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Association of immunosuppressive agents and cytomegalovirus infection with de novo donor-specific antibody development within 1 year after renal transplantation

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ABSTRACT

The association between immunosuppressive therapy or cytomegalovirus (CMV) infection and detection of de novo donor-specific antibody (dnDSA) at 1 year after transplantation was evaluated. The impact of dnDSA positivity at 1 year after transplantation on long-term death-censored renal graft survival was also evaluated. One hundred and sixty adults receiving living renal allografts were studied. Inclusion criteria were renal graft survival for at least 1 year and a standard regimen of immunosuppressive therapy with tacrolimus, mycophenolate mofetil (MMF), steroids, and basiliximab. DSA were measured retrospectively by the Luminex assay. The coefficient of variation (CV) was calculated and receiver operating characteristic (ROC) analysis was employed to clarify the association of tacrolimus with development of dnDSA. Seven of the 160 patients (4.4%) were positive for dnDSA. The intra-patient minimum trough level of tacrolimus (cutoff value: 3.2 ng/mL) was associated with development of dnDSA. Discontinuation of MMF and treatment of CMV infection were more frequent in patients with dnDSA than in those without dnDSA. In multivariate analysis, a low trough level of tacrolimus, discontinuation of MMF, and treatment of CMV infection within 1 year after transplantation were independently associated with detection of dnDSA at 1 year. In patients with or without dnDSA at 1 year, the 10-year allograft survival rate was 51.4 versus 87.9%, respectively ($P = 0.002$). A lower tacrolimus trough level, discontinuation of MMF, and treatment of CMV infection were associated with dnDSA positivity. Further investigation is needed to determine whether a new immunosuppressive regimen that avoids these factors can reduce dnDSA positivity.

1. Introduction

Donor-specific antibodies (DSA) targeting human leukocyte antigens (HLA) play a significant role in graft loss after renal transplantation [1,2]. Aubert et al. [3] reported that patients who developed antibody-mediated rejection (AMR) showed worse graft survival if they had *de novo* DSA (dnDSA) compared to patients with preexisting DSA. Accordingly, dnDAS may be associated with a higher risk of allograft loss compared to preexisting DSA [3,4]. Despite many reports about treatment for dnDSA, no specific intervention has yet been established as effective [4–8]. Therefore, it is important to identify risk factors for dnDSA production after renal transplantation and reduce these risks.

Many risk factors for dnDSA have been reported, including a younger recipient age, frequent nonadherence, early blood transfusion, viral infections, and suboptimal immunosuppressive therapy [9–17]. O'Leary et al. [15] reviewed the risks associated with each immunosuppressive agent and their preventive effect on production of dnDSA. They concluded that early withdrawal of calcineurin inhibitor (CNI) was not recommended if patients had known risk factors for development of dnDSA. On the other hand, they considered that early withdrawal of steroid did not increase the risk of developing dnDSA, and that available data did not show a specific effect of mycophenolic acid (MPA) on production of dnDSA [15]. Furthermore, an increase of DSA has been noted during viral infection [12]. Cytomegalovirus (CMV) infection is an independent factor associated with worse graft survival, and this virus has a considerable impact on the immune

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response [13]. Infection with BK polyomavirus (BKPyV) is also associated with dnDSA positivity [14]. Against this background, the primary aim of the present study was to determine the impact of immunosuppressive agents and viral infection on dnDSA positivity within 1 year after renal transplantation in patients receiving standard immunosuppressive therapy with tacrolimus, mycophenolate mofetil (MMF), steroids, and basiliximab.

Screening for dnDSA at more than 1 year after transplantation has potential limitations, including the cost, the low dnDSA incidence rate, and the absence of effective therapy [18]. Testing for anti-HLA antibodies after transplantation was not previously covered by health insurance in Japan. Therefore, screening renal transplant recipients for dnDSA was not routine at many centers. Until 2014, anti-HLA antibodies were detected at our center by complement-dependent cytotoxicity crossmatch (CDCXM) with or without flow cytometry crossmatch (FCXM) before transplantation, and no screening was done after transplantation. Therefore, the secondary objective of this study was to retrospectively investigate the influence of dnDSA positivity at 1 year after transplantation on long-term death-censored graft survival in patients without prospective dnDSA screening.

