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Authorship

Hiroki Ishihara: designed the work, performed the analysis and interpretation of data for the work, and

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Hideki Ishida: designed the work and revised critically for important intellectual content

KU: collected data and pathological work

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KO: collected data

TS: pathological work

KT: revised the work critically for important intellectual content, and contributed important regents

Abbreviations

MVI, microvascular inflammation; DSA, donor-specific anti-HLA antibodies; eGFR, estimated

glomerular filtration rate; AMR, antibody-mediated rejection; TMR, T-cell mediated rejection

Abstract

Background: In ABO-incompatible kidney transplantation, the diagnostic criteria for antibody-mediated rejection remain controversial because C4d deposition is commonly observed. Thus, we investigated microvascular inflammation (MVI score ≥ 2) within one year as a predictor of graft outcome.

Methods: A total of 148 recipients without preformed or *de novo* donor-specific anti-HLA antibody were stratified based on MVI score < 2 (n = 117) and MVI score ≥ 2 (n = 31).

Results: We found that 5-year graft survival was significantly lower ($p = 0.0129$) in patients with MVI (89.8 %) than in patients without MVI (97.0 %). Graft function, as characterized by serum estimated glomerular filtration rate, was also significantly worse for patients with MVI than it was for patients without MVI, between 3 months and 10 years after transplantation ($p = 0.048$). Multivariate analysis indicated that HLA class II mismatch ($p = 0.0085$) was an independent marker of MVI.

Conclusions: MVI score ≥ 2 is significantly associated with poor graft outcome following ABO-incompatible kidney transplantation. We suggest that MVI score ≥ 2 in ABOi transplantation be used as a basis to diagnose AMR.

Introduction

A serious shortage of organ donors has persisted in Japan over several decades despite widespread efforts to promote kidney donation at death [1-3]. To overcome this issue, transplantation across the ABO blood type barrier has been attempted in Japan [2] and throughout the world [4], and we have devised immunosuppressive regimens and protocols to ensure effectivity and safety [3,5-7]. Recent systematic reviews also suggest that the modern preconditioning therapies, including administration of rituximab, immunoadsorption, and apheresis therapy, contribute to the achievement of graft outcomes equivalent to those achieved by ABO compatible transplantation [8,9]. In the end, ABO-incompatible (ABOi) kidney transplantation promotes increased use of limited resources.

Anti-blood type antibodies were previously considered a lethal factor driving hyper-acute rejection or graft-loss in cases of ABOi transplantation. However, over the course of developing immunosuppressive regimens, neither preoperative nor postoperative anti-blood type antibodies were observed to influence graft outcome in ABOi transplantation[10,11]. Rather than anti-blood type antibodies, we suggested that donor-specific anti-HLA antibodies (DSA) were a significant factor driving development of antibody-mediated rejection (AMR), and a predictor of inferior graft outcome in ABOi transplantation[12]. Bentall et al. also suggested that positive crossmatch kidney transplantations had significantly worse graft outcomes than ABOi transplantations[13]. Moreover, an immunohistochemical study of a human ABOi transplant sample revealed that the endothelium injury

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due to blood-type antigen did not impact the long-term graft outcome [14].

AMR is a critical, well-known determinant of graft survival [6,12,15-17], and is typically diagnosed according to the Banff criteria, which include microvascular inflammation (MVI), C4d deposition, and DSA [18,19]. When diagnosing AMR in transplantation of standard immunological risk, the presence of C4d deposition is regarded as an effective marker [20,21]. However, because C4d deposition is commonly observed in ABOi transplantation, its diagnostic value remains controversial [6,17,22]. Couzi et al.[23] reported that 13/30 (43.3%) patients with C4d deposition have no rejection of ABOi transplantation. Using 89 biopsies from ABOi transplantations, Setoguchi et al. [6] reported C4d deposition in 94% of the studies patients (C4d 3 was in 66%), although AMR occurs for only 27% of these patients. Moreover, a novel immunosuppressive agent such as eculizumab, which is an antibody against C5 that blocks the terminal complement cascade, may cause difficulty in using C4d deposition to diagnose AMR [24,25]. In this context, the role of C4d deposition as an indicator of AMR seems unclear.

Thus, MVI score ≥ 2 may be a more important indicator than C4d presence for diagnosing AMR in the ABOi transplant population. MVI, which typically presents as glomerulitis and peritubular capillaritis, is considered a strong indicator, with MVI score ≥ 2 sometimes used to diagnose rejection even in the absence of C4d deposition [21] as long as DSAs are also detected[19]. MVI score ≥ 2 with circulating DSA correlates with transplant glomerulopathy independently of C4d deposition. MVI score ≥ 2 is also associated with other pathologies such as glomerulonephritis [21,26]. Accordingly, MVI

score ≥ 2 is regarded as a significant predictor of graft outcome [19-21,27,28]. This impact has been demonstrated for standard immunological risk, but not ABOi transplantations.

To clearly define the diagnostic value of MVI score ≥ 2 regardless of C4d deposition, we investigated the relationship between MVI score ≥ 2 and graft outcome in ABOi patients without preformed or *de novo* DSA.

Materials and Methods

Study design and patients

Between January 2001 and January 2015, a total of 227 patients at our department and affiliated institutions received ABOi kidney transplants from living relatives. Several patients were excluded due to preformed (n = 55) and *de novo* (n = 1) DSA within one year after transplantation, as indicated by fluorescence intensity > 1,000 on a Luminex single-antigen beads assay (One Lambda Inc., Canoga, Park, CA, USA). Patients without allograft biopsy within one year after transplantation (n = 20), were also excluded, as were three patients with missing data. During follow-up, *de novo* DSA developed in one patient after 15 months (DR13), and in another patient after 33 months (DQ6). These two patients were included in the analysis, which was based on pathological findings within one year following transplantation.

The remaining 148 recipients were stratified into two groups based on MVI score < 2 (n=117) and

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MVI score ≥ 2 (n = 31, Figure 1). The threshold ≥ 2 was selected, because the aim was to evaluate the

impact of MVI on graft outcome independent of C4d deposition [19], which was defined to be focal or

diffuse C4d score ≥ 2. Clinical and laboratory data were extracted from an electronic database and

from medical records. If multiple biopsies were obtained from a patient, the highest score was used to

determine MVI status. The MVI score was calculated as the sum of g (glomerulitis) and ptc

(peritubular capillaritis) scores. Recipients who lost grafts were excluded from analysis at timepoints

beyond loss of the graft. All study procedures were approved by the Institutional Review Board of

Tokyo Women's Medical University (ID: 3824), and were in accordance with the Declaration of

Helsinki and Istanbul 2008.

