Percentage of CD19$^+$ Cells in Peripheral Blood Lymphocytes After Rituximab-Based Desensitization as a Predictor of Acute Antibody-Mediated Rejection in ABO-Incompatible Kidney Transplantation

T. Hirose, D. Iwami*, K. Hotta, H. Sasaki, H. Higuchi, and N. Shinohara

Department of Renal and Genitourinary Surgery, Graduate School of Medicine, Hokkaido University, Sapporo, Japan

ABSTRACT

Background. Rituximab (RIT) is effective as a part of the desensitization therapy before ABO-incompatible kidney transplantation (ABOi-KTx), and a single dose of RIT at 375 mg/m^2 or less is recommended. However, adequate RIT dose recommendations have not yet been established for individual recipients. Therefore, we evaluated the relationship between the proportion of B cells in peripheral blood and acute antibody-mediated rejection (AAMR).

Methods. Forty-four consecutive ABOi-KTx recipients were enrolled in this retrospective study. Before transplantation, subjects were treated with RIT at various doses, ranging from 65 to 400 mg/body (46–263 mg/m^2), followed by plasmapheresis and intravenous immunoglobulin as a desensitization therapy. The percentage of CD19$^+$ cells in the total peripheral blood lymphocytes population (%CD19) was determined the day before transplantation. Transplant recipients were divided into 2 groups according to pretransplant %CD19, as follows: low %CD19 group, $\leq$ 1.2% (n = 35) and high %CD19 group, $>$ 1.2% (n = 9). The relationship between %CD19 and incidence of AAMR was evaluated, and the predicting factors for AAMR incidence were determined by univariate and multivariate analyses.

Results. The incidence of AAMR was significantly higher in the high %CD19 group than in the low %CD19 group (44.4% vs 5.7%, $P = .006$). Furthermore, multivariate analysis showed that %CD19 $>$ 1.2% was the only independent factor to predict AAMR, with an odds ratio of 14.31 ($P = .038$).

Conclusion. High %CD19 values after rituximab administration in ABOi-KTx recipients implies insufficient depletion of B cells, which can lead to AAMR.

ABO-INCOMPATIBLE kidney transplantation (ABOi-KTx) is well known to have a high risk of acute antibody-mediated rejection (AAMR) that can also cause allograft loss due to anti–blood type antibodies (Abs) in the recipient's serum. After the first successful ABOi-KTx procedure was reported by Alexandre et al, preconditioning protocols have included plasmapheresis (PP) to eliminate ABO Abs and removal of the spleen, a major organ where antibody-secreting cells reside [1]. In the past decade, administration of the anti-CD20 monoclonal Ab rituximab (RIT) has been found to be a safe and effective component of desensitization therapy, replacing splenectomy, in ABOi-KTx patients [2–4]. Depletion of B cells by RIT is more effective than splenectomy with regard to the reduction in the number of cells that have the potential to differentiate into Ab-secreting cells.

In Japan, desensitization of ABOi-KTx recipients by rituximab administration is an approved treatment, with a recommended dose $\leq$ 375 mg/m^2 in guidelines proposed by the Japan Society for Transplantation. An RIT dose of 375

*Address correspondence to Daiki Iwami, Kita 15-jo Nishi 7-chome, Kita-ku, Sapporo 060-8638, Hokkaido, Japan. Tel: (+81) 11 706 5966; Fax: (+81) 11 7067853. E-mail: iwamidaiki@ybb.ne.jp

0041-1345/19
https://doi.org/10.1016/j.transproceed.2019.01.127

mg/m^2 has also been reported to be adequate in many previous studies [2–5]. However, excessive reduction of B cells may lead to adverse events, such as susceptibility to infectious diseases [6]. It has been reported that cytomegalovirus infection and pneumonia occur more frequently in RIT-treated recipients than in the recipients without RIT treatment [7]. In addition, RIT treatment can cause severe neutropenia, as well as a prolonged recovery time of B cells in the peripheral blood for several months to more than 1 year [8]. Thus, some institutes have applied a fixed single dose of RIT at 100 or 200 mg/body as a desensitization regimen for ABOi-KTx recipients [9–11]. However, an appropriate indicator of the correct RIT dose has not been identified because of a lack of suitable methods to precisely evaluate B-cell depletion and biomarker levels to estimate the Ab-producing potential of recipients after RIT treatment. If the optimal number of B cells in the peripheral blood is determined, it would be possible to identify those patients who need additional RIT administration to further reduce the B-cell population to prevent AAMR.

