OPEN

Association Between Peripheral Blood CD19-Positive Rate and Antibody-Mediated Rejection Following Rituximab Administration in Kidney Transplant Recipients

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Background. Rituximab is used widely for desensitization in ABO-incompatible and donor-specific antibody-positive kidney transplantation. However, data about the effects of individual differences in rituximab-induced B-cell suppression on antibody-mediated rejection (AMR) remain unknown. We aimed to assess the association between CD19-positive rate and AMR following rituximab administration after kidney transplantation. **Methods.** Overall, 42 patients who underwent rituximab therapy for pretransplant desensitization in ABO-incompatible (n = 33) and donor-specific antibody-positive (n = 15) kidney transplantation were observed retrospectively. To predict AMR incidence, the peripheral blood CD19-positive rate was determined and classified into short- and long-acting groups. AMR incidence, allograft function, complications, and rituximab dose were compared. **Results.** Eight patients (19%) had AMR within 39.2 months after transplantation. The CD19-positive rate cutoff value to predict AMR incidence was 4.4%, 6.4%, and 7.7% at 6, 12, and 18 months after transplantation, respectively. When comparing the short- and long-acting groups stratified according to the CD19-positive rate cutoff value, AMR incidence was significantly higher in the short-acting group than in the long-acting group at 6 (71.4% vs 8.6%), 12 (70.0% vs 3.1%), and 18 (58.3% vs 3.3%) months after transplantation. The CD19-positive rate for all patients with AMR exceeded the cutoff value 6, 12, or 18 months. Conversely, serum creatinine level, tacrolimus trough-level, cytomegalovirus antigenemia-positive rate, neutropenia incidence rate, and total dose of rituximab before transplantation showed no significant differences between the 2 groups. **Conclusions.** The risk of AMR was higher in patients with short-term B-cell suppression following rituximab administration. Additional rituximab administration after transplantation may prevent AMR in patients with a CD19-positive rate higher than the cutoff value.

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Advances in immunosuppressive therapies and desensitization techniques have improved the outcomes for ABO-incompatible[1-6] and donor-specific antibody (DSA)-positive[7-10] kidney transplantation. However, antibody-mediated rejection (AMR) remains the most common cause of renal allograft failure since 12 months after transplantation.[11,12] Recent studies reported that chronic AMR is difficult to treat because of less response to conventional treatment regimens combined with plasmapheresis, intravenous immunoglobulin, and rituximab.[13,14] Therefore, the most important measure for renal allograft survival is prevention of DSA by long-term B-cell depletion before development to chronic AMR.

Rituximab, a chimeric murine/human anti-CD20 antibody, directly inhibits B-cell proliferation by 3 mechanisms: antibody-dependent cell–mediated cytotoxicity, complement-mediated cytotoxicity, or activation of the apoptotic pathways.[15] Clinically, rituximab is applied widely for B-cell depletion as desensitization before ABO-incompatible and DSA-positive kidney transplantation.[13,16,17] However, little is known about the effects of individual differences in rituximab-induced B-cell suppression on organ transplantation outcomes.

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Therefore, we hypothesized that individual differences in rituximab-induced B-cell suppression affect AMR incidence after ABO-incompatible and DSA-positive kidney transplantation. First, we determined the peripheral blood CD19-positive rate cutoff value to predict AMR. Second, we compared the AMR incidence and patient outcomes between differences in the CD19-positive rate.

MATERIALS AND METHODS

Subjects, Study Design, and End Points

Between March 2013 and March 2017, 131 consecutive patients with end-stage renal disease underwent kidney transplantation at our hospital. Among 131 kidney transplant recipients, we performed a retrospective observational study of 42 who were administered rituximab as desensitization for ABO-incompatible and DSA-positive kidney transplantation.