Graft function

Graft function was compared between groups based on serum estimated glomerular filtration rate

(eGFR) at various timepoints after transplantation. eGFR was calculated according to the formula

developed by the Japanese Society of Nephrology [29], in which eGFR (mL/min/1.73m^2) = 194 $\times$ [Serum

creatinine (mg/dL)]$^{-1.094}$ $\times$ [Age (year)]$^{-0.287}$ ($\times$ 0.739, if female).

Immunosuppressive and desensitization protocols

Multiple immunosuppressive and desensitization protocols, shown in Figure 2, are used in our

institutions. Immunosuppressive regimens, in which recipients were administered tacrolimus, mycophenolate mofetil, and methylprednisolone, have been described in detail previously [3,5]. After 2002, recipients also received basiliximab perioperatively [7]. Splenectomy was performed during transplantation until 2004, after which point rituximab was administered instead, at an intravenous dose of 200 mg per person 5-7 days before transplantation [30,31].

Removal of anti-blood type antibodies

To remove anti-blood type antibodies, recipients underwent 3-4 sessions of double plasmapheresis before transplantation [3,5,11]. Plasmapheresis was performed using OP-05H (ASAHI Medical Co. Ltd., Osaka, Japan) and Evaflux 2A (Kuraray Co Ltd., Osaka, Japan) plasma separators until titers decreased to 1:32 or below.

Anti-blood type IgG is difficult to detect by hemagglutination of pentameric IgM. Thus, we have developed an ELISA assay specific for anti-blood type IgG. However, indirect Coomb's test was used instead to measure IgG titers every other month, as the ELISA assay is not yet accepted worldwide. IgM against blood type A and B was quantified using saline and/or Bromerin agglutination techniques, as specified in our protocol.

Routine and for-cause biopsy

Written informed consent for biopsy was obtained from all patients. As previously described [6],

patients with stable allograft function underwent biopsy 1, 3, 6, and 12 months after transplantation,

and then yearly, if possible.

All patients underwent monthly follow-up blood examinations. For-cause biopsy was performed only

if serum creatinine is measured at < 2 mg/dL, proteinuria less than 1 g/day is observed, and kidney

function is unstable, as indicated by > 15 % variation in serum creatinine two weeks before and after

biopsy. That is, variation in post biopsy serum creatinine, or decreasing serum creatinine, are not

always indications for additional biopsy.

Two core biopsies were obtained under ultrasound guidance using a spring-loaded 16-gauge biopsy

gun. One biopsy was snap-frozen, while the other was fixed in 10 % formalin, embedded in paraffin,

serially sectioned at 3 μm, and stained with hematoxylin and eosin, Masson trichrome, periodic acid-

Schiff, and periodic acid-methenamine silver. Tissues were examined under a light microscope by the

same blinded pathologist, and scored using Banff'09 [18] and '13 classification [19]. A biopsy with seven

or more glomeruli and at least one arterial cross-section was considered adequate, and unsatisfactory

specimens were excluded from analysis.

C4d staining in the peritubular capillaries

C4d staining was routinely performed on all renal allograft biopsies by indirect immunofluorescence

on cryostat sections. The staining used a mouse monoclonal anti-human C4d antibody (Quidel, San

Diego, CA) at 1:40 dilution, followed by fluorescein isothiocyanate-conjugated goat anti-mouse IgG.

Peritubular capillaritis C4d stain extent and intensity were evaluated semi-quantitatively as follows:

negative (< 25% of ptc C4d stained), focal C4d positive ($\geq$ 25% of specimen but < 75%) or diffuse

and bright C4d staining (nearly all ptc in the cortex and medulla). We also examined pre-transplant

biopsies by immunofluorescence microscopy.

Statistical analysis

Continuous variables were analyzed by Mann-Whitney U-test, and categorical variables were

analyzed by χ^2 or Fisher's exact test. Graft survival was calculated using Kaplan-Meier methods, and

compared using the log-rank test and Wilcoxon's test, respectively. Graft survival after transplantation

was defined as the time between transplantation and graft loss due to any cause.. Univariate and

multivariate logistic regression were used to identify predicators of MVI score Graft function

according to mean serum eGFR was compared using a mixed-effects model for repeated-measures

data $\geq$ 2. Risk was expressed in odds ratios and 95 % confidence intervals. An independent statistical

data center (STATZ Institute, Tokyo, Japan) performed the analysis using JMP® 11 (SAS Institute Inc.,

Cary, NC, USA), and SAS 9.1 (SAS Institute Inc., Cary, NC, USA) software packages. Differences

with p-value < 0.05 were considered statistically significant.

Results

Patient characteristics

Patient characteristics with (MVI score ≥ 2) or without MVI (MVI score < 2) are summarized in

Table 1. There were no significant differences in clinical characteristics including recipient age and

sex, kidney disease, duration of dialysis, donor age and sex, and follow-up period (all $p > 0.05$). There

were also no significant differences in immunological parameters such as ABO group ($p = 0.498$),

HLA class I mismatch, and baseline, preoperative, and postoperative anti-blood group IgG (all $p >$

0.05). However, HLA class II mismatch was significantly more frequent in patients with MVI score ≥

2 ($p = 0.0072$). In addition, all patients also appeared to have received comparable immunosuppressive

treatments, and there were no significant differences in basiliximab induction, rituximab induction,

splenectomy status, and preoperative double plasmapheresis (all $p > 0.05$). However, patients with

MVI score ≥ 2 underwent significantly more allograft biopsies ($p < 0.0001$). In addition, one patient

from each group had *de novo* DSA after the first post-transplantation year (1 patient out of 31, or

3.23%, for the MVI ≥ 2 group; for the MVI < 2 group. 1/117, or 0.85%).

Graft survival

Graft survival in patients with high or low MVI score is plotted as Kaplan-Meier curves in Figure 3.