2. Materials and methods

2.1. Subjects

Prospectively collected serum samples from a single-center patient cohort were subjected to retrospective analysis. One hundred and seventy-nine adult patients underwent living kidney transplantation at our hospital between July 2004 and September 2013. Inclusion criteria for this study were as follows: (1) graft survival for at least 1 year after transplantation; (2) serum sample available for detecting DSA both before and 1 year after transplantation; (3) standard tacrolimus-based immunosuppressive regimen throughout the first year after transplantation; and (4) data on tacrolimus from at least blood 3 samples for calculating the coefficient of variation (CV) from3-6 months and 6-12 months after transplantation. Two out of 179 patients died with a functional graft, while 3 patients changed residence and were lost to follow-up within 1 year of transplantation. Fourteen patients did not provide blood samples at 1 year after transplantation. The remaining 160 patients were eligible (Fig. 1). All recipients and donors provided informed consent for transplant-associated clinical and laboratory examinations, and the Ethics Committee of our institute gave approval for this retrospective observational study.

2.2. Serum sample and HLA typing

Serum samples were collected before transplantation and at 1 year after transplantation and were stored at -80°C until analysis. DNA was extracted from the samples by using a QIAamp Blood kit (Qiagen, Hilden, Germany). Genotyping of patients and donors for HLA class I (A, B, C) and class II (DRB1, DQA1, DQB1, DPB1) alleles was done retrospectively by the polymerase chain reaction with a sequence-specific oligonucleotide probe, using WAKFlow® HLA typing kits (Wakunaga Pharmaceutical Co., Hiroshima, Japan) and LABType™ SSO (One Lambda, Inc., Canoga Park, CA, USA).

2.3. Detection of dnDSA

Retrospective testing for DSA was done with Luminex-based SAB kits (LABSCreen® PRA and LABSCreen® Single Antigen, One Lambda, Inc.) according to the manufacturer's instructions, and the date were analyzed with HLA Fusion™ software (One Lambda Inc.). A judgment of positive or negative was based on the normalized mean fluorescence intensity (MFI), which was calculated using the negative control beads and negative serum control. The normalized MFI cutoff values of PRA and single tests were set at 500 and 1,000, respectively. Serum samples collected 1 year after transplantation were tested for antibodies targeting HLA-A*, -B*, -Cw*, -DR*, –DQ*, or -DP*. If DSA were found, we documented the number, class, specificity, and MFI.

If patients had DSA in serum samples from 1 year after transplantation, DSA positivity was also analyzed in serum samples stored before transplantation. Detection of DSA only in the sample from 1 year after transplantation was defined as dnDSA, while detection in both samples was defined as persistent preexisting DSA.

2.4. Immunosuppression

Initial immunosuppressive therapy with tacrolimus and MMF was started 2 days prior to surgery. The daily dose of tacrolimus and the whole-blood trough target level were the same as reported previously [19]. Eighty-seven patients received tacrolimus twice daily (Tac-BID) and 73 patients received tacrolimus once daily (Tac-QD).

The initial dosage of MMF was 1,500 mg/day, which was divided into two equal dosed and administered every 12 hours. Methylprednisolone was given at 500 mg on the day of surgery, and was tapered to 10 mg/day of prednisolone by the fourth week postoperatively. All patients received intravenous basiliximab (20 mg) on the day of surgery and also on postoperative day 4. Patients with ABO incompatibility and those undergoing second transplantation received MMF starting from 21 days prior to surgery and tacrolimus starting from 7 days before surgery, with rituximab (200 mg/body) given intravenously at 21 days before surgery.

2.5. Tacrolimus and MPA trough levels and CV tacrolimus

Tacrolimus blood concentrations were determined with a chemiluminescent magnetic microparticle immunoassay, as reported previously [20]. Plasma concentrations of MPA were measured by high-performance liquid chromatography (HPLC) [21]. Variability of the blood level of tacrolimus was estimated by calculating the coefficient of

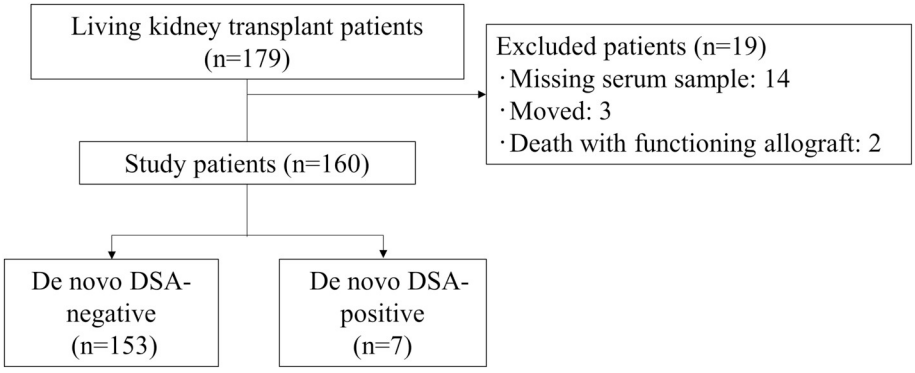


Fig. 1. Patients with and without *de novo* donor-specific antibodies (dnDSA) at 1 year after transplantation.

variation (CV) with the following equation:

CV(%) = (standard deviation/mean concentration) × 100.

