm.2016.11.009.

[17] A. Sicard, S. Ducreux, M. Rabeyrin, L. Couzi, B. McGregor, L. Badet, J.Y. Scoazec, T. Bachelet, S. Lepreux, J. Visentin, P. Merville, Detection of C3d-binding donor-specific anti-HLA antibodies at diagnosis of humoral rejection predicts renal graft loss, J. Am. Soc. Nephrol. 26 (2015) 457–467, https://doi.org/10.1681/ASN.2013101144.

[18] P. Comoli, M. Cioni, A. Tagliamacco, G. Quartuccio, A. Innocente, I. Fontana, A. Trivelli, A. Magnasco, A. Nocco, C. Klersy, L. Rubert, Acquisition of C3d-binding activity by de novo donor-specific HLA antibodies correlates with graft loss in nonsensitized pediatric kidney recipients, Am. J. Transplant. 16 (2016) 2106–2116, https://doi.org/10.1111/ajt.13700.

[19] J.J. Kim, O. Shaw, C. Martin, G. Michaelides, R. Balasubramaniam, N.J. Sebire, N. Mamode, A. Dorling, R. Vaughan, S.D. Marks, Clinical risk stratification of paediatric renal transplant recipients using C1q and C3d fixing of de novo donor-specific antibodies, Pediatr. Nephrol. 33 (2018) 167–174, https://doi.org/10.1007/s00467-017-3772-7.

[20] S.H. Sacks, W. Zhou, The role of complement in the early immune response to transplantation, Nat. Rev. Immunol. 12 (2012) 431–442, https://doi.org/10.1038/nri3225.

[21] E.G. Kamburova, B.W. Wisse, I. Joosten, W.A. Allebes, A. Van Der Meer, L.B. Hilbrands, M.C. Baas, E. Spierings, C.E. Hack, F.E. Van Reekum, A.D. Van Zuilen, Pretransplant C3d-fixing donor-specific anti-HLA antibodies are not associated with increased risk for kidney graft failure, J. Am. Soc. Nephrol. 29 (2018) 2279–2285, https://doi.org/10.1681/ASN.2018020205.

[22] M. Meneghini, E. Melilli, J. Martorell, I. Revuelta, E. Rigol-Monzó, A. Manonelles, N. Montero, D. Cucchiari, F. Diekmann, J.M. Cruzado, S. Gil-Vernet, Combining sensitive crossmatch assays with donor/recipient human leukocyte antigen eplet matching predicts living-donor kidney transplant outcome, Kidney Int. Rep. 3 (2018) 926–938 (doi:j.ekir.2018.03.01).

[23] D. Okada, M. Okumi, Y. Kakuta, K. Unagami, J. Iizuka, T. Takagi, H. Ishida, K. Tanabe, Outcome of the risk-stratified desensitization protocol in donor-specific antibody-positive living kidney transplant recipients: a retrospective study, Transpl. Int. 31 (2018) 1008–1017, https://doi.org/10.1111/tri.13269.

[24] A. Bentall, L.D. Cornell, J.M. Gloor, W.D. Park, M.J. Gandhi, J.L. Winters, M.F. Chedid, P.G. Dean, M.D. Stegall, Five-year outcomes in living donor kidney transplants with a positive crossmatch, Am. J. Transplant. 13 (2013) 76–85, https://doi.org/10.1111/j.1600-6143.2012.04291.x.

[25] M. Okumi, D. Toki, T. Nozaki, T. Shimizu, H. Shirakawa, K. Omoto, M. Inui, H. Ishida, K. Tanabe, ABO-incompatible living kidney transplants: evolution of outcomes and immunosuppressive management, Am. J. Transplant. 16 (2016) 886–896, https://doi.org/10.1111/ajt.13502.

[26] K. Masutani, A. Tsuchimoto, K. Kurihara, Y. Okabe, H. Kitada, M. Okumi, K. Tanabe, M. Nakamura, T. Kitazono, K. Tsuruya, Histological analysis in ABO-compatible and ABO-incompatible kidney transplantation by performance of 3- and 12-month protocol biopsies, Transplantation 101 (2017) 1416–1422, https://doi.org/10.1097/TP.0000000000001324.

[27] J.M. Yabu, J.P. Higgins, G. Chen, F. Sequeira, S. Busque, D.B. Tyan, C1q-fixing human leukocyte antigen antibodies are specific for predicting transplant glomerulopathy and late graft failure after kidney transplantation, Transplantation 91 (2011) 342–347, https://doi.org/10.1097/TP.0b013e318203fd26.

[28] S.M. Sutherland, G. Chen, F.A. Sequeira, C.D. Lou, S.R. Alexander, D.B. Tyan, Complement-fixing donor-specific antibodies identified by a novel C1q assay are associated with allograft loss, Pediatr. Transplant. 16 (2012) 12–17, https://doi.org/10.1111/j.1399-3046.2011.01599.x.

[29] G. Chen, F. Sequeira, D.B. Tyan, Novel C1q assay reveals a clinically relevant subset of human leukocyte antigen antibodies independent of immunoglobulin G strength on single antigen beads, Hum. Immunol. 72 (2011) 849–858, https://doi.org/10.1016/j.humimm.2011.07.001.

[30] J.H. Lan, K. Tinckam, Clinical utility of complement dependent assays in kidney transplantation, Transplantation 102 (2018) S14–S22, https://doi.org/10.1097/TP.0000000000001819.

[31] G. Claisse, L. Absi, F. Cognasse, E. Alamartine, C. Mariat, N. Maillard, Relationship between mean fluorescence intensity and C1q/C3d-fixing capacities of anti-HLA antibodies, Hum. Immunol. 78 (2017) 336–341, https://doi.org/10.1016/j.humimm.2017.02.003.

[32] M.D. Stegall, T. Diwan, S. Raghavaiah, L.D. Cornell, J. Burns, P.G. Dean, F.G. Cosio, M.J. Gandhi, W. Kremers, J.M. Gloor, Terminal complement inhibition decreases antibody-mediated rejection in sensitized renal transplant recipients, Am. J. Transplant. 11 (2011) 2405–2413, https://doi.org/10.1111/j.1600-6143.2011.03757.x.

[33] D. Glotz, G. Russ, L. Rostaing, C. Legendre, G. Tufveson, S. Chadban, J. Grinyó, N. Mamode, P. Rigotti, L. Couzi, M. Büchler, Safety and efficacy of eculizumab for the prevention of antibody-mediated rejection after deceased-donor kidney transplantation in patients with preformed donor-specific antibodies, Am. J. Transplant. (2019), https://doi.org/10.1111/ajt.15397 (Epub ahead of print).