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ORIGINAL ARTICLE

Influence of a low-dose tacrolimus protocol on the appearance of *de novo* donor-specific antibodies during 7 years of follow-up after renal transplantation

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ABSTRACT

Background. Tacrolimus (TAC) is a key immunosuppressant drug for kidney transplantation (KTx). However, the optimal serum trough level of TAC for good long-term outcomes remains unclear. This study aimed to investigate the relationship between the maintenance TAC trough level and the appearance of *de novo* donor-specific anti-human leukocyte antigen (HLA) antibodies (*dn*DSAs).

Methods. A total of 584 KTx recipients were enrolled in this study, of whom 164 developed *dn*DSAs during the follow-up period and 420 did not.

Results. We found no significant relationship between TAC trough level during the follow-up period and *dn*DSA incidence. Patients who developed *dn*DSAs had a significantly greater number of HLA-A/B/DR mismatches (3.4 ± 1.3 versus 2.8 ± 1.5; $P < 0.001$), were more likely to have preformed DSAs (48.2% versus 27.1%; $P < 0.001$) and showed poor allograft outcome.

Conclusions. There was no clear relationship between TAC trough level and *dn*DSA incidence for KTx recipients whose TAC trough levels were kept within the narrow range of 4–6 ng/mL during the immunosuppression maintenance period.

Keywords: clinical research/practice, *de novo* donor-specific anti-HLA antibodies, kidney transplantation/nephrology, rejection, tacrolimus

INTRODUCTION

With advances in immunosuppressive therapy and complication management, long-term graft survival after kidney transplantation (KTx) has improved in recent years. Therefore, lifelong immunosuppressive management is required to prevent allograft rejection and nonimmunological complications such as chronic allograft dysfunction, cardiovascular diseases, infectious diseases, malignancy, hypertension, dyslipidemia and diabetes mellitus [1, 2]. These nonimmunological complications are deeply related to long-term administered immunosuppressive medications such as tacrolimus (TAC) and steroids.

TAC is a current key drug mainly used as an immunosuppressant for KTx recipients [3]. In general, lower TAC concentration due to patient nonadherence or inadequate immunosuppression is one of the causes of acute rejection [4–6] through the development of *de novo* donor-specific anti-human luekocyte antigen (HLA) antibodies (*dn*DSAs) [7], especially during the early phase after transplantation, leading to deterioration of graft function.

On the other hand, high TAC trough levels during the maintenance period may cause progressing arteriolar hyalinosis, arteriosclerosis and interstitial fibrosis and tubular atrophy, likewise leading to the deterioration of graft function. Therefore the appropriate concentration of TAC for long-term use remains a subject of controversy.

At our institution, a TAC protocol using a lower trough concentration has been in use since the 2000s. The aim of this study is to investigate the relationship between maintenance trough levels of TAC and the rate of *dn*DSA appearance during long-term follow-up after KTx. We conducted a retrospective review and statistical analysis assessing the relationship between TAC trough concentration and *dn*DSA incidence over an average period of 7 years.

MATERIALS AND METHODS

Patient population

We evaluated a total of 994 patients who received a transplant at the Department of Urology of Tokyo Women's Medical

KEY LEARNING POINTS

What is already known about this subject?

- Tacrolimus (TAC) is a current key drug mainly used as an immunosuppressant for kidney transplant recipients. In general, a lower TAC concentration is one of the causes of acute rejection through the development of *de novo* donor-specific anti-human leukocyte antigen (*dn*DSA) antibodies, especially during the early phase after kidney transplantation (KTx), leading to the deterioration of allograft function.

What this study adds?

- The aim of this study was to investigate the relationship between maintenance trough levels of TAC and the appearance of *dn*DSA during long-term follow-up after KTx. We retrospectively reviewed and statistically analyzed the association of low trough concentrations of TAC and *dn*DSA incidence for a period of 7 years.

What impact this may have on practice or policy?

- There was no clear relationship between TAC trough level and the incidence of *dn*DSAs for KTx recipients within a rather narrow range of TAC levels, such as between 4 and 6 ng/mL, during the immunosuppression maintenance period.

University between 2000 and 2015 (Figure 1). Of these patients, 410 were excluded: pediatric recipients, deceased transplant recipients, extremely highly sensitized recipients such as complement-dependent cytotoxicity T-test positive and/or desensitization treatment–resistant (highly sensitized patients such as desensitization treatment–reactive patients were included [8]), recipients who did not undergo regular follow-up [regular renal biopsies and/or regular monitoring for DSAs by single-antigen bead assay (SABA)] and recipients who underwent follow-up at unknown hospitals. Thus 584 patients were included in this study.

Data were extracted from the Japan Academic Consortium of Kidney Transplatation study II [University Hospital Medical Information Network (UMIN) Clinical Trials Registry number: UMIN 000033449]. The study protocol was approved by the institutional research ethics committee (approval number 4460) and was consistent with the guidelines of the Declaration of Helsinki. All patients provided written informed consent.