2.6. Genotyping of CYP3A5 genetic polymorphisms

Genotyping of *CYP3A5 6968A > G* was performed by the polymerase chain reaction-restriction fragment length polymorphism methods with the primers reported previously [20].

2.7. Diagnosis and treatment of rejection

Clinical and subclinical T-cell-mediated rejection (TMR) and AMR were diagnosed from clinical and pathological findings according to Banff 1997 and 2007 criteria [22,23]. Intravenous methylprednisolone pulse therapy was given for both TMR and AMR, with intravenous immunoglobulin therapy and plasmapheresis being added for AMR.

2.8. Diagnosis of CMV and VZV infection

CMV infection was diagnosed on the basis of positivity for CMV antigenemia, and treatment of CMV infection was defined as administration of valganciclovir and/or ganciclovir. Patients requiring treatment for herpes zoster were defined as being treated for varicella zoster virus (VZV) infection.

2.9. Statistical analysis

Results are reported as the mean ± standard deviation (SD), median with a range, or median (quartile 1-3) for continuous variables, while the number and percentage are reported for discrete variables. The Mann-Whitney *U* test was used to compare non-parametric quantitative data, while the chi-square test or Fishers' exact test was employed to assess differences of categorical data. Groups were dichotomized according to various categories. Multivariate logistic regression analysis was also performed. Graft survival data were censored at the time of death. Renal graft survival rates were subjected to Kaplan-Meier analysis and were compared between groups with the log-rank test. The optimum cutoff value of the intra-patient minimum trough level of tacrolimus at 3 to 12 months after transplantation for predicting dnDSA was obtained from ROC analysis. In all analyses, $P < 0.05$ was considered statistically significant. Analyses were performed with SPSS 19.0 statistical software (SPSS Japan Inc., Tokyo, Japan).

3. Results

3.1. Study population

Table 1 lists the pretransplant clinical characteristics of the 160 recipients and their living donors. The majority of the study population comprised male recipients (64.4%) with female donors (62.5%). The median follow-up period was 8.8 years (range: 1.6 to 14.8 years). At 1 year after transplantation, we identified dnDSA in 7 (4.4%) of the 160 recipients (Fig. 1) (Table 2).

3.2. Comparison between dnDSA-positive and -negative patients

Treatment of CMV infection with ganciclovir and/or valganciclovir, but not CMV antigenemia positivity, was more frequent in patients with dnDSA than in those without dnDSA. Other clinical characteristics showed no significant differences between patients with or without dnDSA positivity (Table 2). Clinical and subclinical TMR or AMR were not associated with dnDSA (Table 3).

Table 1
Characteristics of the patients and their living donors at transplantation.

Number of patients	160
Recipient age at transplantation (years)	49 [21–70]
Male recipients (%)	103 (64.4)
BMI	22.7 ± 3.7
Donor age	60 [26–80]
Male donors (%)	60 (37.5)
HLA serotype mismatch	
-Class I & II	5.39 ± 2.54
-A	0.84 ± 0.70
-B	1.24 ± 0.61
-C	1.19 ± 0.66
-DR	1.12 ± 0.66
-DQ	1.01 ± 0.64
Full HLA serotype match (%)	7 (4.4)
ABO incompatibility (%)	36 (22.5)
Second transplantation (%)	8 (5.0)

Values are expressed as the median [range] or mean ± SD. BMI, body mass index; HLA, human leukocyte antigen.

Table 2
Clinical characteristics of patients with or without *de novo* DSA.

Characteristic	De novo DSA-negative	De novo DSA-positive	*P*-value
	At 1 yr	At 1 yr	
Number of patients	153	7	
Recipient age at transplantation (years)	49 [38–57]	47 [31–60]	0.802
Male recipients (%)	97 (63.4)	6 (85.7)	0.217
BMI (kg/cm^2)	22.7 ± 3.7	22.2 ± 2.4	0.920
Donor age at transplantation (years)	59 [52–65]	60 [52–67]	0.767
Male donors, male (%)	58 (37.9)	2 (28.6)	0.474
HLA serotype mismatch			
-Total	5.35 ± 2.53	6.29 ± 2.93	0.481
-A	0.82 ± 0.70	1.14 ± 0.69	0.233
-B	1.24 ± 0.62	1.43 ± 0.54	0.443
-C	1.17 ± 0.67	1.43 ± 0.54	0.336
-DR	1.12 ± 0.66	1.14 ± 0.90	0.860
-DQ	1.01 ± 0.64	1.14 ± 0.69	0.584
Full HLA serotype match (%)	7 (4.6)	0 (0)	0.727
ABO incompatibility (%)	34 (22.2)	2 (28.6)	0.494
Second transplantation (%)	7 (4.6)	1 (14.3)	0.307
Husband donors (%)	21 (13.7)	1 (14.3)	0.735
Clinical and subclinical rejection	67 (43.8)	4 (57.1)	0.376
CMV viremia (%)	73 (47.7)	5 (71.4)	0.201
Treatment for CMV infection (%)	44 (28.8)	5 (71.4)	0.028
Treatment for VZV infection (%)	18 (11.8)	0 (0)	0.426
Follow-up period	8.8 [6.8–11.5]	8.5 [7.3–10.2]	0.623