Graft survival over five years was significantly lower in patients with MVI score ≥ 2 than it was in patients with low MVI scores, according to Wilcoxon's test (89.8 % vs. 97.0 %, $p = 0.0129$). The log-rank test indicated only a marginal graft survival difference between these two groups ($p = 0.0878$). Grafts were lost during this period in three recipients with MVI score < 2, and in three recipients with MVI ≥ 2. In the former, grafts were lost at 1786 days, 3293 days, and 253 days, respectively, due to recurrent focal segmental glomerulosclerosis, choric rejection, and vascular rejection. In the latter, grafts were lost at 20 and 1 day due to hyper-acute rejection, and at 325 days due to choric rejection. For the MVI score ≥ 2 treatment, we performed steroid pulse, intravenous immunoglobulin therapy, and plasmapheresis for 29 (93.5%), 6 (19.4%), and 3 (9.68%) patients, respectively. We administered additional rituximab, deoxyspergualin, and muromonab-CD3 for 4 (12.9%), 6 (19.4%), and 3 (9.68%) patients, respectively.

Pathological findings

A total of 292 biopsies were obtained, of which 188 were routine, and 104 were for-cause. We compared Banff scores and pathological diagnosis rates between patients with MVI score ≥ 2 and those with MVI score < 2. These comparisons focused on T-cell mediated (TMR) and interstitial fibrosis/tubular atrophy within the first year following transplantation; we defined these pathologies according to the Banff criteria[32].

In Table 2, pathological findings based on Banff criteria are compared between patients with high or low MVI scores. All Banff scores were significantly higher in patients with MVI score ≥ 2 (all $p <$ 0.05). C4d deposition (C4d score ≥ 2) was observed in a total of 126 recipients (85.1%), with comparable frequency between patients with low (83.8 %) and high MVI scores (90.3 %, $p = 0.570$). However, very high C4d scores (> 3) were significantly more frequent in patients with MVI score ≥ 2 (51.6 %) than in patients with MVI score < 2 (19.7 %, $p = 0.0009$), along with TMR ($p = 0.0001$) and interstitial fibrosis/tubular atrophy ($p = 0.0143$).

Graft function

Figure 4 illustrates mean serum eGFR for the two groups. Mean serum eGFR at 3 months was 40.0 mL/min/1.73m^2 in the MVI score ≥ 2 group and 45.6 mL/min/1.73m^2 in the MVI score < 2 group. Mean serum eGFR between 3 months and 10 years was reduced by 3.7 mL/min/1.73m^2 in the MVI score ≥ 2 group and increased by 5.5 mL/min/1.73m^2 in the MVI score < 2 group. Throughout the study, serum eGFR was significantly inferior for patients with MVI score ≥ 2, relative to those with MVI score < 2 ($p = 0.048$).

Graft survival according to MVI localization in patients with MVI score ≥ 2

To evaluate the impact of MVI localization on graft survival, we divided the patients into two

subgroups as follows: patients with peritubular capillaritis only (ptc score $\geq$ 2) and patients with glomerulitis (g score $\geq$ 1) and peritubular capillaritis (ptc score $\geq$ 1). Among 31 patients with MVI score $\geq$ 2, peritubular capillaritis was observed in 9 patients, while glomerulitis and peritubular capillaritis were both observed in the remaining 22. There were no significant differences in 5-year graft survival (Figure 5) between these patients (100.0 % vs. 85.9 %, Log-rank test $p = 0.268$; Wilcoxson's test $p = 0.269$). No patient presented glomerulitis alone.

Graft function according to MVI localization in patients with MVI score $\geq$ 2

Figure 6 illustrates mean serum eGFR for the two groups. Mean serum eGFR at 3 months was 40.6 mL/min/1.73m^2 in patients with peritubular capillaritis alone and 39.7 mL/min/1.73m^2 in patients with both glomerulitis and peritubular capillaritis. Mean serum eGFR between 3 months and 8 years was increased by 8.5 mL/min/1.73m^2 in patients with peritubular capillaritis alone and decreased by 3.0 mL/min/1.73m^2 in patients with both glomerulitis and peritubular capillaritis. Throughout the study, serum GFR did not differ significantly between these groups ($p = 0.788$).

Prognostic indictors for MVI score $\geq$ 2

As shown in Table 3, univariate logistic regression indicated that predicators of MVI score $\geq$ 2 include HLA class II mismatch (OR 4.85, $p = 0.0038$), C4d score (OR 2.45, $p = 0.0039$), and number

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of biopsies (OR 11.4, $p < 0.0001$). Multivariate analysis also indicated that HLA class II mismatch (OR 5.10, $p = 0.0093$) was an independent predictor after adjusting for number of biopsies (OR 10.1, $p < 0.0001$). High C4d scores were also independently associated with MVI score, but not to a statistically significant extent ($p = 0.0627$).

Discussion

Previous studies have identified MVI score ≥ 2 as a risk factor for poor graft outcomes [19-21,27,28]. For example, Sis et al. [21] reported that MVI in late biopsies may indicate AMR, whereas MVI in early biopsies often indicated antibody-independent pathologies, including TMR and acute tubular necrosis. Accordingly, they proposed a decision tree algorithm that includes C4d deposition as a marker to help diagnose AMR in early biopsies. However, this algorism is not suitable for use in ABOi transplantation, because C4d deposition is frequently observed [6,17,22]. Notably, they also found that MVI score may predict graft loss independently of time, C4d deposition, and transplant glomerulopathy. Subsequently, de Kort et al. [20] demonstrated that high MVI scores were significantly associated with poor graft survival and *de novo* DSA. However, the diagnostic value of MVI score ≥ 2 independent of preformed or *de novo* DSA is not clear for ABOi recipients.

In this analysis, we demonstrate for the first time that MVI score ≥ 2 was significantly associated with poor graft outcome in patients who received kidney transplantation from ABOi living relatives,

regardless of C4d deposition. We note, however, that a high C4d score, *i.e.*, C4d score 3, was

associated with MVI score ≥ 2. In any case, graft survival and function were significantly lower soon

after and years after transplantation in patients with MVI score ≥ 2, as indicated by Banff scores and

other parameters, in line with Gupta et al. [33], who demonstrated that high MVI scores are associated

with choric injury and inflammation. We also found that these patients were also more likely to develop

TMR and interstitial fibrosis/tubular atrophy, as noted by Setoguchi et al.[6], who reported that

interstitial inflammation associated with interstitial fibrosis/tubular atrophy may negatively impact

long-term graft outcome. Finally, we noted high i and t scores (tubulointerstitial inflammation) in

patients with MVI score ≥ 2, perhaps confirming the observation by Couzi et al.[23], who suggested that

C4d deposition due to tubulointerstitial inflammation (i > 0 with or without tubulitis) was strongly

associated with choric graft dysfunction in ABOi transplants. To further evaluate the impact of MVI

score on graft outcome, we compared graft survival between patients with MVI score 0 and those with

score 1. The influence of low magnitude MVI score such as 1 is unclear. We did not observe any

significant differences between these two subgroups (data not shown).