Here, we hypothesized that the pretransplant proportion of CD19$^+$ B cells in the peripheral blood lymphocyte population may be a marker to predict the incidence of AAMR in ABOi-KTx patients. In this study, we aimed to determine the usefulness of monitoring the B-cell population after RIT-based desensitization therapy.

Table 1. Baseline Patient Characteristics

	Total (n = 44)
Age, y	54 (14–69)
Sex, M/F	28/16
Body mass index (kg/m^2)	22.0 (15.7–27.7)
Duration of HD, y	2.0 (0.1–14.2)
PEKT	9
Second KTx	2
Primary renal disease	
Diabetic nephropathy	10
ADPKD	7
IgA nephropathy	7
Nephrosclerosis	4
Congenital hypoplastic kidney	3
Obstructive nephropathy by urolithiasis	2
FSGS	1
Unknown	10
Relationship with donor	
Parent	13
Sibling	5
Spouse	26
Anti–blood type IgG Ab titer at referral	1:16 (1:2–1:2048)
Anti–blood type IgM Ab titer at referral	1:16 (1:4–1:256)

Where applicable, data are expressed as median (range).
Abbreviations: ADPKD, autosomal dominant polycystic kidney disease; Ag, antigen; CNI, calcineurin inhibitor; CsA, cyclosporine A; FSGS, focal segmental glomerulosclerosis; HD, hemodialysis; KTx, kidney transplantation; PEKT, preemptive kidney transplantation; TAC, tacrolimus.

PATIENTS AND METHODS

Patient Characteristics

Forty-four consecutive ABOi-KTx recipients (28 men and 16 women) were enrolled in this retrospective study. Those who had simultaneously performed donor-HLA specific Abs were excluded. Table 1 shows that the median age at transplantation was 54 years, and that the median pretransplant dialysis period was 2.0 years, as 9 recipients had undergone pre-emptive kidney transplantation (KTx). The primary disease of study subjects and the relationship of the recipients to the donors are described in Table 1. Median anti–blood type IgG Ab titer at referral was 1:16 (1:4–1:2048), and the median anti–blood type IgM Ab titer at referral was 1:16 (1:2–1:256). Approval was obtained from the institutional review board for the current retrospective study (Approval No. 016-0457).

Desensitization Protocol

The desensitization protocol is summarized in Fig 1. As the dose of RIT for ABOi-KTx recipients had changed historically in our institute, patients included in this study were treated with RIT doses ranging from 65 to 400 mg/body (46–263 mg/m^2) 2 weeks before transplantation, followed by PP and intravenous immunoglobulin (5 g/body for 3 consecutive days, starting on the day before KTx) as desensitization therapy. Exceptions to this protocol occurred in 6 recipients who had longer intervals between RIT treatment and KTx, ranging from 6 to 12 weeks, because of a very high titer of anti–blood type IgG Ab at referral (1:256–1:2048) in 4 patients or postponement of KTx in 2 patients.

All recipients were given basiliximab as an induction therapy and received triple-immunosuppressant maintenance therapy consisting of a calcineurin-inhibitor (CNI; tacrolimus or cyclosporine A), mycophenolate mofetil, and methylprednisolone. Thirty-three recipients underwent pretransplant PP, while the remaining 11 recipients did not undergo PP because of low titers of anti–blood type Ab (median, 1:8; range, 1:4–1:32).

Examination of Anti–Blood Abs and %CD19

An isohemagglutinin assay was performed using serial double dilutions of serum in test tubes to measure anti–blood type A and B Ab titers. The percentage of CD19$^+$ cells in the total peripheral blood lymphocyte population (%CD19) was examined the day before transplantation.

Diagnosis of AAMR

All incidence of AAMR were confirmed by allograft biopsy or diagnosed according to a remarkable rise in serum creatinine and anti–blood type Ab titers, as well as deterioration of graft perfusion, confirmed by ultrasonography. All pathologic diagnoses were made by pathologists in our institute based on the Banff criteria.