To identify the period of the peripheral blood CD19 (as B-cell marker)–positive rate to predict AMR, this rate was measured at some periods (–14, –1, and 7 days, and 6, 12, 18, 24, 36, and 48 months after transplantation) and compared between patients with and without AMR. In each period, the peripheral blood CD19-positive rate cutoff value was determined using receiver operating characteristic (ROC) curve. Patients in whom the peripheral blood CD19-positive rates were higher than or lower than the cutoff values were classified into the short- and long-acting groups, respectively.

The primary end point was AMR incidence rate during the observation period. Secondary end points were serum creatinine level and tacrolimus trough-level at 1 year after transplantation, cytomegalovirus antigenemia-positive and neutropenia incidence rates during the observation period, and total dose of rituximab before transplantation. All end points were compared between the 2 groups.

This study was conducted in accordance with the principles of the Declarations of Helsinki and Istanbul and approved by the institutional review board.

Detection of Anti-A/B Antibody Titer and DSA

Anti-A/B immunoglobulin (Ig) M titer and IgG titer was measured using the saline agglutination technique and indirect Coombs test, respectively. DSAs were analyzed by lymphocyte crossmatch using complement-dependent cytotoxicity and flow cytometry and panel reactive antibody assay for screening of anti-human leukocyte antigen antibodies. A single-antigen beads test was performed to determine donor specificity of anti-human leukocyte antigen antibodies. The anti-A/B antibody titers and DSAs were measured at 1 month before transplantation. The anti-A/B antibody titer for the desensitization protocol was determined by the greater titers of IgM and IgG. Anti-A/B antibody titer and DSAs were also measured for auxiliary diagnosis of AMR after transplantation.

Desensitization Protocol

Desensitization for ABO-incompatible and DSA-positive recipients was performed before transplantation, through 0–4 sessions of plasmapheresis (double-filtration plasmapheresis or plasma exchange) and rituximab administration 1–2× at 100-mg dose, according to the quantity of antibody. For an anti-A/B antibody titer of ×128 or more and DSA-positive finding, plasmapheresis was performed at 6, 4, 2, and 1 days before transplantation and rituximab was administered at 14 and 1 days before transplantation. For anti-A/B antibody titers of ×64 and ×32, plasmapheresis was performed at 2 and 1 days before transplantation and rituximab was administered at 1 day before transplantation. For an anti-A/B antibody titer ×16 or less, rituximab was administered at 1 day before transplantation alone without plasmapheresis.

All ABO-incompatible and DSA-positive recipients received combined tacrolimus (0.1 mg/kg/day), mycophenolate mofetil (20 mg/kg/day), and methylprednisolone (20 mg/day) starting 7 days before transplantation for an anti-A/B antibody titer ×128 or more and DSA-positive finding, or 5 days before transplantation for an anti-A/B antibody titer ×64 or less.

Immunosuppressive Therapy

All ABO-incompatible and DSA-positive recipients received induction immunosuppressive therapy consisting of tacrolimus (0.1 mg/kg/day), mycophenolate mofetil (30 mg/kg/day), methylprednisolone (500 mg/day, subsequent decrease by half), and basiliximab (20 mg/day) at 0 and 4 days after transplantation. All recipients were maintained on the triple-drug combination of tacrolimus (trough-level, 3–5 ng/mL), mycophenolate mofetil (1000 mg/day), and methylprednisolone (4 mg/day) since 3 months after transplantation.

Diagnosis and Treatment of AMR

AMR was suspected based on elevated serum creatinine levels or urine protein levels above baseline and elevated anti-A/B antibody titer or presence of DSAs. Diagnosis of AMR was confirmed by 2 different pathologists using allograft biopsy according to the revised Banff 2017 classification.[18]

AMR was treated with bolus methylprednisolone (500 mg/day for 2 or 3 days) and 2 sessions of plasmapheresis (double-filtration plasmapheresis or plasma exchange). For steroid-resistant AMR, 1.5 mg/kg/day of rabbit anti-thymocyte globulin was administered over 12 hours for 5 days.