Meanwhile, we observed that localization of MVI either as peritubular capillaritis alone or in

combination with glomerulitis, was not significantly associated with graft outcome, although this

result is based on a relatively small size ($N = 29$). Nevertheless, we observed that graft outcome had a

tendency to be worse among patients with peritubular capillaritis and glomerulitis, perhaps suggesting

that glomerulitis is a marker of graft dysfunction. Indeed, Couzi et al. [23] concluded that even mild

glomerulitis was associated with poor graft function in ABOi transplants. Therefore, larger studies,

ideally prospective, are needed to fully evaluate the impact of glomerulitis on graft outcome.

Multivariate analysis revealed that HLA class II mismatch is an independent predictor of MVI score

≥ 2, after adjustment for the number of biopsies. It is not surprising that the number of biopsies is a

significant factor, because patients with high MVI (i.e., inferior graft function due to rejection) tend

to receive more biopsies for diagnosis of rejection and further treatment. We removed the potential

bias of this confounding factor by performing multivariate analysis, and HLA class II mismatch

remained a significant predictor of high MVI. HLA class II mismatch may trigger class II DSA, and

lead to AMR, however, this cohort was originally designed to exclude any effects of DSA. Indeed, de

novo DSA following the first post-transplantation year was quite rare in this analysis. We believe that

this rarity was caused by long-term B-cell depletion effects from our immunosuppressive protocols;

our previous study indicated that B-cell depletion therapies, such as splenectomy or rituximab

administration significantly reduced rates of de novo DSA and chronic AMR development 2 years

after transplantation [34]. In spite of this setting, HLA class II mismatch contributes to graft injury with

high MVI, which leads to graft deteriorations such as interstitial fibrosis and tubular atrophy. One

possibility is that mismatched HLA class II molecules elicit activation of CD4Th cells [35,36], resulting

in TMR or graft injury. Indeed, TMR was more frequent among patients with MVI score ≥ 2,

highlighting the potential relationship between TMR and MVI outside and/or inside vessels in

transplanted organs. For instance, Sis et al. [37] recently reported that in patients who were not highly

sensitized, isolated endarteritis was a marker of acute TMR, and that isolated endarteritis was

associated with poor graft outcome, as was endarteritis with TMR. In the end, high MVI scores

represent heavy infiltration of immune cells into the microvasculature, tubules, and interstitium of the

graft, so that such scores, in combination with TMR, eventually result in poor graft outcome, as seen

in cases of mixed acute rejection. Indeed, our data might indicate that MVI score ≥ 2 captures some

of the effects of TMR. This result is consistent, at least in part, with previous reports [21,37]. In other

words, it can be difficult to definitely distinguish between AMR and TMR, particularly during the

early ABOi post-transplant period. According to Couzi et al.[23], C4d deposition with tubulointerstitial

inflammation in ABOi transplantation is associated with graft dysfunction, although these authors

regard isolated C4d deposition and tubulointerstitial inflammation as benign. When high MVI is

observed in ABOi transplantation, it is possible that even T-cell processes, supposedly moderate

compared to AMR, mediate rejection, and should be treated accordingly with T-cell depletion

therapies such as thymoglobulin.

Multivariate analysis revealed that C4d score tends to predict MVI score. We observed C4d

deposition in almost all patients (85.1 %) regardless of MVI score, in line with previous reports [6,17,22].

Indeed, the high frequency of C4d deposition precludes its use as a marker for antibody-mediated

rejection in ABOi recipients. Accordingly, previous studies demonstrated that C4d deposition was not a key predictor of graft survival [20,21]. Nevertheless, we demonstrate for the first time that a high C4d score, in particular C4d score 3, might be an independent predictor of MVI score ≥ 2. Thus, C4d scores should be carefully evaluated for its potential ability to effectively predict graft outcome in ABOi transplantation.

Finally, our data revealed that anti-blood group IgG titers were not associated with MVI score ≥ 2, in line with our previous studies [10,11]. However, other groups reported that early postoperative titers were biomarkers of antibody-mediated rejection [38]. This discrepancy might be due to differences in analytical techniques. For example, we measure titers by agglutination and ELISA assays [39] while Tobian et al. [38] at John Hopkins Hospital used Marsh scoring and agglutination. Moreover, the mechanism of binding between carbohydrate antigens and antibodies remains unclear.

This study was limited first by the retrospective design and the relatively small sample size. Second, the highest Banff and MVI scores observed within 1 year after transplantation were used, even though such scores were not necessarily obtained at the same time in some cases. Third, we did not evaluate non-HLA antibodies such as MHC class I chain-related gene A or B, and therefore, we cannot exclude the effects of these antibodies. Forth, we did not evaluate post-treatment changes to MVI score. Because the present study used only the maximum MVI score, it did not include subsequent MVI scores, which always decreased after treatment, or were unknown due to lack of a follow-up biopsy.

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Further investigation of the relationship between post-treatment MVI score changes and graft outcome could improve treatments for high MVI after ABOi transplantation. Finally, because the present study was retrospective, we missed some biopsies following the first year post-transplantation (1-2 y post-transplantation, the biopsy skip rate was 114/144 or 79.2%; 2-5 y, 98/134 or 73.1%; and > 5 y, 55/82 or 67.1%). These missing long-term follow-up data may cause unavoidable bias, or mask detection of pathological diagnosis such as subclinical rejection.

In summary, we found that MVI score ≥ 2 within 1 year after ABOi transplantation was a useful predictor of poor graft outcome, regardless of C4d deposition. Moreover, MVI score ≥ 2 was significantly associated with HLA class II mismatch, and tended to correlate with high C4d scores. Quick and proper treatment is required for acute rejection with MVI score ≥ 2, especially in the early ABOi post-transplant period. Because some impaired grafts with MVI score ≥ 2 exhibit cellular rejection, a new therapeutic approach such as T-cell system depression may be necessary. Thus, we suggest that MVI score ≥ 2 supports a diagnosis of AMR in ABOi transplantation. This conclusion should be confirmed for large patient populations from different transplant centers.