Values are expressed as the median [25–75 percentile] or mean ± SD. DSA, donor-specific antibody; BMI, body mass index; HLA, human leukocyte antigen; CMV, cytomegalovirus; VZV, varicella zoster virus.

3.3. Influence of tacrolimus on dnDSA positivity

The tacrolimus formulations administered and *CYP3A5* polymorphism did not influence the development of dnDSA (Fig. 2A). The mean tacrolimus trough levels or CV values at 3 to 6 months (Fig. 2B and C) or 6 to 12 months (Fig. 2D and E) after transplantation were also not associated with dnDSA. Regarding the intra-patient minimum trough level of tacrolimus at 3 to 12 months after transplantation, a lower trough level (cutoff value: 3.2 ng/mL) was associated with dnDSA positivity (Fig. 3A). The ROC curve used to determine the cutoff values is shown in Fig. 3B. The area under the ROC curve was 0.74 (95%CI, 0.48-0.99).

Table 3
Influence of TMR and AMR on de novo DSA positivity within 1 year after transplantation.

	No. of patients	TMR	*P*-value[a]	AMR	*P*-value[a]
De novo DSA-negative (%)	153	65 (42.5)		12 (6.1)	
De novo DSA-positive (%)	7	4 (57.1)	0.350	1 (14.3)	0.480

TMR, T-cell-mediated rejection; AMR, antibody-mediated rejection.
[a] Fischer's test.

A

		n	dnDSA-negative	dnDSA-positive	*p*-value
Tacrolimus formulation	BID	87	83	4	0.597
	QD	73	70	3	
CYP3A5 polymorphisms	**1/*1+*1/*3*	73	72	1	0.092
	**3/*3*	87	81	6	