Statistics

For all data, results are expressed as medians with ranges. A receiver operating characteristic (ROC) curve was plotted for the relationship between AAMR incidence and %CD19. Determination of significance was performed using a Mann-Whitney U test or χ^2 test (GraphPad Prism; GraphPad, La Jolla, Calif, United States), with a value of $P < .05$ considered significant. Univariate and multivariate analyses were performed using logistic regression analysis (JMP 13; SAS Institute Inc, Cary, NC, United States) with $P < .05$ considered significant.

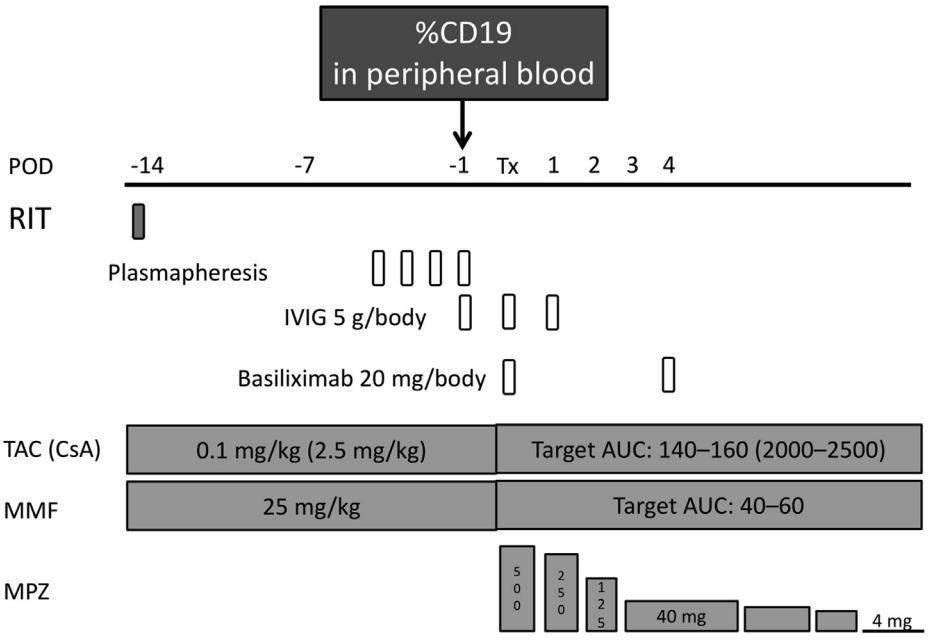

Fig 1. Desensitization protocol for ABO-incompatible kidney transplantation. %CD19, percentage of $CD19^+$ cells in the total peripheral blood lymphocytes population; AUC, area under the concentration curve; CsA, cyclosporine A; IVIG, intravenous immunoglobulin; MMF, mycophenolate mofetil; MPZ, methylprednisolone; POD, postoperative day; RIT, rituximab; TAC, tacrolimus; Tx, transplant.

RESULTS

Incidence of AAMR and Outcome of Antirejection Therapy

AAMR was observed in 6 recipients (13.6%). Five out of the 6 recipients were pathologically diagnosed as AAMR by allograft biopsy along with a rise in serum creatinine from post-transplant day 1 to 7 (median, 3 days). In the sixth recipient with AAMR, diagnosis was based on a remarkable rise in serum creatinine on post-transplant day 2 and deteriorated graft perfusion observed by ultrasonography without graft biopsy. All patients with AAMR were successfully treated with multimodal antirejection therapy, consisting of a steroid pulse, PP, RIT, intravenous immunoglobulin, and bortezomib. No allograft was lost during the course of antirejection therapies or during the observational periods.

Comparison of Pre–RIT-Treatment Clinical Characteristics Between Groups With and Without AAMR

In the comparison of clinical characteristics between groups divided by AAMR occurrence, there was no statistical difference in the age at transplant, sex, the number of mismatched HLA, CNI agent (tacrolimus or cyclosporine A), or incompatible blood (Table 2). Although there was no statistical difference between the groups, pretransplant %CD19 tended to be higher in the 6 recipients who developed AAMR than in recipients without AAMR (median, 1.5% vs 0.4%; $P = .1692$).

Evaluation of Risk Factors for AAMR

In the next investigation, we focused on %CD19 and, using ROC, assessed its ability to predict the risk of AAMR incidence. The ROC curve showed that a %CD19 value of 1.2% was the highest point of sensitivity and specificity for predicting AAMR incidence (Fig 2). Therefore, we adopted 1.2% as the %CD19 cutoff point for predicting the incidence of AAMR.