Immunosuppressive regimen

Beginning a week before transplantation, all patients were treated following a triple immunosuppressive protocol including TAC (0.1 mg/kg/day), mycophenolate mofetil (MMF; 1500 mg/day if body weight was $<$50 kg, 2000 mg/day if $\geq$50 kg) and methylprednisolone (MP; 20 mg/day). Recipients whose transplants took place after 2002 also received basiliximab as an induction immunosuppression therapy,

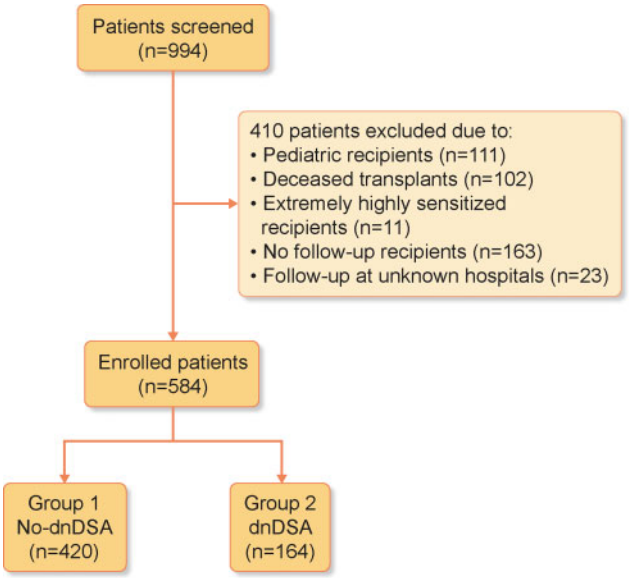

FIGURE 1: Flow diagram. Among the 994 patients who underwent transplantation in our institution within the study period, 584 patients were enrolled in this study. For the exclusion criteria, 'extremely highly' sensitized patients represent, for example, complement-dependent cytotoxicity T-test-positive and/or desensitization treatment-resistant recipients.

administered on the day of transplantation and on postoperative Day 4 [9]. Beginning in 2005, a single dose of rituximab (200 mg) was administered within 3–4 days before the operation if the transplant was ABO incompatible, the patient was highly sensitized or the etiology of end-stage renal disease (ESRD) was immunoglobulin A (IgA) nephropathy.

Plasma exchange (PE) was performed according to a previously reported protocol used for ABO-incompatible transplantation or before transplantation in patients with a history of sensitization [10]. This protocol involves a total of two or three sessions before surgery to reduce the anti-A/B antibody titer to $\geq$1:32 [11]. Some patients also received preoperative PE therapy to prevent recurrence of their original kidney disease, such as focal segmental glomerulosclerosis (FSGS). Intravenous immunoglobulin (IVIG) therapy was also performed for highly sensitized cases. Antithymocyte globulin was not used for induction in this study.

Patients received either a twice-daily dose of TAC (Prograf; Astellas Fujisawa, Osaka, Japan) or a once-daily dose of a prolonged-release formulation (Graceptor; Astellas Fujisawa). The dose was adjusted to maintain a trough level of TAC in whole blood of 8–12 ng/mL during the first month after KTx, 7–9 ng/mL during the second and third months and 4–6 ng/mL thereafter. The trough concentration of TAC was measured and analyzed before KTx; 2 weeks after KTx; 1, 3 and 6 months after and yearly from 1 to 10 years after. Routine TAC level measurements were performed in our institution by chemiluminescence immunoassay. When the results were 2 standard deviations (SDs) above or below normal, the assay was repeated and the average was taken as the value to be recorded. TAC levels given in this study are the average values for 3 months around each observation time.

The dose of MMF (Cellcept; Roche, Nutley, NJ, USA) was decreased to 1000–1500 mg/day ∼2 weeks after KTx, then to 1000 mg/day ∼1 month after KTx. The dose was also reduced in some patients who experienced side effects, such as diarrhea and viral infection. MP was administered intravenously at doses of 500 mg on the day of transplantation, 250 mg the day after the operation and 125 mg 2 days after the operation. MP was orally started 5 days after the operation at a dose of 20 mg/day; the dose was then tapered to 4 mg/day within 1 month after transplantation.

Graft function was evaluated according to estimated glomerular filtration rate (eGFR), calculated using the Modification of Diet in Renal Disease equation, which was measured and analyzed at our hospital before KTx; 2 weeks after KTx; 1, 3 and 6 months after and yearly from 1 to 10 years after.

Assessment of preformed DSAs and dnDSAs

Serum collected within 6 months before transplantation was analyzed by SABA (FlowPRA; One Lambda, Canoga Park, CA, USA) as previously reported [10]. Luminescence was read on a LABScan 100 Luminex system (One Lambda) and a mean fluorescence intensity >1000 was considered a positive result.

Polymerase chain reaction sequence-specific oligonucleotide technology (LABType XR; One Lambda) was used for HLA typing of recipients and donors (HLA-A, -B, -C, -DQ and -DR). Until 2016, routine HLA typing was performed only for HLA-A, HLA-B and HLA-DR; HLA-C and HLA-DQ were typed only if antibodies against them were detected by SABA, whereupon typing was performed to determine whether the antibodies were DSAs or non-DSAs. Patients with anti-HLA antibodies that recognized and cross-reacted with epitope groups specific to donor-mismatched HLAs were considered to have DSAs.

Serum analysis by SABA was again performed 6 months after KTx and then followed up annually. SABA was also performed when a patient underwent for-cause biopsy or when rejection was suspected on clinical evaluation.

During the follow-up period, *dn*DSAs sometimes disappeared and reappeared. Patients who tested positive for *dn*DSAs even once during this period were included in the *dn*DSA group.

Diagnosis and treatment of rejection

Protocol allograft biopsies were performed within 6 months and around 1-year post-KTx and, if possible, annually thereafter. When rejection was suspected, an episode biopsy was performed. The type of rejection was classified as T-cell-mediated rejection (TCMR) or antibody-mediated rejection (ABMR) according to the Banff 2015 criteria. All biopsy specimens were evaluated using light microscopy and the specimens obtained were evaluated for C4d using immunofluorescence staining. Two or three core biopsy samples were obtained using a spring-loaded 16-gauge biopsy gun under ultrasound guidance. The diagnosis of rejection was made by pathologists from our institution's pathology department.

