Title	ABO Blood Incompatibility Positively Affects Early Graft Function: Single-Center Retrospective Cohort Study
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Introduction

Kidney transplantation is the renal replacement therapy that provides the greatest benefit for patients with end-stage renal failure.[1,2] ABO-incompatible kidney transplant (ABO-I KTx) have been performed to compensate for the low rate of deceased-donor organ transplants because of the increased demand for organs. ABO-I is only performed in living-donor kidney transplants because it requires extensive preconditioning therapy before transplant. Until about 2000, short-term graft survival was significantly poorer in ABO-I KTx because of the high incidence of early graft loss caused by acute antibody-mediated rejection. Preconditioning for desensitization and immunosuppressive such as splenectomy or rituximab with plasma exchange (PE) significantly decreased the incidence of acute antibody-mediated rejection in the setting of ABO-I KTx and contributed to favorable long-term kidney outcomes that were comparable to those of ABO-compatible (ABO-C) KTx.[3,4] A recent meta-analysis suggested that continual humoral immunosuppression by rituximab might lead to comparable or even better long-term graft survival for ABO-I KTx than that for ABO-C KTx.[5] However, the relationship between post-transplant early graft function and ABO incompatibility remains unclear.

Delayed graft function (DGF), which requires dialysis within the first week after transplantation, is known to be associated with poor clinical outcomes, increased rates of acute

rejection, prolonged hospital stay, and poor long-term graft survival. [6-11] DGF is uncommon in living-donor kidney transplant settings. Most living-donor graft recipients obtain favorable early graft function, although a very few recipients do not.

Slow graft function (SGF), which is defined as an allograft with slowly decreasing serum creatinine level (sCr) post-transplant but with renal functioning sufficient for avoiding dialysis,[12] has not received as much attention as DGF. However, the absence of DGF may not guarantee good graft function and survival rates.[12-15]

Recent retrospective cohort studies reported that SGF in living-donor kidney recipients might be associated with severe ischemic reperfusion/acute kidney injury (IR/AKI) caused by longer ischemic time or longer time to anastomosis.[15-19] Immune cells such as neutrophils, dendritic cells, macrophages, and lymphocytes contribute to the process of IR/AKI,[20] suggesting that the immune profile of the patient or the immunosuppression strategy might affect IR/AKI and post-transplant early graft function.

In this study, we used a single-institution database to assess the effect of ABO-I KTx on the incidence of SGF and early graft function.

Materials & Methods

Patients

For this retrospective cohort study, we evaluated 104 patients who had undergone living-donor kidney transplants at our hospital from May 2009 to July 2019. The following exclusion criteria were used: prerenal acute kidney injury, acute rejection, and aged < 18 years. Finally, we included 95 patients in this study. Twenty-four-hour Cr clearance values of all donors were obtained by collection of urine 2 days before the transplant procedure. The surgical records of both the donors and recipients; the sCr and estimated glomerular filtration rate (eGFR) on postoperative day (POD) 3; and eGFR at 1, 3, 6, and 12 months post-transplant were obtained until March 31, 2020. The eGFR values were estimated based on the following Japanese $eGFR_{creat}$ formula: eGFR (mL/min/1.73 m^2) = 194 x $sCr^{-1.094}$ x $age^{-0.287}$ x 0.739 (if female).[21]

Immunological testing was performed before the transplant. HLA typing at loci A, B, C, DR, and DQ was performed by the Luminex 200 system (Luminex, Inc., Austin, TX, USA). Alloantibody binding was measured by the LABScreen single antigen bead assay (Luminex, Inc.). Beads with a mean fluorescent index of > 500 were considered positive on donor-specific antibody (DSA) or cross-reactive group antigens (CREG). For all transplants, grafts were perfused with the Euro-Collins solution (KYOWA CritiCare Co., Ltd., Kanagawa, Japan).

This study was approved by the ethics board of our institution (research ID; 1911-006). Opt-out consents were obtained from every patient. The study procedures were carried out in accordance with the Declaration of Helsinki.

Preconditioning for desensitization and immunosuppression

Preconditioning for desensitization and immunosuppression were demonstrated in Figure. 1. The strategy was based on risks of an immunological graft rejection and recurrence of original disease in patients with focal segmental glomerulosclerosis (FSGS). Patients were classified into group 1 according to the following criteria: major ABO mismatch; a pair of ABO blood groups in which antibodies in the recipient's plasma bind and react with donor derived antigens (from a type-A donor to a type-B recipient, -A to -O, -B to -O, -AB to -O, -AB to -A, and -AB to -B), or positive for DSA, positive for CREG, or with FSGS. Patients with a minor ABO mismatch; a pair of different ABO blood types in which antibodies in the recipient's plasma do not bind or react with antigens from the donor (type-O donor to a type-A recipient, -O to -B, -O to -AB, -A to -AB, and -B to -AB) and otherwise not applicable to group 1 were classified as group 2. Patients with a low risk of graft rejection and otherwise not applicable to group 1 and group 2 were classified into group 3.