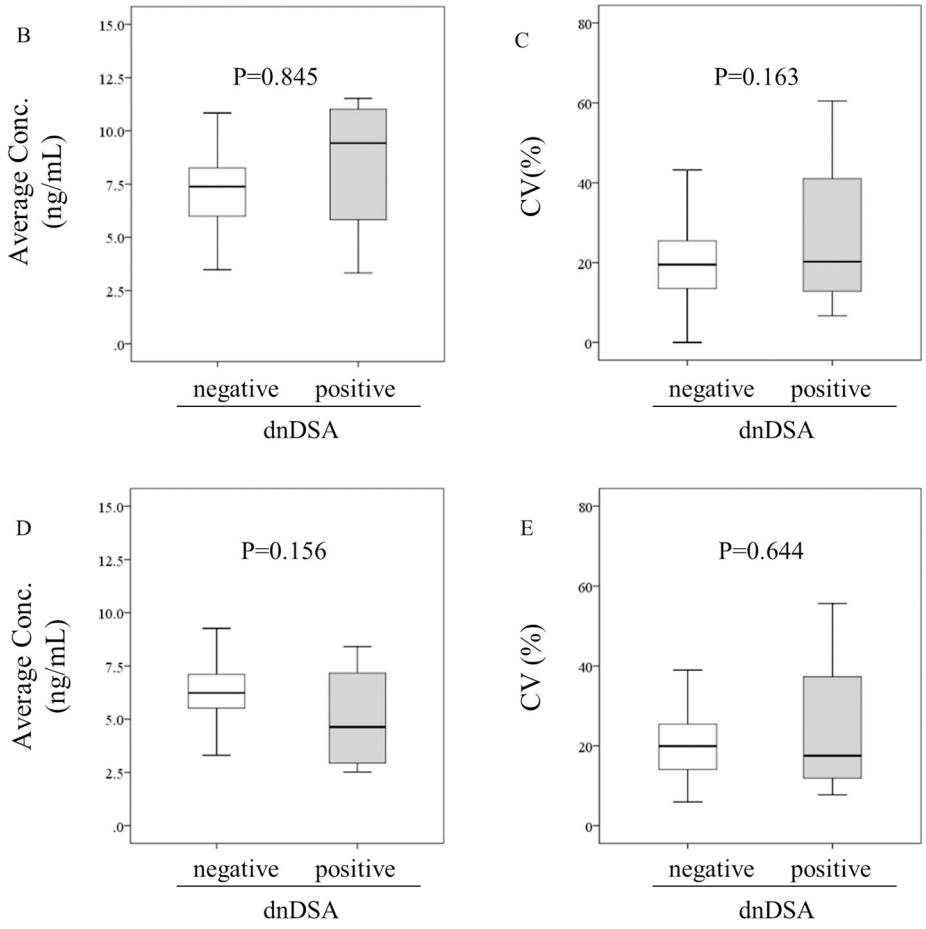


Fig. 2. Influence of tacrolimus on development of *de novo* DSA (dnDSA): (A) tacrolimus formulation and *CYP3A5* polymorphism, (B) mean trough level of tacrolimus and (C) coefficient of variation (CV) of tacrolimus at 3 to 6 months after transplantation, (D) mean trough level of tacrolimus and (E) CV of tacrolimus at 6 to 12 months after transplantation.

3.4. Influence of MMF on dnDSA positivity

Discontinuation of MMF within 1 year after transplantation was associated with dnDSA positivity at 1 year. MMF was replaced by another antimetabolite, mizoribine, in all patients with MMF discontinuation. Reducing the MMF dose from 1,500 to 1,000 or 500 mg/day was not associated with dnDSA positivity (Fig. 4A). There were no differences of the absolute MMF dose (Fig. 4B) or weight-adjusted MMF dose (Fig. 4C) at 1 year after transplantation between the patients with and without dnDSA.

3.5. Influence of prednisolone on dnDSA positivity

Withdrawal of prednisolone within 1 year after transplantation did

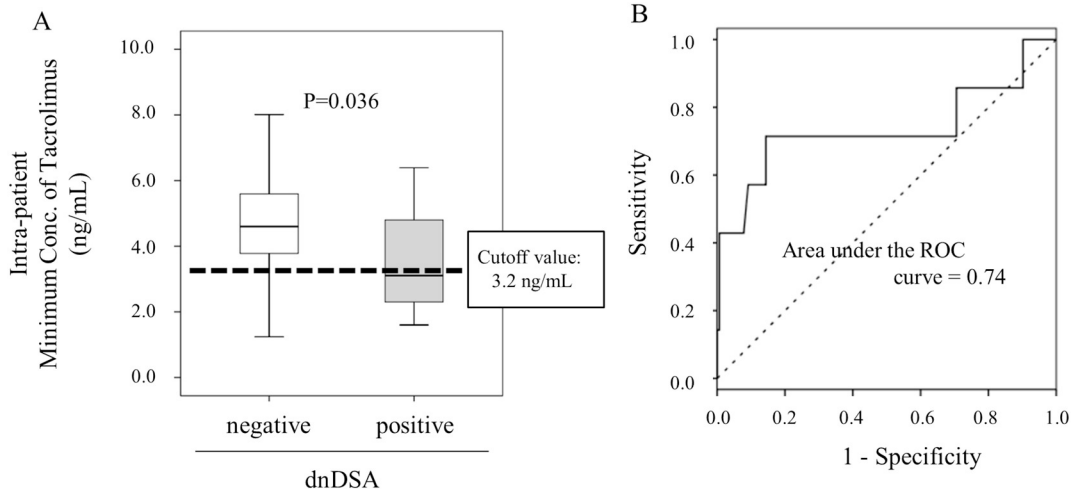


Fig. 3. Intra-patient minimum trough level of tacrolimus (Tac) at 3 to 12 months after transplantation: (A) cutoff value (3.2 ng/mL) for development of *de novo* DSA (dnDSA), (B) receiver operating characteristics curve for optimum cutoff value of the intra-patient minimum trough level of tacrolimus at 3 to 12 months after transplantation. The solid line represents the results from panel A.