All patients showing TCMR or ABMR received bolus MP (500 mg) intravenously for 2 days. Patients diagnosed as having acute or chronic active ABMR were treated with PE and/or IVIG and administered rituximab. Patients diagnosed with acute or chronic active TCMR were treated with antithymocyte globulin.

Statistical analyses

Statistical analyses were performed with SAS version 9.4 (TS1M5; SAS Institute, Cary, NC, USA). One-way analysis of variance was used to compare normally distributed continuous variables, while the chi-square test was used to compare nominal variables; a two-tailed P-value <0.05 was considered statistically significant. A logistic regression analysis was also performed, with P-values <0.05 considered statistically significant.

RESULTS

Demographics and baseline characteristics

A total of 584 transplant recipients were enrolled in this study, of whom 164 developed *dn*DSAs during the follow-up period and 420 did not. Thereafter we divided the enrolled patients into two groups (Group 1, no *dn*DSA, $n = 420$; Group 2, *dn*DSAs, $n = 164$). Patient demographics are given in Table 1. Patients were followed up for an average of 7.4 ± 2.6 years, without significant differences between the two groups.

In Group 2, *dn*DSAs appeared at 812 ± 102 days on average after KTx (Figure 2). Class II *dn*DSAs are more common than Class I (140 cases [85.4%] and 50 cases [30.5%], respectively) (Table 1). There were no significant differences between the two groups with regard to sex, dialysis duration or renal function before KTx. With regard to the etiology of ESRD, the incidence of IgA nephropathy was significantly higher in Group 1 than in Group 2. There were no significant differences between the groups with regard to patients' medical history of KTx, pregnancy or blood transfusion.

Donor and transplant details are given in Table 2. There were no significant differences between the groups with regard to donor profiles (age, sex and blood relationship with recipients). The number of mismatches in HLA-A/B/DR was more significant in Group 2 than in Group 1 (3.4 ± 1.3 in Group 2 versus 2.8 ± 1.5 in Group 1; $P < 0.001$). Patients with preformed DSA also showed a higher incidence of *dn*DSA production (48.2% in Group 2 versus 27.1% in Group 1; $P < 0.001$). Details regarding preformed DSAs are given in Table 3; some patients had several types. There were no significant differences between the groups with regard to the immunosuppressant regimen used.

The

TAC dosage and trough level

There were no significant differences between the two groups with regard to mean TAC trough concentration during the follow-up period (Figure 3). Furthermore, there were no significant differences in the mean TAC dosage per kilogram during the follow-up period, except at 6 months and 1 year after KTx (Figure 4). For each group as a whole, the mean trough

Table 1. Baseline characteristics of all patients

Variable	Total	Group 1, no *dn*DSA	Group2, *dn*DSA	P-value
Patients, *n*	584	420	164	–
*dn*DSA	164	0	164	–
Class I, *n* (%)	50 (8.6)	–	50 (30.5)	–
Class II, *n* (%)	140 (24.0)	–	140 (85.4)	–
Age at transplant (years), mean ± SD	45.2 ± 13.4	44.5 ± 13.3	47.1 ± 13.6	0.036
Recipient sex, *n* (%)				
Male	379 (64.9)	263 (62.6)	116 (70.7)	0.065
Female	205 (35.1)	157 (37.4)	48 (29.3)	
Duration of dialysis (months), median (range)	30 (13–64)	29 (13–63)	32 (13–74)	0.33
Serum creatinine (mg/dL), mean ± SD	10.8 ± 3.1	10.8 ± 3.2	10.7 ± 2.9	0.923
eGFR (mL/min/1.73 m^2), mean ± SD	4.9 ± 2.0	4.9 ± 2.1	4.9 ± 1.9	0.751
Etiology of ESRD, *n* (%)				
Chronic glomerulonephritis	118 (20.2)	74 (17.6)	44 (26.8)	0.008
Diabetic nephropathy	68 (11.6)	46 (11.0)	22 (13.4)	–
IgA nephropathy	120 (20.5)	98 (23.3)	22 (13.4)	–
Polycystic kidney disease	35 (6.0)	28 (6.7)	7 (4.3)	–
Hypoplastic kidney	8 (1.4)	7 (1.7)	1 (0.6)	–
FSGS	31 (5.3)	25 (6.0)	6 (3.7)	–
Nephrosclerosis	22 (3.8)	17 (4.0)	5 (3.0)	–
Other	85 (14.6)	65 (15.5)	20 (12.2)	–
Unknown	96 (16.4)	59 (14.0)	37 (22.6)	–
Past history, *n* (%)				
KTx	45 (7.7)	33 (7.9)	12 (7.3)	0.826
Pregnancy	107 (18.3)	78 (18.6)	29 (17.7)	0.803
Blood transfusion	172 (29.5)	120 (28.6)	52 (31.7)	0.356

Bold values are statistically significant at $P < 0.05$.