In group 1, low dose (200 mg/body) of rituximab was administered 2 weeks before surgery. Group 1 also underwent 2 or 3 sessions of double filtration plasmapheresis (DFPP) and PE. They received oral tacrolimus, mycophenolate mofetil for 2 weeks and prednisolone for one week before transplant. 20 mg/body of Basiliximab, an anti-CD 25 monoclonal antibody, was administered on the day of transplant surgery before skin incision and on POD 4 (Fig. 1A). In group 2, patient received 200 mg/body of rituximab and 20 mg/body of basiliximab in a similar way. They received oral tacrolimus, mycophenolate mofetil for 4 days before transplant. Prednisolone started on the day of transplant (Fig. 1B). In group 3, immunosuppression is the same as that of group 2 except administration of rituximab (Fig. 1C).

Definition of graft function

To our knowledge, the definition of SGF has not been clearly established. Various definitions have been employed in studies, and the previous studies have included deceased-donor kidney transplant cases.[12,22-24] Since incidence of DGF is more frequent in deceased-donor than in living donor, we should include only living-donor kidney transplants population to compare incidence of SGF as well. In this study, 2 definitions of SGF were adopted for the purpose of a better clinical meaning. Two definitions were used for SGF as follows: POD 3 sCr > 3 mg/dL and eGFR < 20

mL/min./1.73 m^2. Immediate graft function (IGF) was defined as one without SGF or DGF.

Outcomes

The primary outcome of this study was the association between ABO-I KTx and the incidence of SGF. Secondary outcomes were the risk factors that contributed to SGF and the differences of the eGFR at 1, 3, 6, and 12 months after transplant between SGF and IGF.

Statistical analysis

We used EZR software (Saitama Medical Center, Jichi Medical University, Saitama, Japan) for statistical analysis. Data were expressed as medians and range. The characteristics of recipients of ABO-I KTx and ABO-C KTx were evaluated by the Fisher exact test for nominal variables and the Mann-Whitney U test for continuous variables. Multivariable analysis with logistic regression was used to identify risk factors associated with SGF according to the 2 definitions which were based either on a specified sCr or eGFR on POD3. The following covariates were used in regression analysis: ABO incompatibility and CIT. CIT were converted to nominal variables. The cut-points were set at median values. CIT was defined as the time from the start of cold perfusion of the graft and to unclamping after vascular anastomosis. CIT was selected as variable based on

previous reports [15-19] and clinical experience. After the results based on the first definition of SGF were statistically analyzed, the results based on the second definition of SGF were analyzed. The differences of mean eGFR at 1, 3, 6, and 12 months after transplant between SGF and IGF were evaluated using Mann-Whitney U test. All *P* values were two-sided, and $P < 0.05$ considered to indicate statistical significance**.**

Results

A total of 95 recipients were evaluated in this study. Nine patients were excluded (acute rejection n = 3, aged younger than 18 years n = 3, circulatory failure n = 2, and dehydration caused by diarrhea n = 1). A total of 34 patients received ABO-I KTx. The clinical characteristics of patients are shown in Table 1. The Mann-Whitney U test showed that the sCr and eGFR values on POD3 of the ABO-I patients were significantly lower and significantly higher, respectively, than those values of the ABO-C patients. Table 2 shows the distribution of preconditioning for desensitization for the ABO-I and ABO-C KTx recipients. According to our protocol, low dose (200mg/body) of rituximab and PE were administered to all ABO-I patients (major mismatch). All patients with a minor mismatch were administered low dose of rituximab. Figure 2 shows the distribution of patients with SGF according to sCr > 3 mg/dL or eGFR < 20 mL/min./1.73 m^2 on POD3. Nineteen and 21 patients were found to have SGF for elevated sCr or decreased eGFR on

POD3, respectively. None of the patients required dialysis after undergoing the transplant. The associations between the tested covariates and incidence of SGF are shown in Table 3. For patients with POD3 sCr > 3 mg/dL, ABO incompatibility was associated with a significantly decreased risk for SGF by multivariate analysis (OR, 0.15; 95% CI, 0.03-0.7; P = 0.02). CIT > 150 minutes led to significantly increased risks of SGF (OR, 6.5; 95% CI, 1.7-25; P = 0.006). For patients with POD3 eGFR < 20 mL/min./1.73 m^2, ABO incompatibility was associated with a significantly decreased risk for SGF (OR, 0.13; 95% CI, 0.03-0.63; P = 0.011). The ORs (95% CI) of prolonged CIT for incidence of SGF was 5.25 (1.55-17.8; P < 0.008).

Figure 3 shows the changes in mean eGFR over time in patients who underwent transplant with subsequent SGF and IGF. The Mann-Whitney U test showed that the mean eGFR in patients with SGF was significantly lower than the eGFR in patients with IGF, as assessed by POD3 sCr > 3 mg/dL and eGFR < 20 mL/min./1.73 m^2, over the first 6 months after transplant surgery, while statistical significance had disappeared by 12 months after surgery. No graft loss was identified within 1 year after transplant in entire population.