A

		n	dnDSA-negative	dnDSA-positive	*P*-value
MMF	Maintenance	97	96	1	
	Dose reduction*	56	53	3	
	Discontinuation**†	7	4	3	<0.001
	Maintenance + Dose reduction	153	149	4	
	Discontinuation	7	4	3	0.002

* Maintenance vs. Dose reduction, p=0.139
** Maintenance vs. Discontinuation, p<0.001
† Dose reduction vs. Discontinuation, p=0.015

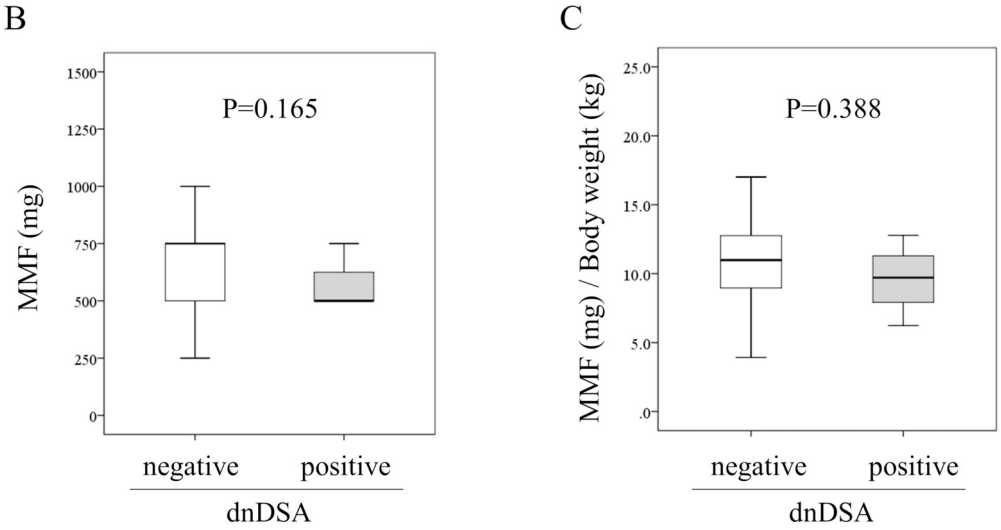


Fig. 4. Influence of MMF on development of *de novo* DSA (dnDSA): (A) patients with maintenance or discontinuation of MMF treatment within 1 year after transplantation, (B) absolute dose of MMF at 1 year after transplantation, (C) weight-adjusted dose of MMF at 1 year after transplantation.

A

		n	dnDSA-negative	dnDSA-positive	*P-value*
Prednisolone	Maintenance	142	135	7	
	Withdrawal	18	18	0	0.426

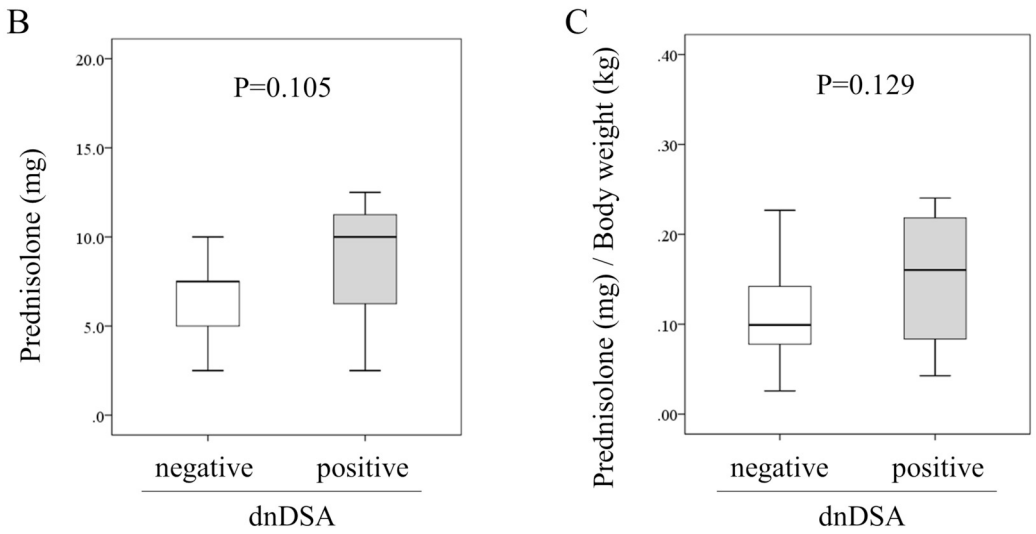


Fig. 5. Influence of prednisolone on development of *de novo* DSA (dnDSA): (A) patients continuing or withdrawn from prednisolone within 1 year after transplantation, (B) absolute doses of prednisolone at 1 year after transplantation, (C) weight-adjusted dose of prednisolone at 1 year after transplantation.

not influence dnDSA positivity (Fig. 5A). There were no differences of the absolute prednisolone dose (Fig. 5B) or weight-adjusted prednisolone dose (Fig. 5C) between the patients with and without dnDSA.