Statistical Analysis

For correlation analysis between the peripheral blood CD19-positive rate and count, we used the Spearman rank correlation coefficient. To identify the period of the peripheral blood CD19-positive rate to predict AMR, we used the univariate logistic regression analysis. In comparison between patients with and without AMR, the period of the peripheral blood CD19-positive rate with a statistically significant risk factor of AMR ($P < 0.05$) was determined to predict AMR. In each period to predict AMR, the CD19-positive rate cutoff value to predict AMR was most effectively determined using ROC curve analysis. In each period, the short- and long-acting groups were divided by the CD19-positive rate cutoff value.

Continuous variables are presented as means with SDs or medians with ranges, as appropriate. Categorical variables are presented as number of patients and percentages. Continuous variables were assessed using unpaired or paired *t* tests. Categorical variables were compared using the Fisher exact test. A statistically significant difference was determined when the 2-tailed *P* value was <0.05. All statistical analyses were performed using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan),[19] which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria).

TABLE 1.

Demographics of patients receiving rituximab as desensitization

	N = 42
Female, n (%)	16 (38.1)
Recipient age at transplantation, y, mean (SD)	49.0 (12.2)
Dialysis duration, day, median (range)	614 (0–5525)
Donor age at transplantation, y, mean (SD)	58.9 (10.1)
Unrelated donor, n (%)	24 (57.1)
ABO incompatible, n (%)	33 (78.6)
DSA positive, n (%)	15 (35.7)
Frequency of apheresis (n), mean (SD)	2.1 (1.4)
Dose of rituximab, mg, mean (SD)	159.5 (49.7)
Period after transplantation, day, median (range)	1151 (496–1966)
AMR, n (%)	8 (19.1)
Acute	5 (11.9)
Chronic	2 (4.8)
Acute and chronic	1 (2.4)
CMV IgG positive before transplantation, n (%)	31 (73.8)
CMV antigenemia-positive after transplantation, n (%)	25 (59.5)
Neutropenia after transplantation, n (%)	26 (61.9)

AMR, antibody-mediated rejection; CMV, cytomegalovirus; DSA, donor-specific antibody; IgG, immunoglobulin G; SD, standard deviation.

RESULTS

Patient Demographics

Demographics of 42 patients receiving rituximab as desensitization are shown in Table 1. AMR, acute AMR, and chronic AMR occurred in 8 (19.1%), 6 (14.3%, including one acute and chronic), and 3 (7.1%, including one acute and chronic) patients at 289 (5–1176), 98 (5–356), and 1147 (478–1176) days after transplantation, respectively. In patients with AMR, the median g score (quantitative criteria for glomerulitis) was 3 (0–3) and median ptc score (quantitative criteria for peritubular capillaritis) was 2 (1–3) using Banff lesion grading system. All allografts except one were functioning during the observation period. One ABO-incompatible recipient lost the allograft at 108 days after transplantation due to acute AMR.

Changes of the Peripheral Blood CD19-Positive Rate and Count

The peripheral blood CD19-positive rate and count promptly decreased after the first rituximab administration 14.5 ± 8.2% to 0.54 ± 0.72% ($P < 0.001$) and from 1185.6 ± 916.7/μL to 48.3 ± 64.6/μL ($P < 0.001$), respectively. Thereafter, the CD19-positive rate and count rebounded in some patients within 3–6 months after transplantation; conversely, in some patients, the CD19-positive rate and count were maintained at a low level over 12 months (Figure 1). A strong positive correlation was found between the peripheral blood CD19-positive rate and count in each period ($r = 0.87–0.97$).

Period of the Peripheral Blood CD19-Positive Rate to Predict AMR

On univariate logistic regression analysis, the peripheral blood CD19-positive rate at 6, 12, and 18 months after transplantation was a risk factor for AMR (Table 2). As a result, each of these periods after transplantation was determined for the period of the peripheral blood CD19-positive rate to predict AMR.