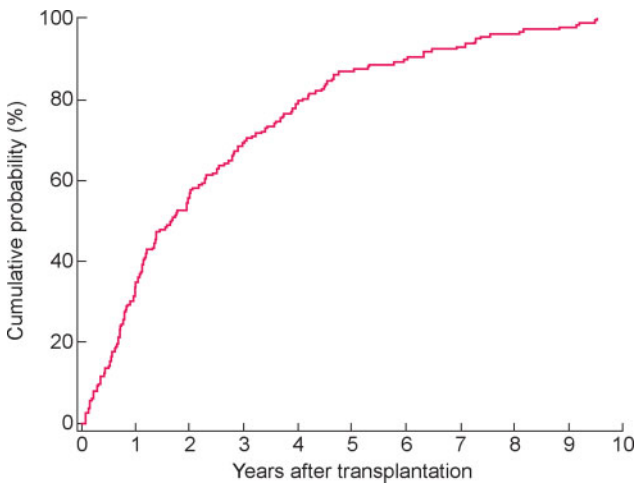

FIGURE 2: Kaplan–Meier estimates for time to developing *dn*DSAs. Recipients who developed *dn*DSAs only.

concentration of TAC was within set target ranges throughout the study period. At every follow-up appointment in the outpatient clinic we monitored the TAC trough level and adjusted the dose of TAC to be administered until the next appointment. However, intrapatient variability in TAC exposure may occur as a result of nonadherence to treatment, abnormal absorption after gastrointestinal tract surgery or pharmacological interaction with other drugs, and certain genetic polymorphisms may also have an effect [12, 13]; accordingly, some patients showed TAC trough levels outside the target ranges [14]. The percentage of patients with trough levels outside the target ranges at each time point (2 weeks; 1, 3 and 6 months; 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 years after KTx) were 29.0, 37.8, 45.4, 27.7, 23.9, 24.1, 25.3, 28.1, 26.2, 28.1, 25.3, 27.3, 29.3 and 25.1% in Group 1 and 31.2, 37.4, 42.5, 29.6, 24.5, 29.5, 23.2, 19.2, 23.8, 24.5, 22.4, 21.2, 25.6 and 15.1% in Group 2, respectively. Thus TAC levels in the majority of patients were maintained within our specified target ranges.

Graft outcomes

Biopsy-proven rejection was observed in 158 patients (Table 4). The incidence of ABMR was more significant in Group 2 than in Group 1 [acute ABMR, 35/420 (8.3%) in Group 1 versus 48/164 (29.3%) in Group 2, $P < 0.001$; chronic ABMR, 22/420 (5.2%) in Group 1 versus 58/164 (35.4%) in Group 2, $P < 0.001$].

Regarding graft function, eGFR 1–4 years and 6–7 years after KTx was significantly worse in Group 2 than in Group 1 (Figure 5). There were no significant differences at 2 weeks, 1, 3 or 6 months or 5, 8, 9 or 10 years after KTx.

The rate of death-censored graft failure was also significantly worse in Group 2 than Group 1 [18/420 (4.3%) in Group 1 versus 28/164 (17.1%) in Group 2; $P < 0.001$]. Patients in Group 2 also had a higher incidence of proteinuria when compared with patients in Group 1 (data not shown). There was no significant difference between groups in the rate of death with a functioning graft.

Relationship between dnDSA incidence and TAC trough level

The 584 patients were divided into three groups according to their average TAC trough concentrations during the

Table 2. Donor and transplantation information

Variable	Total	Group 1, no dnDSA	Group 2, dnDSA	P-value
	584	420	164	
Donor information				
Age at transplant (years), mean ± SD	57.7 ± 9.4	57.8 ± 9.5	57.3 ± 9.4	0.592
Donor sex, *n* (%)				
Male	185 (31.7)	134 (31.9)	51 (31.1)	0.851
Female	399 (68.3)	286 (68.1)	113 (68.9)	
Donor type, *n* (%)				
Father	73 (12.5)	54 (12.9)	19 (11.6)	0.177
Mother	193 (33.0)	148 (35.2)	45 (27.4)	–
Sibling	78 (13.4)	58 (13.8)	20 (12.2)	–
Child	5 (0.9)	2 (0.5)	3 (1.8)	–
Spouse	219 (37.5)	147 (35.0)	72 (43.9)	–
Other blood relative	8 (1.4)	6 (1.4)	2 (1.2)	–
Non-blood relative	8 (1.4)	5 (1.2)	3 (1.8)	–
Transplantation information				
Histocompatibility				
ABO incompatibility, *n* (%)	186 (31.8)	134 (31.9)	52 (31.7)	0.963
HLA-A/B/DR mismatches, mean ± SD	2.9 ± 1.5	2.8 ± 1.5	3.4 ± 1.3	<0.001
Preformed DSA, *n* (%)	193 (33.0)	114 (27.1)	79 (48.2)	<0.001
Immunosuppressive therapy, *n* (%)				
TAC	584 (100)	420 (100)	164 (100)	
MMF	553 (94.7)	399 (95.0)	154 (93.9)	0.595
MP	584 (100)	420 (100)	164 (100)	
Basiliximab	458 (78.4)	324 (77.1)	134 (81.7)	0.228
Rituximab	350 (59.9)	250 (59.5)	100 (61.0)	0.772
DFPP/PE	290 (49.7)	205 (48.8)	85 (51.8)	0.528
IVIG	14 (2.4)	8 (1.9)	6 (3.7)	0.247

Bold values are statistically significant at $P < 0.05$.
For the donor type, 'other blood relative' represents, for example, aunts, cousins, nephews and nieces (no uncles in this study) and 'non-blood relative' represents, for example, fathers, mothers, brothers, sisters and sons-in-law. DFPP, double filtration plasmapheresis.

Table 3. Preformed DSA information

Variable	Class 1			Class 2		
Preformed DSA ($n = 193$)	93			123		
Type of HLA locus	A	B	Cw	DP	DQ	DR
	52	45	5	2	35	86

maintenance period, calculated based on 11 time points (6 months after KTx and yearly from 1 to 10 years after KTx) as follows: low, ≤4 ng/mL ($n = 39$); medium, 4–6 ng/mL ($n = 488$); and high, >6 ng/mL ($n = 57$; Table 5). There were no significant differences among these groups with regard to *dn*DSA incidence, eGFR (Figure 6), donor sex and age, HLA-A/B/DR mismatches, preformed DSA, medical history or the incidence of biopsy-proven rejection. In all three groups, patients' TAC trough concentrations stayed within the group range throughout the follow-up period (Figure 7).