Disclosure

We have no conflicts of interest to declare.

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Table 1: Patient characteristics.

Variable	All (n = 148)	MVI score < 2 (n = 117)	MVI score ≥ 2 (n = 31)	*p*-value
Recipient age	46.6 ± 14.0	46.6 ± 14.1	46.7 ± 13.7	0.991
Recipient sex				0.833
Male	96 (64.9%)	75 (64.1%)	21 (67.7%)	
Female	52 (35.1%)	42 (35.9%)	10 (32.3%)	
Kidney disease				0.986
Diabetes	22 (14.9%)	17 (14.5%)	5 (16.1%)	
ADPKD	8 (5.41%)	6 (5.13%)	2 (6.45%)	
IgA nephropathy	28 (18.9%)	21 (17.9%)	7 (3.23%)	
FSGS	7 (4.73%)	7 (5.98%)	0	
Reflux nephropathy	6 (4.05%)	5 (4.27%)	1 (3.23%)	
Others	22 (14.9%)	20 (17.1%)	2 (6.45%)	
Unknown/CGN	55 (37.2%)	41 (35.0%)	14 (45.2%)	
Duration of dialysis	44.6 ± 54.9	44.5 ± 54.1	45.1 ± 59.0	0.506
Donor age	59.4 ± 9.34	59.3 ± 9.31	60.0 ± 9.59	0.814
Donor sex				0.280
Male	47 (31.8%)	40 (34.2%)	7 (22.6%)	
Female	101 (68.2%)	77 (65.8%)	24 (77.4%)	
ABO groups				0.498
A to B	28 (18.9%)	21 (17.9%)	7 (22.6%)	
AB to B	14 (9.46%)	11 (9.40%)	3 (9.68%)	
A to O	45 (30.4%)	34 (29.1%)	11 (35.5%)	
B to A	11 (7.43%)	11 (9.40%)	0	
AB to A	17 (11.5%)	15 (12.8%)	2 (6.45%)	
B to O	31 (20.9%)	23 (19.7%)	8 (25.8%)	
AB to O	2 (1.35%)	2 (1.71%)	0	
HLA class I mismatch				0.663
≥ 3	46 (31.1%)	35 (29.9%)	11 (35.5%)	
< 3	102 (68.9%)	82 (70.1%)	20 (64.5%)	
HLA class II mismatch				0.0072
≥ 1	105 (70.9%)	77 (65.8%)	28 (90.3%)	
0	43 (29.1%)	40 (34.2%)	3 (9.68%)	
BSX induction				0.493
With	134 (90.5%)	107 (91.5%)	27 (87.1%)	

Without	14 (9.50%)	10 (8.55%)	4 (12.9%)	
RTX induction				0.176
With	123 (83.1%)	100 (85.5%)	23 (74.2%)	
Without	25 (16.9%)	17 (14.5%)	8 (25.8%)	
SPX				0.209
With	30 (20.3%)	21 (17.9%)	9 (29.0%)	
Without	118 (79.7%)	96 (82.1%)	22 (71.0%)	
Preoperative DFPP				0.655
≥ 4	42 (28.4%)	32 (27.4%)	10 (32.3%)	
< 4	106 (71.6%)	85 (72.6%)	21 (67.7%)	
Baseline anti-blood group IgG				0.156
≥ 1: 128	36 (24.3%)	25 (21.4%)	11 (35.5%)	
< 1: 128	112 (75.7%)	92 (78.6%)	20 (64.5%)	
Preoperative anti-blood group IgG				0.578
≥ 1: 32	23 (15.5%)	17 (14.5%)	6 (19.4%)	
< 1: 32	125 (84.5%)	100 (85.5%)	25 (80.6%)	
Postoperative anti-blood group IgG				0.362
≥ 1: 64	13 (8.78%)	9 (7.69%)	4 (12.9%)	
< 1: 64	135 (91.2%)	108 (92.3%)	27 (87.1%)	
Total biopsies				<0.0001
≥ 3	26 (17.6%)	10 (8.55%)	16 (51.6%)	
< 3	122 (82.4%)	107 (91.5%)	15 (48.4%)	
Years of follow-up	5.16 ± 4.06	5.09 ± 4.01	5.41 ± 4.32	0.790

MVI, microvascular inflammation; ADPKD, autosomal dominant polycystic kidney disease;   FSGS, focal segmental glomerulosclerosis; CGN, chronic glomerular nephritis; HLA, human leukocyte antigen; BSX, basiliximab; RTX, rituximab; SPX, splenectomy; DFPP, double filtration plasmapheresis

Table 2: Correlation of MVI score with Banff scores and pathological diagnosis rate

Pathological parameter	All (n = 148)	MVI score < 2 (n = 117)	MVI score ≥ 2 (n = 31)	*p*-value
v	0.14 ± 0.45	0.068 ±0.34	0.39 ±0.67	<0.0001
i	0.65 ±0.80	0.49 ±0.69	1.29 ±0.90	<0.0001
t	0.38 ± 0.73	0.21 ± 0.55	1.00 ±0.97	<0.0001
C4d	2.06 ±0.76	1.97 ±0.74	2.39 ±0.76	0.0019
cg	0.061±0.29	0	0.29 ±0.59	<0.0001
cv	0.081 ±0.38	0.051 ± 0.32	0.19 ± 0.54	0.0375
ct	0.54 ±0.78	0.36 ±0.59	1.19 ± 1.01	<0.0001
ci	0.55 ±0.76	0.38 ±0.60	1.19 ±0.95	<0.0001
ah	0.52 ±0.72	0.45 ± 0.71	0.77 ± 0.72	0.0094
TCMR	18 (12.2%)	8 (6.84%)	10 (32.2%)	0.0001
IF/TA	37 (25.0%)	24 (20.5%)	13 (41.9%)	0.0143

MVI, microvascular inflammation; TCMR, T cell-mediated rejection; IF/TA, interstitial fibrosis and tubular atrophy

Table 3: Univariate and multivariate logistic regression analyses of risk factors associated with MVI score ≥ 2.