Peripheral Blood CD19-Positive Rate Cutoff Value to Predict AMR and Classification Into the Short- and Long-Acting Groups

At 6, 12, and 18 months after transplantation, the peripheral blood CD19-positive rate cutoff value to predict AMR most effectively using ROC curves was 4.4%, 6.4%, and 7.7%, respectively (Figure 2). The patients were divided into 2 groups according to the each rate cutoff value. As a result, the numbers of patients classified into the short- and long-acting groups were 7 and 35, 10 and 32, and 12 and 30, respectively, at 6, 12, and 18 months after transplantation, respectively.

Comparison Between the Short- and Long-Acting Groups

At 6, 12, and 18 months after transplantation, AMR incidence rate was significantly higher in the short-acting group than in the long-acting group (Table 3). The peripheral blood CD19-positive rate of all patients with AMR exceeded the cutoff value at 6, 12, or 18 months after transplantation. Conversely, serum creatinine level and tacrolimus trough-level at 1 year after transplantation, cytomegalovirus antigenemia-positive and neutropenia incidence rates during the observation period, and total dose of rituximab before transplantation were comparable between the groups at each time point (Table 3).

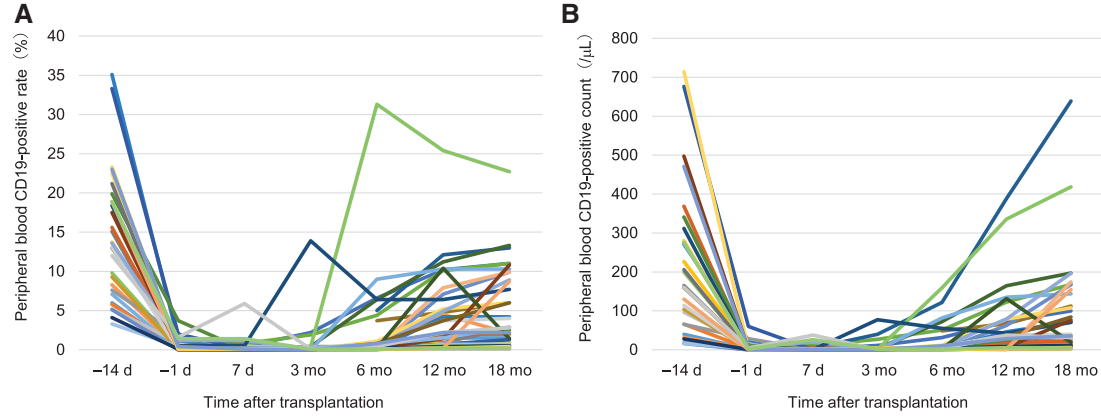


FIGURE 1. Time course of the peripheral blood CD19-positive rate (normal range, 10.0%–18.9%) (A) and the peripheral blood CD19-positive count (B) in all patients. CD19-positive count = white blood cell count (/μL) × %lymphocyte/100 × %CD19-positive cell/100.

TABLE 2.

Period of the peripheral blood CD19-positive rate to predict AMR

Time after transplantation	OR	95% CI	*P*
–14 day	1.01	0.88–1.17	0.86
–1 day	0.54	0.11–2.70	0.45
7 day	0.88	0.31–2.48	0.81
6 mo	1.51	1.08–2.10	0.015
12 mo	1.54	1.17–2.02	0.002
18 mo	1.46	1.13–1.89	0.004
24 mo	1.24	0.97–1.58	0.091
36 mo	1.36	0.98–1.88	0.069
48 mo	1.03	0.82–1.30	0.78

AMR, antibody-mediated rejection; CI, confidence interval; OR, odds ratio.