Risk factors for dnDSA development

We performed a logistic regression analysis to determine the expected risk factors for development of *dn*DSAs. The average TAC trough concentration of each patient during the maintenance period was calculated based on 11 time points, as previously described. Preformed DSA ($P = 0.0001$) and HLA-A/B/DR mismatches ($P = 0.0005$) were found to be risk factors for the development of *dn*DSAs, but the average TAC trough concentration was not ($P = 0.328$; Figure 8).

DISCUSSION

TAC as a calcineurin inhibitor is a key drug for mainstream immunosuppression following KTx [3], along with antimetabolite medicine and steroids. TAC contributes to the prevention of allograft rejection through suppression of T-cell activity. Thus the management of TAC concentration is important for successful immunosuppressive therapy both before and after KTx.

In general, patients who develop *dn*DSAs after KTx have a higher rate of rejection [15], decreased graft function and poorer graft survival rates than those who do not [16–22]. In some recent studies, lower TAC trough levels were associated with the production of *dn*DSAs [7], leading to ABMR and graft function deterioration [5]. However, these findings apply only to the early phases after KTx, e.g. within 5 years, and there are few reports dealing with the relationship between TAC trough concentration and long-term graft outcomes.

Higher TAC immunosuppression may be associated with complications such as chronic calcineurin inhibitor toxicity, including arteriosclerosis, hyalinosis and ischemic changes, leading to deterioration, infection, malignancy, hypertension, dyslipidemia, diabetes mellitus and other problems. These factors may affect graft and patient survival in the long term. Thus post-KTx management using a lower TAC level may be acceptable and

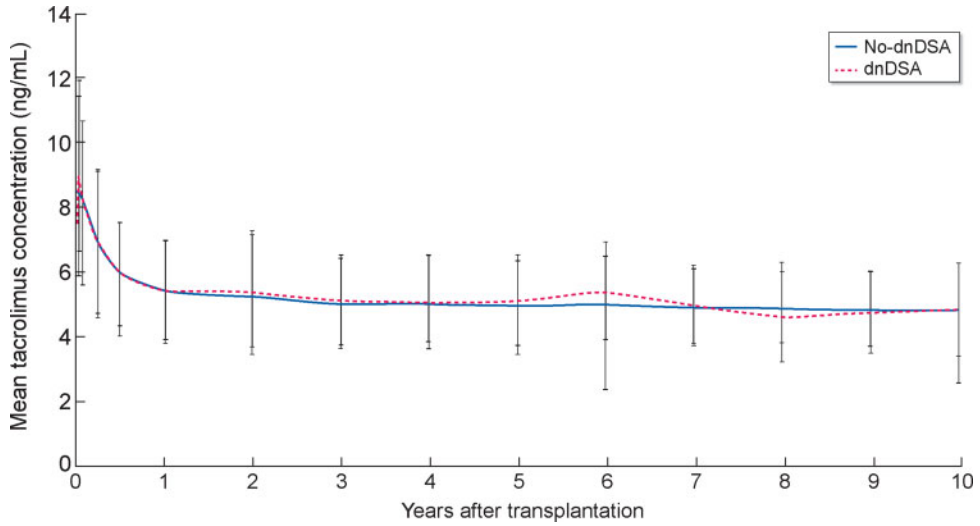

FIGURE 3: Mean TAC concentrations of each group in the different follow-up durations (mean ± SD).

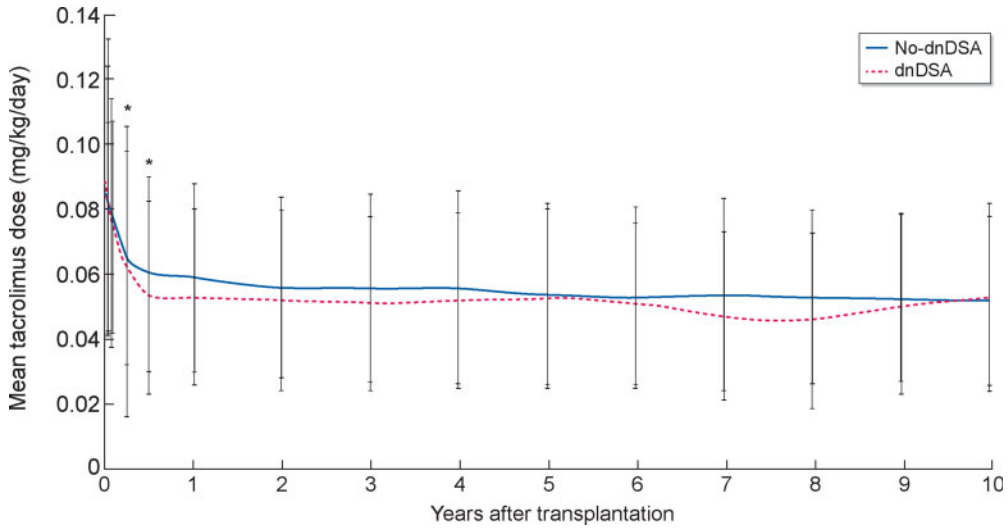

FIGURE 4: Mean TAC doses of each group in the different follow-up durations (mean ± SD).
*P < 0.05 calculated with Student's *t*-test.