3.6. Risk of dnDSA positivity within 1 year after transplantation

Stepwise multiple liner regression analysis (step-up procedure) was performed using data on treatment of CMV infection, *CYP3A5* polymorphisms, intra-patient minimum trough levels of tacrolimus, discontinuation of MMF, prednisolone withdrawal, full HLA serotype match, second transplantation, and husband donors. This analysis revealed that a low intra-patient trough level of tacrolimus (< 3.2 ng/mL) at 3 to 12 months, discontinuation of MMF, and treatment of CMV infection within 1 year after transplantation were independently associated with the detection of dnDSA at 1 year (Table 4).

3.7. Influence of dnDSA on long-term graft survival

The death-censored renal graft survival rates of patients with or without dnDSA were 85.7 vs. 98.0% (6 years), 68.6 vs. 95.5% (8 years), and 51.4 vs. 87.9% (10 years). The graft survival rate was significantly lower in recipients with dnDSA at 1 year after transplantation (Fig. 6).

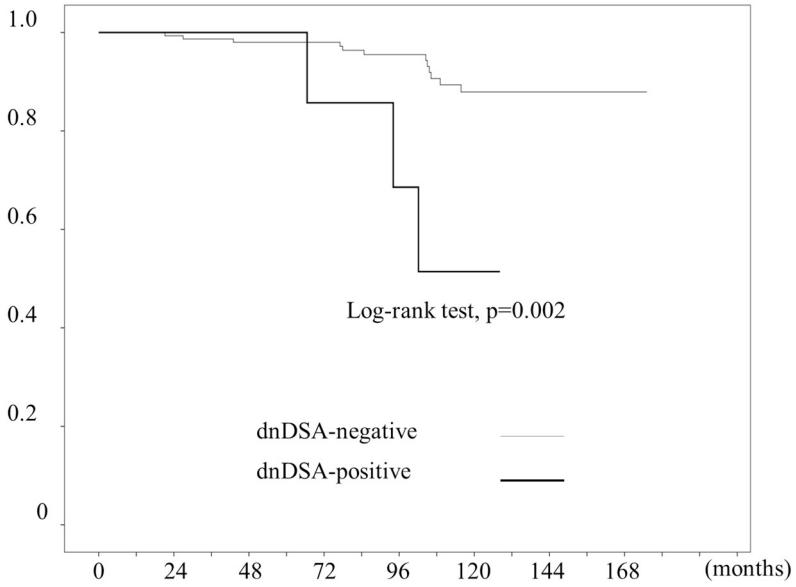
Table 4
Multivariable analysis of risk factors for development of *de novo* DSA within 1 year after transplantation.

	Hazard ratio (95% CI)	*P*-value
Intra-patient minimum trough level of tacrolimus (< 3.2 ng/mL)	16.47 (3.03–89.55)	0.006
Discontinuation of MMF	32.20 (4.79–216.73)	0.003
Treatment for CMV infection	9.72 (1.59–59.44)	0.039

4. Discussion

This study demonstrated that a low intra-patient trough level of tacrolimus (< 3.2 ng/mL) at 3 to 12 months, discontinuation of MMF, and treatment for CMV infection within 1 year after transplantation were independently associated with detection of dnDSA at 1 year. It has been reported that the incidence of dnDSA is increased in kidney transplant patients not receiving CNIs or with early switching to mammalian target of rapamycin inhibitor (mTORi) [15,24,25]. Administration of CNIs in the early posttransplantation period seems to be protective against development of dnDSA [15]. In addition, the frequency of dnDSA was reported to be lower in patients treated with tacrolimus than in those using cyclosporine [15,26]. Moreover, a lower tacrolimus trough level is associated with development of dnDSA [15,27,28], and marked intra-patient variability of the tacrolimus trough levels may be a major risk factor for dnDSA [16,29]. In the present study, the mean trough levels or the CV of tacrolimus at 3 to 6 months or 6 to 12 months after transplantation was not associated with development of dnDSA. However, the intra-patient minimum trough level of tacrolimus (cut-off value: 3.2 ng/mL) at 3 to 12 months after transplantation was independently associated with detection of dnDSA. Kaneku et al. [30] have previously reported that a tacrolimus trough level < 3 ng/mL is a risk factor for dnDSA after liver transplantation.

There have been no reports of an association between MPA and development of dnDSA [15]. A pilot study indicated that escalation of MPA improved long-term graft survival without CMV or BKPyV infections in stable DSA-positive patients [31]. Filler et al. [17] reported that MPA trough levels < 1.3 mg/L after transplantation were associated with the development of DSA in pediatric recipients. In the present study, we did not measure MPA trough levels. There were various reasons for discontinuation or dose reduction of MMF in our study population, such as diarrhea, myelosuppression, and infections. After discontinuation of MMF, the administration of another antimetabolite (mizoribine) was started. Neither the mean dose nor the weight-



dnDSA-negative	Rate	1.000	0.993	0.980	0.980	0.955	0.879	0.879	0.875
	No. at risk	153	149	145	128	91	57	30	8
dnDSA-positive	Rate	1.000	1.000	1.000	0.857	0.686	0.514		
	No. at risk	7	7	7	6	4	2		

Fig. 6. Death-censored graft survival rates of patients with or without *de novo* DSA (dnDSA) at 1 year after transplantation.