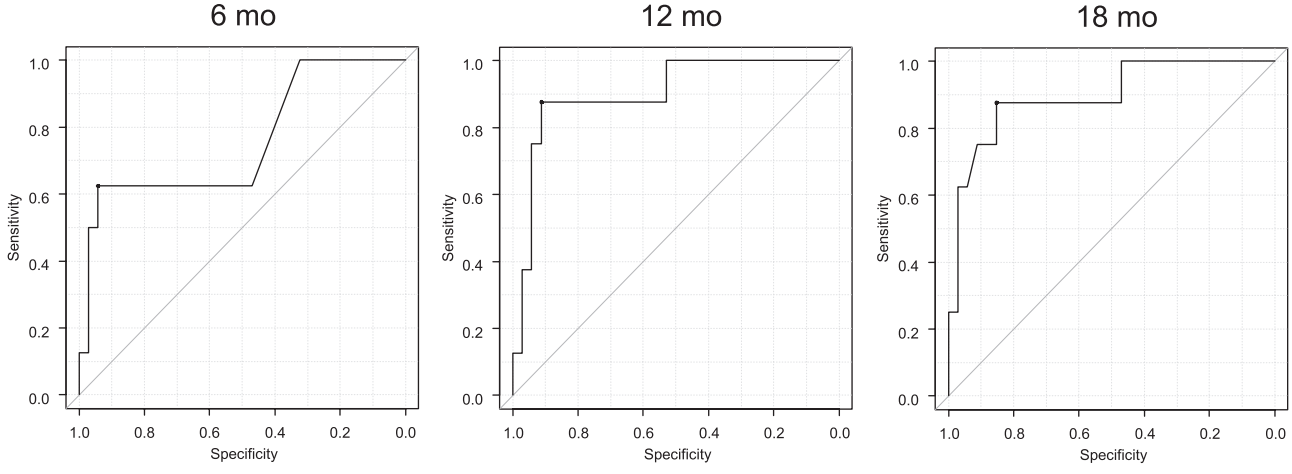
DISCUSSION

We demonstrated important clinical observations for AMR following rituximab administration in kidney transplant recipients and clarified the association between peripheral blood CD19-positive rate and AMR following rituximab administration in ABO-incompatible and DSA-positive kidney transplant recipients. Our findings proved a hypothesis that individual differences in rituximab-induced B-cell suppression affect AMR incidence after ABO-incompatible and DSA-positive kidney transplantation.

The risk of AMR after ABO-incompatible and DSA-positive kidney transplantation was higher in patients with short-term B-cell suppression following rituximab administration. Therefore, the peripheral blood CD19-positive rate should be measured regularly to predict AMR after kidney transplantation following rituximab administration as desensitization. If the peripheral blood CD19-positive rates were higher than the cutoff values, namely the short-acting group in this study, patients should be observed carefully for AMR. Moreover, additional rituximab administration may be considered to prevent AMR for B-cell suppression in patients with a CD19-positive rate higher than the cutoff value.

The mechanism of individual differences in rituximab-induced B-cell suppression in kidney transplant recipients is unknown. In our study, the total rituximab dose before transplantation did not affect the individual differences in rituximab-induced B-cell suppression. The host immunologic environment may be more influential than dose of rituximab administration. In the fields of hematology and autoimmune disease, recent studies have reported the association of some gene polymorphisms with the response to rituximab treatment in some diseases, such as *Fc-γ receptors* in non-Hodgkin lymphoma.[20-22] In organ transplantation, to our knowledge, only one study reported the effect of *Fc-γ receptor* polymorphism on rituximab-mediated B-cell depletion for complications after ABO-incompatible liver transplantation.[23] The analysis of gene polymorphism also in kidney transplant recipients before desensitization using rituximab may be useful for prediction of the AMR risk after kidney transplantation.

Several limitations of this study should be acknowledged. This was a retrospective and small study. Therefore, the possibility of unintentional selection bias and lack of analysis power cannot be completely excluded. Moreover, the



	6 mo	12 mo	18 mo
Cutoff value (%)	4.4	6.4	7.7
AUC	0.756	0.901	0.895
95% CI	0.538–0.973	0.779–1.000	0.762–1.000
Specificity (%)	94.1	91.2	85.3
Sensitivity (%)	62.5	87.5	87.5

FIGURE 2. Analysis of the peripheral blood CD19-positive rate cutoff value to predict antibody-mediated rejection using receiver operating characteristic curve at 6, 12, and 18 mo after transplantation. AUC, area under the curve; CI, confidence interval.