Next, we analyzed the risk factors of AAMR incidence, including %CD19 > 1.2%, using univariate and multivariate analyses. Age, CNI agent, anti–blood type IgG and IgM Ab titer at referral, anti–blood type IgG and IgM titer just before KTx, the period after RIT administration, the number of PP treatments, and RIT dose (mg/body or mg/m^2) were not significant predictive factors for AAMR. %CD19 > 1.2% was the only factor to predict AAMR incidence ($P = .018$, Table 3). Moreover, multivariate analysis also showed that %CD19 > 1.2% was the only independent factor to predict AAMR, with an odds ratio of 14.31 ($P = .038$).

Table 2. Comparison of Clinical Parameters

	AAMR (n = 6)	No AAMR (n = 38)	*P* Value
Age, y	43.9 (14.3–60)	54.6 (15.8–69.3)	.0857*
Sex, M/F	5/1	23/15	.2805†
HLA mismatch, n/6	2.5 (2–5)	3 (0–6)	.6498*
CNI agent, TAC/CsA	4/2	19/19	.4475†
Incompatible blood type Ag			.3737†
A	3	27	
B	3	9	
A and B	0	2	

Abbreviations: AAMR, acute antibody-mediated rejection; Ag, antigen; CNI, calcineurin inhibitor; CsA, cyclosporine A; TAC, tacrolimus.
*Mann-Whitney *U* test.
†χ^2 test.

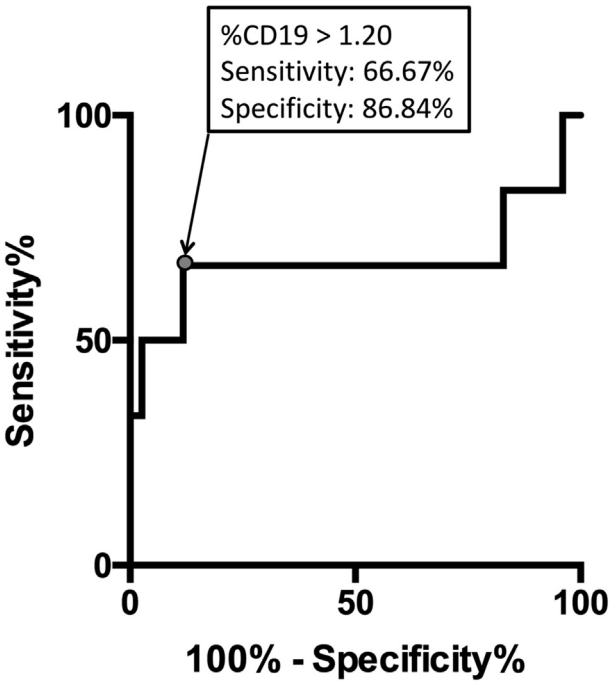

Fig 2. ROC curve to define the value for the division of high and low %CD19 B cells after rituximab treatment. %CD19, percentage of $CD19^+$ cells in the total peripheral blood lymphocytes population.

Discussion

CD20 is known as a B-cell surface antigen that is expressed only on pre-B and mature B cells. However, detection of CD20 is affected by RIT because RIT binds CD20 and interferes with the reaction of the reagents used in flow cytometric analysis. Another B-cell marker, CD19, is expressed on almost all B cells but is lost at later stages as B cells differentiate into plasma cells that are negative for both CD20 and CD19. Kamburova et al showed that the percentage of $CD19^+$ cells after treatment with a single 375 mg/m^2 dose of RIT on the day of KTx was equal to the percentage of $CD3^-CD4^-CD8^-CD14^-CD16^-CD56^-$ cells, which they defined as B cells, suggesting that CD19 is a useful B-cell marker after RIT administration [12].

B cells differentiate into plasma cells, which potently secrete Abs. Plasma cells reside in lymphoid organs, such as the lymph nodes, spleen, and bone marrow, and are barely detectable in the peripheral blood [13–15]. In addition, B 1 B cells expressing CD20 also secrete Abs against blood-type antigens, which mainly present in the peritoneal and pleural cavities and, much less commonly, in the peripheral blood [16,17]. Therefore, it seems difficult to precisely define a patient's potential to produce Abs by monitoring biomarkers obtained only from peripheral blood. However, our data suggested that, to some extent, the proportion of B cells surviving in the peripheral blood reflects the patient's potential to produce anti–blood type Abs.