Table 4. Incidence rates of rejection and outcomes

Variable	Total	Group 1, no *dn*DSA	Group 2, *dn*DSA	P-value
	584	420	164	
Follow-up duration (years), mean ± SD	7.4 ± 2.6	7.3 ± 2.7	7.5 ± 2.5	0.569
Biopsy-proven rejection, *n* (%)				
Acute rejection	158 (27.1)	84 (20.0)	74 (45.1)	<0.001
A-TCMR	104 (17.8)	63 (15.0)	41 (25.0)	0.005
A-ABMR	83 (14.2)	35 (8.3)	48 (29.3)	<0.001
Chronic rejection	89 (15.2)	30 (7.1)	59 (36.0)	<0.001
C-TCMR	14 (2.4)	9 (2.1)	5 (3.0)	0.52
C-ABMR	80 (13.7)	22 (5.2)	58 (35.4)	<0.001
Outcomes, *n* (%)				
Death-censored graft failure	46 (7.9)	18 (4.3)	28 (17.1)	<0.001
Death with functioning graft	12 (2.1)	11 (2.6)	1 (0.6)	0.124

Bold values are statistically significant at P < 0.05.
A-TCMR, acute T-cell-mediated rejection; A-ABMR, acute antibody-mediated rejection; C-TCMR, chronic T-cell-mediated rejection; C-ABMR, chronic antibody-mediated rejection.

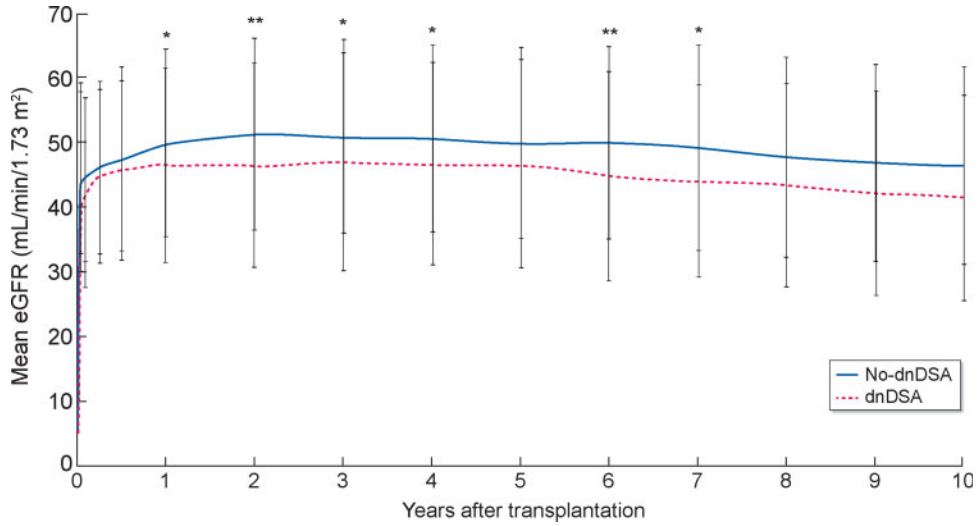

FIGURE 5: Mean eGFR of each group in the different follow-up durations (mean ± SD).
*P < 0.05, **P < 0.01 calculated with Student's *t*-test.

Table 5. Classifications according to average TAC concentration

TAC concentration (ng/mL)		≤4	4–≤6	>6	
Variable	Total	Low	Medium	High	P-value
	584	39	488	57	
TAC concentration during the follow-up duration (ng/mL), mean ± SD	5.05 ± 0.83	3.67 ± 0.29	4.97 ± 0.50	6.68 ± 0.91	<0.001
*dn*DSA, *n* (%)	164 (28.1)	10 (25.6)	138 (28.3)	16 (28.1)	0.940
Age at transplant (years), mean ± SD	45.2 ± 13.4	45.2 ± 14.0	45.2 ± 13.4	45.1 ± 13.6	0.982
Recipient sex, *n* (%)					0.321
Male	379 (64.9)	26 (66.7)	311 (63.7)	42 (73.6)	
Female	205 (35.1)	13 (33.3)	177 (36.3)	15 (26.3)	
HLA-A/B/DR mismatches, mean ± SD	2.9 ± 1.5	2.7 ± 1.5	2.9 ± 1.5	2.8 ± 1.5	0.436
Preformed DSA, *n* (%)	193 (33.0)	14 (35.9)	159 (32.6)	20 (35.1)	0.862
Past history, *n* (%)					
KTx	45 (7.7)	3 (7.7)	37 (7.6)	5 (8.8)	0.951
Pregnancy	107 (18.3)	7 (17.9)	93 (19.1)	7 (12.3)	0.458
Blood transfusion	172 (29.5)	11 (28.2)	142 (29.1)	19 (33.3)	
Donor information					
Age at transplant (years), mean ± SD	57.7 ± 9.4	58.5 ± 9.1	57.6 ± 9.5	57.6 ± 9.4	0.866
Donor sex, *n* (%)					0.818
Male	185 (31.7)	13 (33.3)	155 (31.8)	17 (29.8)	
Female	399 (68.3)	26 (66.7)	333 (68.2)	40 (70.0)	
Biopsy-proven rejection, *n* (%)					
Acute rejection	158 (27.1)	11 (28.2)	129 (26.4)	18 (31.6)	0.701
A-TCMR	104 (17.8)	8 (20.5)	83 (17.0)	13 (22.8)	0.503
A-ABMR	83 (14.2)	5 (12.8)	70 (14.3)	8 (14.0)	0.966
Chronic rejection	89 (15.2)	8 (15.4)	74 (15.2)	7 (12.3)	0.543
C-TCMR	14 (2.4)	1 (2.6)	11 (22.5)	2 (3.5)	0.841
C-ABMR	80 (13.2)	8 (15.4)	66 (13.5)	6 (10.5)	0.364

there may be many long-term advantages for post-KTx management as long as the patient does not develop *dn*DSAs.