TABLE 3.

Comparison between the short- and long-acting groups

	6 mo after transplantation			12 mo after transplantation			18 mo after transplantation		
	Short-acting group	Long-acting group	*P*	Short-acting group	Long-acting group	*P*	Short-acting group	Long-acting group	*P*
N	7	35		10	32		12	30	
AMR, n (%)	5 (71.4)	3 (8.6)	0.001	7 (70.0)	1 (3.1)	<0.001	7 (58.3)	1 (3.3)	<0.001
Serum creatinine level, mg/dL, mean (SD)	2.32 (3.17)	1.29 (0.41)	0.061	2.00 (2.64)	1.29 (0.43)	0.14	2.03 (2.39)	1.23 (0.37)	0.079
Tacrolimus trough-level, ng/mL, mean (SD)	4.4 (1.9)	3.9 (1.4)	0.48	3.9 (1.8)	4.0 (1.4)	0.90	3.4 (1.8)	4.2 (1.3)	0.13
CMV antigenemia-positive, n (%)	5 (71.4)	20 (57.1)	0.68	7 (70.0)	18 (56.2)	0.49	9 (75.0)	16 (53.3)	0.30
Neutropenia, n (%)	4 (57.1)	22 (62.9)	1.0	7 (70.0)	19 (59.4)	0.72	8 (66.7)	18 (60.0)	0.74
Total dose of rituximab, mg, mean (SD)	171.4 (48.8)	157.1 (50.2)	0.49	180.0 (42.2)	153.1 (50.7)	0.14	158.3 (51.5)	160.0 (49.8)	0.92

AMR, antibody-mediated rejection; CMV, cytomegalovirus; SD, standard deviation.

observation period after transplantation was not long enough to analyze chronic AMR. Further long-term observation is needed to obtain stronger confirmation against our hypothesis. Furthermore, some antibody-producing cells, including noncirculating B cells and CD19-negative plasma cells, were not comprehensively measured in this study. However, CD19 is extensively expressed on B-cell lineage that develops into plasma cells. Thus, CD19 is a useful marker for predicting the antibody production.

In conclusion, we clearly demonstrated that individual differences in rituximab-induced B-cell suppression affect AMR incidence after ABO-incompatible and DSA-positive kidney transplantation. Additional rituximab administration after transplantation may prevent AMR in short-acting rituximab patients with a high CD19-positive rate.