Variable	Univariate OR 95%CI	*p*-value	Multivariate OR 95%CI	*p*-value
Recipient age, years	1.00 (0.97-1.03)	0.962		
Recipient sex		0.705		
Male	Reference			
Female	0.85 (0.52-2.82)			
Donor age, years	1.01 (0.97-1.05)	0.702		
Donor sex		0.206		
Male	Reference			
Female	1.78 (0.74-4.80)			
ABO group		0.326		
A1 or A1B incompatible	1.51 (0.67-3.62)			
Others	Reference			
HLA class I mismatch		0.555		
≥ 3	1.29 (0.55-2.94)			
< 3	Reference			
HLA class II mismatch		0.0038		0.0093
≥ 1	4.85 (1.59-21.1)		5.10 (1.45-25.2)	
0	Reference		Reference	
BSX induction		0.477		
With	0.63 (0.19-2.44)			
Without	Reference			
RTX induction		0.153		
With	0.49 (0.19-1.32)			
Without	Reference			
Splenectomy		0.186		
With	1.87 (0.73-4.56)			
Without	Reference			
Preoperative DFPP		0.593		
≥ 4	1.26 (0.52-2.93)			
< 4	Reference			

Anti-blood group IgG at baseline		0.114		
≥ 1: 128	2.02 (0.84-4.74)			
< 1: 128	Reference			
Anti-blood group IgG at pre-transplant		0.519		
≥ 1: 32	1.41 (0.47-3.80)			
< 1: 32	Reference			
Anti-blood group IgG at post-transplant		0.383		
≥ 1: 64	1.78 (0.45-5.92)			
< 1: 64	Reference			
Total biopsies		<0.0001		<0.0001
≥ 3	11.4 (4.47-30.8)		10.1 (3.73-29.4)	
< 3	Reference		Reference	
C4d score	2.45 (1.31-4.92)	0.0039	1.88 (0.97-3.87)	0.0627

MVI, microvascular inflammation; OR, odds ratio; CI, confidence interval; HLA, human leukocyte antigen; BSX, basiliximab; RTX, rituximab; SPX, splenectomy; DFPP, double filtration plasmapheresis

Figure legends

Figure 1: Patient selection and study design

Figure 2: Immunosuppressive and desensitization protocols for patients undergoing ABO-incompatible transplantation at Tokyo Women's Medical University.

Figure 3: ABO-incompatible kidney graft survival in patients stratified according to MVI score ≥ 2. Survival rate was calculated using the Kaplan-Meier method, and compared using the log-rank test and Wilcoxon's test, respectively ($p = 0.0878, 0.0129$). Solid line and dotted line show the survival rates and 95% confidence intervals, respectively.

Figure 4: Mean serum eGFR in patients stratified according to MVI score ≥ 2. Serum eGFR was significantly inferior for patients with MVI score ≥ 2 relative to eGFR for those with MVI score < 2 throughout the study ($p = 0.048$). Error bars indicate standard deviation. *P*-value was obtained using a mixed model test of trend profile. We excluded 2 patients with MVI ≥ 2 from this analysis because their grafts had been lost prior to 3 months after transplantation.

Figure 5: ABO-incompatible kidney graft survival according to MVI localization in patients with MVI

score ≥ 2. Survival rate was calculated using the Kaplan-Meier method, and compared using the log-rank test and Wilcoxon's test, respectively (p = 0.268, 269). Solid line and dotted line indicate the survival rates and 95% confidence intervals, respectively.

Figure 6: Mean serum eGFR according to MVI localization in patients with MVI score ≥ 2. Serum GFR did not differ significantly between patients with peritubular capillaritis alone and patients with both glomerulitis and peritubular capillaritis (p = 0.788). Error bars indicate standard deviation. *P*-value was obtained using a mixed model test of trend profile. We excluded 2 patients with both glomerulitis and peritubular capillaritis from this analysis because their grafts had been lost prior to 3 months after transplantation.

Figure 1: Patient selection and study design

Patients who underwent ABO-incompatible kidney transplantation between 2001 and 2015 (n = 227)

Exclusion criteria
- Patients with preformed DSA (n = 55)
- Patients without biopsy within 1 year after transplantation (n = 20)
- Patients with *de novo* DSA within 1 year after transplantation (n = 1)
- Patients without detailed clinical data (n = 3)

Study population (n = 148)

MVI score < 2 (n = 117)
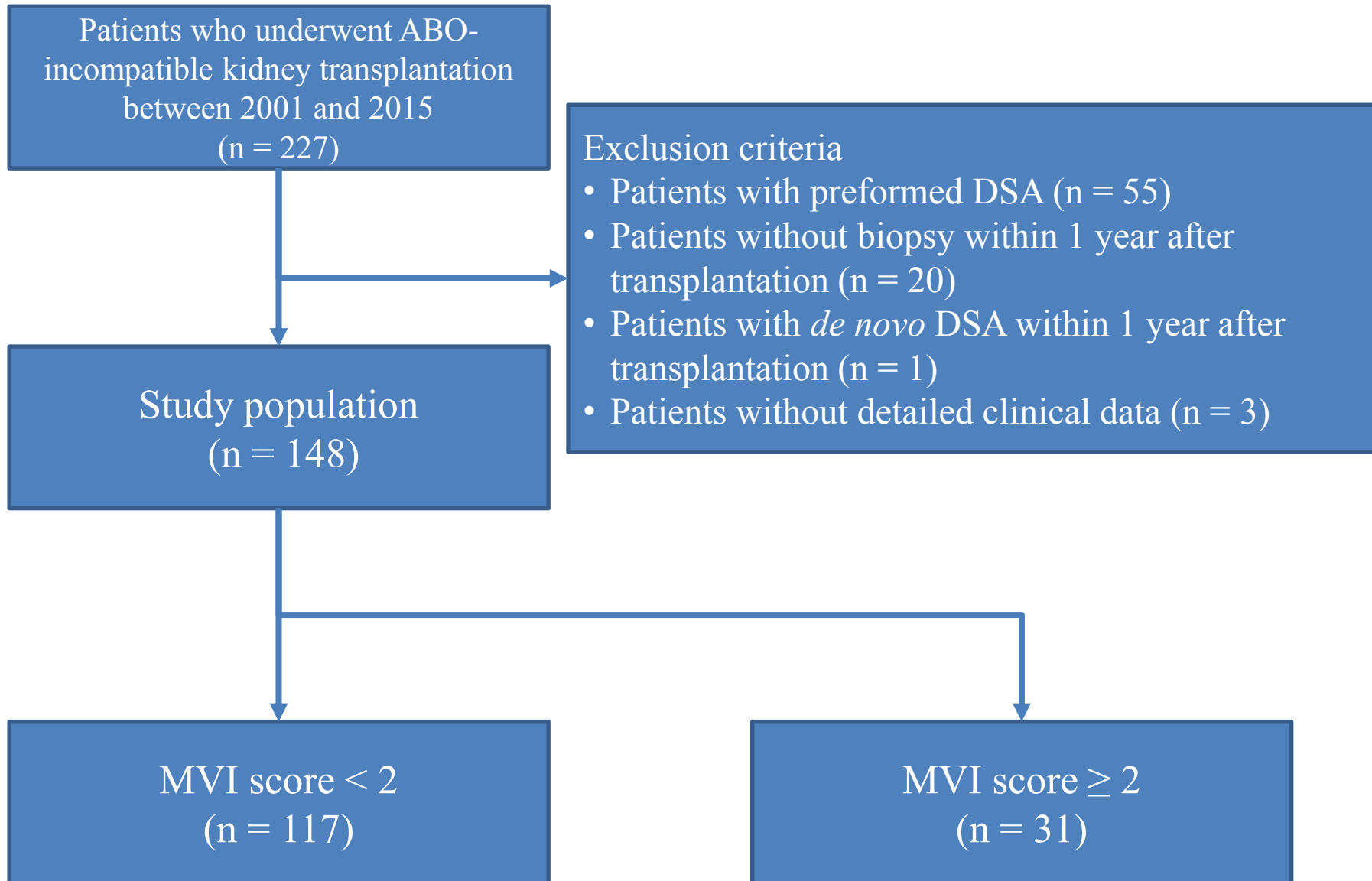
MVI score ≥ 2 (n = 31)