At our institution, a protocol using a lower trough concentration of TAC has been in use since the 2000s. We investigated whether *dn*DSA incidence differs according to the recipient's immunological sensitization, the immunosuppressive regimen used or the TAC trough level during maintenance. All the patients in this study received TAC according to the aforementioned protocol and their TAC levels were well managed within the set target ranges.

Previous studies have reported the prevalence of *dn*DSA development to be approximately 2–10% at 1 year post-KTx, reaching 10–40% by 4–5 years post-KTx [23–27]. In our study, *dn*DSAs were seen in 164 recipients (28.1%) over an average observation period of 7 years. Reports of the median onset of *dn*DSAs vary from 3.8 to 68 months after KTx [23, 27, 28]; in our study, *dn*DSAs appeared at 27.1 ± 2.27 months after KTx. In a previous study we reported the *dn*DSA incidence for ABO-compatible KTx recipients as 13% at 5 years after KTx and the time of onset

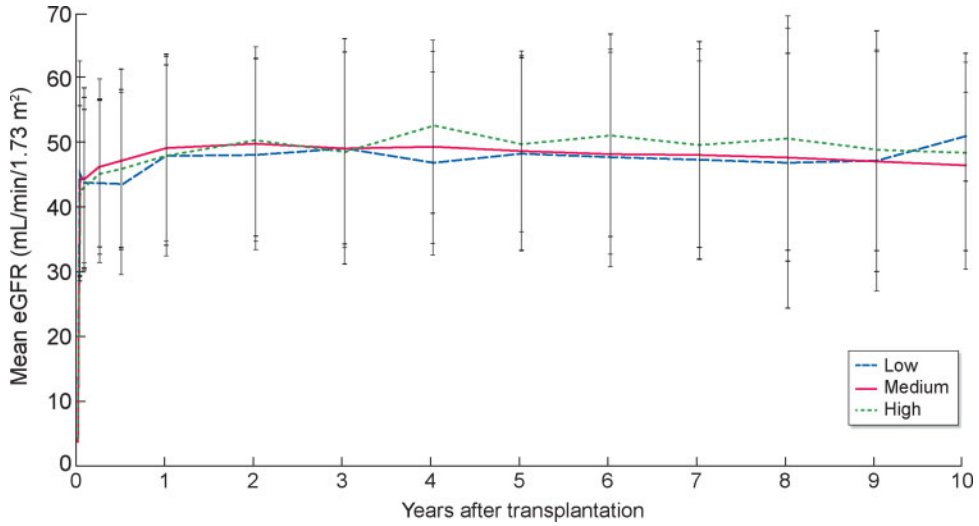

FIGURE 6: Mean eGFRs of the TAC concentration classifications at the different follow-up durations (mean ± SD).

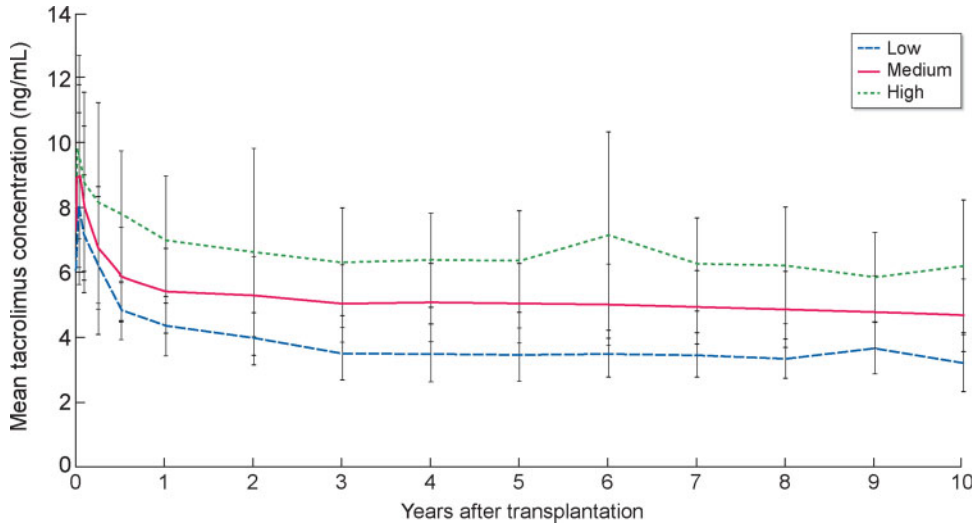

FIGURE 7: Mean TAC doses of the TAC concentration classifications at different follow-up durations (mean ± SD).

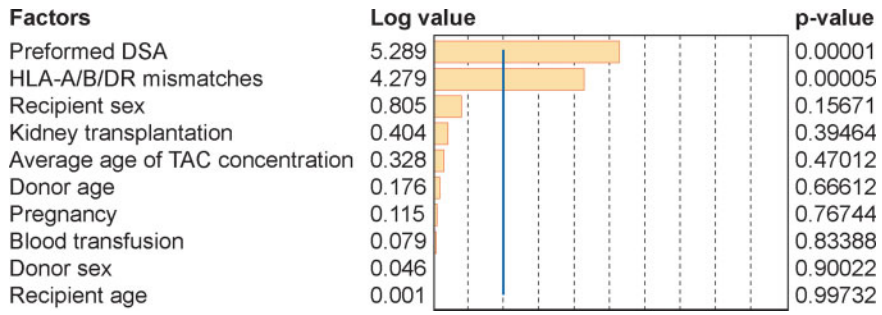

FIGURE 8: Logistic regression analysis of the expected risk factors for the development of *dn*DSAs.

as 46.5 months after KTx [29]. The reason for this discrepancy could be that in the previous study, antibodies against HLA-C and HLA-DQ were not counted as DSAs, thus *dn*DSA incidence could have been underestimated.