The data we showed were obtained only from peripheral blood samples, not from lymph nodes, in which the density of B cells is much greater than in peripheral blood. Genberg et al demonstrated that a single 375 mg/m^2 dose of RIT leads to a marked reduction in the number of B cells in the lymph nodes and in peripheral blood, suggesting that a single dose of RIT is sufficient to eliminate B cells [8]. However, RIT treatment at a dose of 375 mg/m^2 induces infectious diseases more frequently than when RIT treatment is not given [7] and it causes severe neutropenia, with a prolonged peripheral B-cell recovery time of several years [8]. Our clinical goal is to prevent AAMR during the "critical period," which is suggested to be as short as 2 to 7 days after ABOi-KTx. After that period, recipients achieve the immunological status known as "accommodation" and, thereafter, do not experience AAMR caused by anti–blood type Abs [18]. Thus, we do not believe that complete or long-term depletion of the B-cell population by RIT is necessary. Therefore, we sought to establish a biomarker to determine the adequate dose of RIT by monitoring the proportion of peripheral B cells.

Table 3. Risk Factors for AAMR Incidence by Univariate and Multivariate Analyses

	Univariate Analysis			Multivariate Analysis		
	OR	95% CI	*P* Value	OR	95% CI	*P* Value
Age, y	0.953	0.897–1.006	.081	1.21	0.015–248.5	.936
%CD19 > 1.2%	9.852	1.133–132.4	.018	14.31	1.153–387.6	.038
CsA (vs TAC)	0.500	0.064–2.883	.443	–	–	–
Anti–blood type IgG Ab titer at referral	1.130	0.812–1.572	.456	–	–	–
Anti–blood type IgM Ab titer at referral	1.010	0.516–1.922	.976	–	–	–
Anti–blood type IgG Ab titer just before KTx	0.836	0.468–1.495	.548	–	–	–
Anti–blood type IgM Ab titer just before KTx	1.006	0.481–2.108	.986	–	–	–
Period after rituximab administration, d	1.050	0.934–1.180	.286	–	–	–
Number of PP	0.816	0.476–1.399	.454	–	–	–
RIT dose, mg/body	0.992	0.977–1.004	.225	–	–	–
RIT dose, mg/m^2/body	0.984	0.958–1.004	.138	–	–	–

Abbreviations: %CD19, percentage of $CD19^+$ cells in the total peripheral blood lymphocytes population; AAMR, acute antibody-mediated rejection; Ab, antibody; CI, confidence interval; CsA, cyclosporine A; KTx, kidney transplantation; OR, odds ratio; PP, plasmapheresis; RIT, rituximab; TAC, tacrolimus.

In the current study, a high percentage of $CD19^+$ B cells among the total peripheral blood lymphocyte population was seen after RIT administration. This implied an insufficient depletion of B cells, which can lead to AAMR in ABOi-KTx recipients. In contrast, the dose of RIT and the anti–blood type IgG and IgM Ab titers were not associated with the incidence of AAMR. Measurement of %CD19 may help to identify the patients who need additional administration of RIT to prevent AAMR. Our findings suggest that additional administration of RIT should be considered if % CD19 is higher than 1.2%. At present, to incorporate results and recommendations from other studies, it seems a good approach to administrate a fixed dose of 200 mg/body initially and then consider additional RIT treatment before ABOi-KTx for patients with high %CD19.

There are limitations in our study. This study was analyzed retrospectively and the number of patients was small. Therefore, the reliability of the results derived from these data may have been impacted by insufficient statistical power. In addition, the RIT dose in our desensitization protocol had a wide range from 65 to 400 mg/body (46–263 mg/m^2). However, there was no difference between AAMR incidence and RIT dose, and we demonstrated that high % CD19 was the only single risk factor for AAMR in ABOi-KTx. Our findings need to be confirmed in a larger cohort undergoing ABOi-KTx.

In conclusion, this is the first report of a clinical study describing the importance of B-cell monitoring with various RIT doses for predicting AAMR in ABOi-KTx recipients. Further investigation in a large cohort is required to confirm our findings and define a more precise %CD19 cutoff point to prevent AAMR incidence.