Figure 2: Immunosuppressive and desensitization protocols for patients undergoing ABO-incompatible transplantation at Tokyo Women's Medical University.
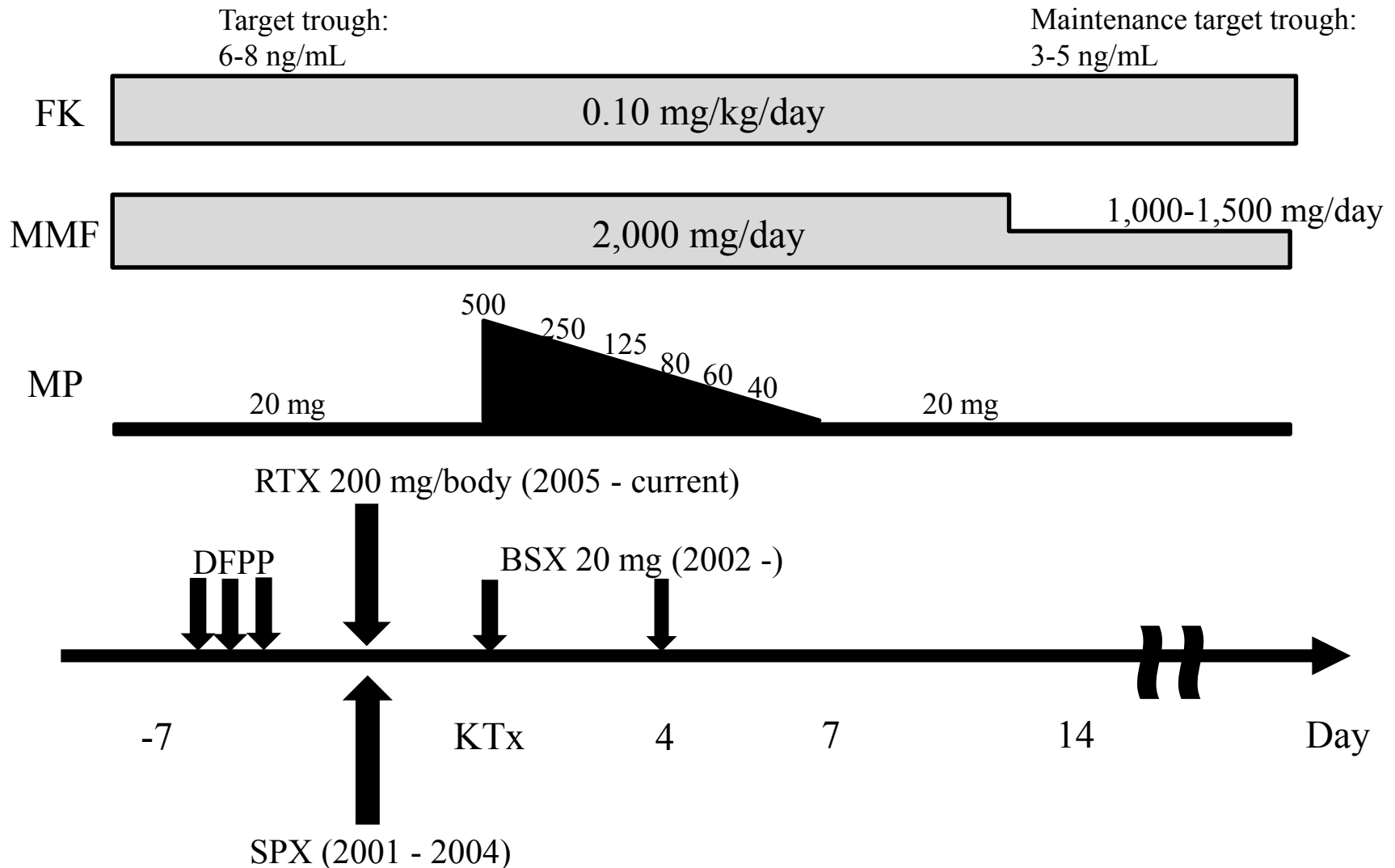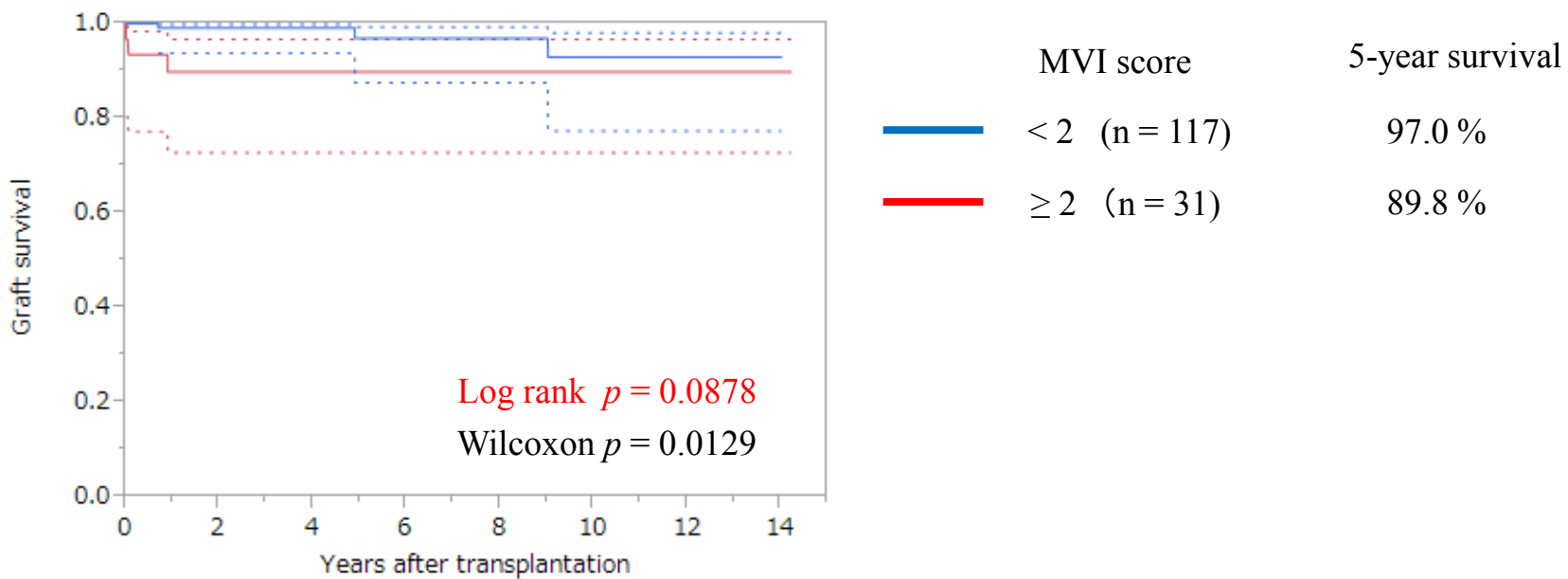
Figure 3: ABO-incompatible kidney graft survival in patients stratified according to MVI score ≥ 2.