Table 5
Influence of CMV infection on MMF treatment within 1 year after transplantation.

	n	CMV infection-negative	CMV infection-positive	*P*-value
Maintenance	104	60	44	
Dose reduction + discontinuation	56	22	34	0.032

adjusted dose of MMF at 1 year after transplantation was associated with dnDSA production. These results suggest that discontinuation, but not dose-reduction, of MMF within 1 year after transplantation is independently associated with development of dnDSA.

In some patients with CMV infection, we reduced the MMF dose or the target trough level of tacrolimus. Accordingly, the frequency of MMF dose reduction or discontinuation was higher in patients with CMV infection than in those without CMV infection (Table 5). Tacrolimus trough levels were not different between these two groups (data not shown). However, discontinuation of MMF and treatment of CMV infection within 1 year after transplantation were factors that showed an independent association with dnDSA positivity at 1 year. There could be several mechanisms leading to an association between treatment of CMV infection and development of dnDSA. Michelo et al. [13] reported that CMV infection enhanced natural killer (NK) cell alloreactivity, which might adversely influence graft survival. Calabrese et al. [32,33] reported that NK cells can promote tolerance after lung transplantation, or can conversely drive rejection through cytotoxic effects on the graft which is recognized as foreign or stressed. They also stated that NK cells are important in the response to infection, particularly by receptor-based mediation of CMV infection [32,33]. Therefore, further evaluation of NK cell alloreactivity should be done in renal transplant patients with CMV infection or receiving treatment for this infection. BKPyV infection is also associated with dnDSA production [14]. In the present study cohort, no patient had BKPyV infection or Epstein-Barr virus infection within 1 year of transplantation. Recent studies have suggested that CMV and BKPyV infections are less frequent in patients treated with CNIs and everolimus compared with those on MMF therapy [34,35].

It was reported that early steroid withdrawal does not affect dnDSA production in standard-risk patients [15]. Also, Monfa et al. [36] reported that steroid withdrawal at 7 months after renal transplantation was not a risk factor for development of DSA, and it has been suggested that steroid may be discontinued in patients with good graft function and no rejection [15]. In our study population, prednisolone was intentionally withdrawal within 1 year of transplantation in patients with well-functioning grafts. Even with this policy, the mean dose and weight-adjusted dose of prednisolone at 1 year after transplantation were not associated with dnDSA production.

We found worse long-term death-censored graft survival in patients with dnDSA positivity at 1 year after transplantation than in those without dnDSA. An impact of dnDSA on long-term graft survival has been reported previously [1–4,15,16,37,38]. In the present study, 7 (4.4%) of the 160 patients had dnDSA at 1 year after transplantation, and the prevalence of dnDSA at 1 year was lower than in previous reports [26,39]. Usefulness of screening stable recipients for dnDSA is controversial. A study of patients receiving prospective annual DSA screening has shown that early treatment of dnDSA associated with subclinical disease resulted in lower graft loss rates than in patients who were not screened [18]. Our findings suggested that dnDSA positivity at 1 year after transplantation was associated with long-term graft loss in patients without periodic screening for DSA. Since April 2018, the costs of annual screening for posttransplant anti-HLA antibodies has been covered by health insurance in Japan and a DSA is also covered if the screening test is positive. Monitoring dnDSA may improve stratification of individual patients for the risk of graft loss [40].

This study had several limitations, since it was performed retrospectively at a single center and the group of patients with or without dnDSA were small. Patient background factors, immunosuppressive regimens, time after transplantation, and other factors may influence the development of dnDSA. Furthermore, we did not assess the impact of preexisting DNA on graft survival and data on MPA trough levels

were not available. Moreover, CMV infection was not diagnosed by measurement of CMV-DSA. Finally, we could not clarify the mechanism by which treatment of CMV infection, but not CMV infection itself, was associated with development of dnDSA.

5. Conclusion

Detection of dnDSA at 1 year after renal transplantation was associated with worse long-term death censored renal graft survival. A lower tacrolimus trough level, discontinuation of MMF, and treatment of CMV infection within 1 year after transplantation were factors associated with dnDSA positivity. Further investigation is required to assess whether a new immunosuppressive regimen that avoid these factors can reduce dnDSA production.

Acknowledgments

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Ethical approval

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Declaration of competing interest

All authors declare no conflicts of interest.

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