REFERENCES

1. Sawada T, Fuchinoue S, Teraoka S. Successful A1-to-O ABO-incompatible kidney transplantation after a preconditioning regimen consisting of anti-CD20 monoclonal antibody infusions, splenectomy, and double-filtration plasmapheresis. *Transplantation.* 2002;74:1207–1210.
2. Sawada T, Fuchinoue S, Kawase T, et al. Preconditioning regimen consisting of anti-CD20 monoclonal antibody infusions, splenectomy and DFPP-enabled non-responders to undergo ABO-incompatible kidney transplantation. *Clin Transplant.* 2004;18:254–260.
3. Sonnenday CJ, Warren DS, Cooper M, et al. Plasmapheresis, CMV hyperimmune globulin, and anti-CD20 allow ABO-incompatible renal transplantation without splenectomy. *Am J Transplant.* 2004;4:1315–1322.
4. Tydén G, Kumlien G, Genberg H, et al. ABO incompatible kidney transplantations without splenectomy, using antigen-specific immunoadsorption and rituximab. *Am J Transplant.* 2005;5:145–148.
5. Fuchinoue S, Ishii Y, Sawada T, et al. The 5-year outcome of ABO-incompatible kidney transplantation with rituximab induction. *Transplantation.* 2011;91:853–857.
6. Takahashi K, Saito K, Takahara S, et al; IDEC-C2B8 ABO-I KTx Study Group. Results of a multicenter prospective clinical study in Japan for evaluating efficacy and safety of desensitization protocol based on rituximab in ABO-incompatible kidney transplantation. *Clin Exp Nephrol.* 2017;21:705–713.
7. Gloor JM, DeGoey SR, Pineda AA, et al. Overcoming a positive crossmatch in living-donor kidney transplantation. *Am J Transplant.* 2003;3:1017–1023.
8. Vo AA, Lukovsky M, Toyoda M, et al. Rituximab and intravenous immune globulin for desensitization during renal transplantation. *N Engl J Med.* 2008;359:242–251.
9. Loupy A, Suberbielle-Boissel C, Zuber J, et al. Combined post-transplant prophylactic IVIg/anti-CD 20/plasmapheresis in kidney recipients with preformed donor-specific antibodies: a pilot study. *Transplantation.* 2010;89:1403–1410.
10. Morath C, Beimler J, Opelz G, et al. Living donor kidney transplantation in crossmatch-positive patients enabled by peritransplant immunoadsorption and anti-CD20 therapy. *Transpl Int.* 2012;25:506–517.
11. Einecke G, Sis B, Reeve J, et al. Antibody-mediated microcirculation injury is the major cause of late kidney transplant failure. *Am J Transplant.* 2009;9:2520–2531.
12. Sellarés J, de Freitas DG, Mengel M, et al. Understanding the causes of kidney transplant failure: the dominant role of antibody-mediated rejection and nonadherence. *Am J Transplant.* 2012;12:388–399.
13. Sood P, Hariharan S. Anti-CD20 blocker rituximab in kidney transplantation. *Transplantation.* 2018;102:44–58.
14. Moreso F, Crespo M, Ruiz JC, et al. Treatment of chronic antibody mediated rejection with intravenous immunoglobulins and rituximab: a multicenter, prospective, randomized, double-blind clinical trial. *Am J Transplant.* 2018;18:927–935.
15. Jordan SC, Pescovitz MD. Presensitization: the problem and its management. *Clin J Am Soc Nephrol.* 2006;1:421–432.
16. Becker YT, Samaniego-Picota M, Sollinger HW. The emerging role of rituximab in organ transplantation. *Transpl Int.* 2006;19:621–628.
17. Barnett AN, Hadjianastassiou VG, Mamode N. Rituximab in renal transplantation. *Transpl Int.* 2013;26:563–575.
18. Haas M, Loupy A, Lefaucheur C, et al. The Banff 2017 Kidney Meeting Report: revised diagnostic criteria for chronic active T cell-mediated rejection, antibody-mediated rejection, and prospects for integrative endpoints for next-generation clinical trials. *Am J Transplant.* 2018;18:293–307.
19. Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. *Bone Marrow Transplant.* 2013;48:452–458.
20. Paiva M, Marques H, Martins A, et al. FcgammaRIIa polymorphism and clinical response to rituximab in non-Hodgkin lymphoma patients. *Cancer Genet Cytogenet.* 2008;183:35–40.
21. Ahlgrimm M, Pfreundschuh M, Kreuz M, et al. The impact of Fc-γ receptor polymorphisms in elderly patients with diffuse large B-cell lymphoma treated with CHOP with or without rituximab. *Blood.* 2011;118:4657–4662.
22. Liu D, Tian Y, Sun D, et al. The FCGR3A polymorphism predicts the response to rituximab-based therapy in patients with non-Hodgkin lymphoma: a meta-analysis. *Ann Hematol.* 2016;95:1483–1490.
23. Sakai H, Tanaka Y, Tazawa H, et al. Effect of Fc-γ receptor polymorphism on rituximab-mediated B cell depletion in ABO-incompatible adult living donor liver transplantation. *Transplant Direct.* 2017;3:e164.