Figure 4: Mean serum eGFR in patients stratified according to MVI score ≥ 2.

n	3m	6m	1y	2y	3y	4y	5y	6y	7y	8y	9y	10y
MVI < 2	117	116	115	103	89	79	61	50	41	32	28	20
MVI $\geq$ 2	29	29	28	28	21	19	18	14	13	12	10	8

Figure 5: ABO-incompatible kidney graft survival according to MVI localization in patients with MVI score $\geq$ 2.

Localization	5-year survival
ptc (n = 9)	100.0 %
g and ptc (n = 22)	85.9 %

Log rank $p = 0.268$
Wilcoxon $p = 0.269$
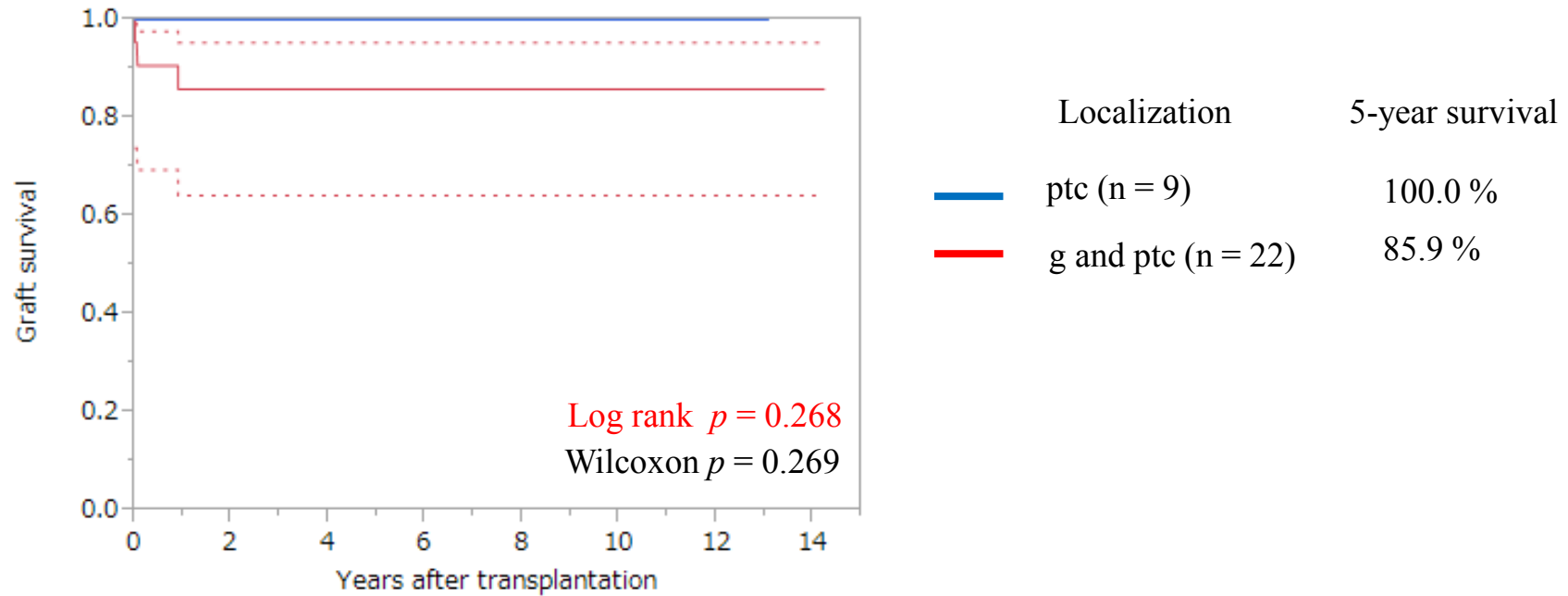
n								
MVI < 2	9	5	4	2	1	1	1	
MVI $\geq$ 2	22	16	12	9	8	7	1	1

Figure 6: Mean serum eGFR according to MVI localization in patients with MVI score ≥ 2.