ACKNOWLEDGMENT

We are thankful to Editage (http://www.editage.com/) for editing a draft of this manuscript for English language.

REFERENCES

[1] Alexandre GJ, De Bruyere M, Squifelet JP, Moriau M, Latinne D, Pirson Y. Human ABO-incompatible living donor renal homografts. Neth J Med 1985;28:231–4.

[2] Tydén G, Kumlien G, Fehrman I. Successful ABO-incompatible kidney transplantations without splenectomy using antigen-specific immunoadsorption and rituximab. Transplantation 2003;76:730.

[3] Genberg H, Kumlien G, Wennberg L, Berg U, Tydén G. ABO-incompatible kidney transplantation using antigen-specific immunoadsorption and rituximab: a 3-year follow-up. Transplantation 2008;85:1745.

[4] Saito K, Nakagawa Y, Suwa M, Kumagai N, Tanikawa T, Nishiyama T, et al. Pinpoint targeted immunosuppression: anti-CD20/MMF desensitization with anti-CD25 in successful ABO-incompatible kidney transplantation without splenectomy. Xenotransplantation 2006;13:111–7.

[5] Barnett NA, Manook M, Nagendran M, Kenchayikoppad S, Vaughan R, Dorling A, et al. Tailored desensitization strategies in ABO blood group antibody incompatible renal transplantation. Transplant Int 2014;27:187–96.

[6] Okada M, Watarai Y, Iwasaki K, Murotani K, Futamura K, Yamamoto T, et al. Favorable results in ABO-incompatible renal transplantation without B cell-targeted therapy: advantages and disadvantages of rituximab pretreatment. Clin Transplant 2017;31: e13071.

[7] Baek C, Yang W, Park K, Han D, Park J, Park S-K. Infectious risks and optimal strength of maintenance immunosuppressants in rituximab-treated kidney transplantation. Nephron Extra 2012;2:66–75.

[8] Genberg H, Hansson A, Wernerson A, Wennberg L, Tyden G. Pharmacodynamics of rituximab in kidney allotransplantation. Am J Transplant 2006;6:2418–28.

[9] Shirakawa H, Ishida H, Shimizu T, Omoto K, Iida S, Toki D, et al. The low dose of rituximab in ABO-incompatible kidney transplantation without a splenectomy: a single-center experience. Clin Transplant 2011;25:878–84.

[10] Nakao T, Ushigome H, Kawai K, Nakamura T, Harada S, Koshino K, et al. Evaluation of rituximab dosage for ABO-incompatible living-donor kidney transplantation. Transplant Proc 2015;47:644–8.

[11] Hatakeyama S, Fujita T, Murakami R, Suzuki Y, Sugiyama N, Yamamoto H, et al. Outcome comparison of ABO-incompatible kidney transplantation with low-dose rituximab and ABO-compatible kidney transplantation: a single-center experience. Transplant Proc 2014;46:445–8.

[12] Kamburova EG, Koenen H, Joosten I, Hilbrands LB. CD19 is a useful B cell marker after treatment with rituximab: comment on the article by Jones et al. Arthritis Rheum 2013;65:1130–1.

[13] Benner R, Hijmans W, Haaijman J. The bone marrow: the major source of serum immunoglobulins, but still a neglected site of antibody formation. Clin Exp Immunol 1981;46:1–8.

[14] Smith K, Light A, Nossal G, Tarlinton D. The extent of affinity maturation differs between the memory and antibody-forming cell compartments in the primary immune response. EMBO J 1997;16:2996–3006.

[15] Nutt SL, Hodgkin PD, Tarlinton DM, Corcoran LM. The generation of antibody-secreting plasma cells. Nat Rev Immunol 2015;15:160–71.

[16] Springer GF, Horton RE. Blood group isoantibody stimulation in man by feeding blood group-active bacteria. J Clin Invest 1969;48:1280–91.

[17] Wuttke NJ, Macardle PJ, Zola H. Blood group antibodies are made by CD5+ and by CD5− B cells. Immunol Cell Biol 1997;75:icb199774.

[18] Takahashi K, Saito K. ABO-incompatible kidney transplantation. Transplantation Rev 2013;27:1–8.