Discussion

This study found that patients who received living-donor ABO-I KTx had a significantly decreased risk of SGF. A CIT > 150 minutes led to significantly increased risk of SGF. In addition, the renal function of recipients with SGF remained reduced up to 6 months after transplant. These results were consistent for both the definition we used to identify SGF (sCr and eGFR on POD 3). To our knowledge, this is the first paper to demonstrate that preconditioning therapy for ABO blood incompatibility positively affect early graft function.

A number of reports on early graft function after kidney transplant surgery were focused primarily on the clinical impact and risk factors of DGF. Very few clinical studies have evaluated the outcomes and predictive factors of SGF among the living-donor kidney transplant population. We excluded patients with postoperative renal dysfunction caused by anything other than IR/AKI to eliminate bias. We analyzed by two covariates including ABO incompatibility and CIT given the small sample size. However, the definition of SGF by an eGFR value allowed to eliminate the effects of gender and age. In addition, our use of 2 methods to define and identify SGF, which included sCr and eGFR on POD 3, increased the reliability of our findings. The innate immune response to the reperfused graft begins within minutes of reperfusion, whereas the adaptive immune response requires days to manifest.[25] Given that rigorous preoperative

immunosuppression of the ABO-I recipients decreased the incidence of SGF, rigorous preoperative immunosuppression should be considered as affecting the innate immune response. Additionally, an in vitro study showed that knockout mice lacking both B and T cells showed decreased severity of injury from IR/AKI, and the adoptive transfer of CD4+ T cells from wild type mice reconstituted the severity of injury.[26] Regulatory T cell have been suggested to be involved in repair after ischemic reperfusion injury. [27,28] Together, these reports support the possibility that preconditioning for desensitization of ABO-I KTx recipients affects the incidence of SGF by means of some immunological mechanism.

According to our results, a CIT > 150 minutes also led to an increased incidence of SGF. A prolonged CIT had been suspected to cause DGF and medical and surgical complications.[18,19] These studies differed from our study in that the participants were kidney transplant recipients mainly from deceased donors, and the CIT cut-points were as long as 1080 minutes or longer. It is conceivable that a prolonged CIT can induce systemic upregulation of cytokines and oxidative stress, and lead to increased severity of IR/AKI. To our knowledge, no clinical studies have investigated the association between CIT and early graft function in the living-donor kidney transplant setting. It is important to maintain CIT for less than 150 minutes to prevent SGF, even in the living-donor kidney transplant setting.

We compared the renal function of recipients with SGF and IGF at 1, 3, 6, and 12 months after transplant. Previous studies reported that the eGFR of patients with SGF was lower than that of patients with IGF and equivalent to that of patients with DGF at 1 month after surgery.[14,29,30] Wang suspected that ischemic reperfusion injury, if severe, would have long-term implications in the case of DGF.[14] A prolonged CIT may have long-term implications also in SGF populations. In this study, the bias affecting post-transplant graft function (e.g., acute rejection) was removed as much as possible. However, our result was similar to the results of the previous reports. Therefore, we are more convinced that our belief that SGF exerts an adverse effect on function outcome up to 6 months after transplant surgery is true.

This study has several limitations. First, the definition of SGF had been poorly established and we created *de novo* definitions for this study. We tried to use rigorous definitions of SGF, since most living-donor kidney transplant recipients obtain better early graft function than deceased kidney transplant recipients do. We adopted a Cr value to define SGF by reference to the other reports,[12,22-24]. A previous report used the relative Cr change as a definition of SGF for KTx including deceased-donor cases.[14] However, it was highly expected to improve renal function as early as 3 days after transplantation owing to better early graft function in living-donor kidney transplantation, even if the preoperative Cr level of recipient was very high. Therefore, the definition of Cr value was more practical in living-donor kidney transplantation. Second,

recipients with prerenal acute kidney injury or acute rejection were excluded from the study. Given that ABO-I KTx recipients have an increased risk for bleeding complications or acute antibody-mediated rejection in the early post-transplant period, the exclusion of the above patients may have affected the primary outcome of the study. Third, there remain unanswered questions why ABO-I KTx was likely to exert beneficial effects on the decreased incidence of SGF. We should have considered more the impact of desensitization therapy, including rituximab, to the IR/AKI and early graft function, rather than ABO incompatibility. Because of the small number of cases, we were not able to perform a stratified analysis, as follows: DSA-positive versus -negative cases and rituximab therapy with or without plasma exchange. We could not conclusively identify which immunosuppressive therapy effectively prevented SGF. Further study is needed to clarify the association between immunosuppression and IR/AKI.

In summary, ABO-I KTx decreased the incidence of SGF. Poor graft function with SGF remains up to 6 months after transplant surgery.

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Conflicts of interest

None declared.

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