In our study, patients with *dn*DSAs had a much higher rate of ABMR (29.3% had acute ABMR, 35.4% had chronic ABMR) than those without. The prevalence of chronic ABMR was particularly high. A reason for this could be the long follow-up period, averaging 7 years. The presence of *dn*DSAs over a prolonged period is associated with chronic ABMR and deteriorated allograft function.

We found the number of mismatches in HLA-A/B/DR and the presence of preformed DSAs to be risk factors for *dn*DSA

development. We did not routinely perform HLA typing for HLA-C and HLA-DQ until 2016, therefore we did not analyze the risk associated with HLA-C and HLA-DQ mismatches, which remains unknown. In addition, patients with preformed DSA showed a higher incidence of *dn*DSA production (48.2% in Group 2 versus 27.1% in Group 1; $P < 0.001$). It can be supposed that for KTx recipients with preformed DSAs, many B cells and long-lived plasma cells are generated as memory cells and reside in the bone marrow and lymphoid tissues throughout sensitization [30, 31]. Therefore, once KTx is performed, these cells react to the allograft, readily producing *dn*DSAs. Thus poor HLA histocompatibility and the existence of preformed DSAs are suggested as main risk factors for *dn*DSA production that may lead to allograft deterioration. With regard to the etiology of ESRD, the incidence of IgA nephropathy was significantly higher in Group 1 than in Group 2. For these patients, we usually administered rituximab as induction immunosuppression in order to prevent recurrence. This may have been somewhat efficacious in preventing *dn*DSA development.

In our study, TAC trough levels were very similar in the two groups throughout the observation period. Even in Group 2, TAC trough concentrations were maintained within the target range for an average of 7 years. As in previous reports, many patients with *dn*DSAs progress to ABMR, leading to allograft deterioration and loss. Thus good graft function and outcome in KTx recipients still depends on preventing *dn*DSA development through immunosuppressive therapy. Managing KTx recipients using lower TAC concentrations is thought to improve long-term outcomes by preventing complications caused by calcineurin inhibitors. Furthermore, our study suggests that the risk of *dn*DSA development may be related not to low TAC concentration, but to other factors such as HLA mismatches and the existence of preformed DSAs. Thus the maintenance of lower TAC concentrations, such as 4–6 ng/mL, may be acceptable for long-term KTx management.

In recent years, the examination of HLA epitope identification has become available using the HLA Matchmaker software [32, 33], and eplet mismatch is known to be a significant risk factor for developing *dn*DSAs [33]. Therefore future studies of immunosuppression with TAC should involve epitope analysis rather than simple serum assays for antibodies against HLA-DQ, HLA-DR or other HLAs. However, epitope identification is not currently widespread; in the future, it should be possible to integrate it with HLA mismatch identification for proper TAC management. In our study, some patients developed *dn*DSAs, while others with about the same TAC concentration did not. Furthermore, some patients did not progress to ABMR despite developing *dn*DSAs. With regard to these patients, we suspect the involvement of eplet mismatches affecting *dn*DSA development and/or progression to ABMR. Further analysis and examination are required.

This study has several limitations. First, TAC levels observed in our patients were generally within a narrow range (4–6 ng/mL). Our discussion applies only to the narrow range evaluated, for which no relationship could be observed between TAC levels and the risk of *dn*DSA production. Concentrations lower than the aforementioned range might still increase the risk of *dn*DSA production. Second, plasma concentration of mycophenolic acid was not routinely measured. In addition to inhibiting the production of *dn*DSAs and the development of rejection, MMF is also an important immunosuppressant. There is currently no clear evidence on the association between plasma concentration of mycophenolic acid and allograft outcome [12]. Finally, ABO-incompatible KTx cases were included in our study. Such cases are likely to be more heavily immunosuppressed as a result of rituximab administration and PE therapy, which may have an impact on the likelihood of developing *dn*DSAs. There were no significant differences between the two groups in our study with regard to the prevalence of ABO incompatibility, rituximab use or PE therapy for desensitization. On the other hand, these cases may affect the deterioration of graft function, although rejection in the setting of ABO incompatibility can occur via other mechanisms independent of ABMR or *dn*DSA.

In conclusion, our institution has used an immunosuppression protocol with lower target TAC concentrations since the 2000s, with no significant differences in *dn*DSA incidence compared with other institutions. There were no clear relationships between *dn*DSA incidence and immunosuppressive regimen or TAC trough level. Most notably, there were no significant differences in TAC concentration during the observation period between patients who developed *dn*DSAs and those who did not. Therefore management of KTx recipients at a lower TAC concentration appears not to be a main risk factor for developing *dn*DSAs and its advantages should be taken into account with regard to long-term outcomes for patients and allografts. However, as development of *dn*DSAs is closely associated with allograft deterioration, immunosuppressive therapy that prevents *dn*DSA development is still required. Thus it is important to use appropriate immunosuppressive therapies that reduce the risk of complications, including those caused by the immunological sensitization of individual recipients.

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AUTHORS' CONTRIBUTIONS

K.U., H.I., T.S. and M.F. participated in the research design, writing the article, performance of the research and data analysis. K.K., T.H., Y.K., M.O., K.T. and K.N. contributed to the design and editing of the article.

CONFLICT OF INTEREST STATEMENT

The authors of this manuscript have no conflicts of interest to disclose as described by the *Nephrology Dialysis Transplantation*.

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