ORIGINAL ARTICLE

Higher calcineurin inhibitor levels predict better kidney graft survival in patients with *de novo* donor-specific anti-HLA antibodies: a cohort study

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SUMMARY

The development of *de novo* anti-HLA donor-specific antibodies (dnDSA) is associated with poorer outcomes in kidney transplant recipients. Despite this, antibody screening post-transplant is not widespread, largely because the optimal management of patients with dnDSA remains undetermined. We hypothesized that in this population, calcineurin inhibitor blood levels would be an independent predictor of graft loss. We analyzed a cohort of unsensitized patients for whom anti-HLA antibody screening was performed prospectively post-transplant. During the screening period between January 2005 and April 2016, 42 patients developed dnDSA. There was no difference in the clinical characteristics or the histological scores of patients biopsied for clinical indication versus those biopsied solely due to detection of dnDSA. Cox modeling revealed a strong relationship between mean tacrolimus levels following dnDSA detection and graft loss, with a hazard ratio of 0.49 (95% CI, 0.33–0.75), which persisted following adjustment for established independent predictors (HR, 0.52, 95% CI, 0.30–0.89). Kaplan–Meier analysis by tertiles of tacrolimus levels and receiver operating curve analysis concurred to show that a threshold of 5.3 ng/ml could be predictive of graft loss. These data suggest that anti-HLA antibody monitoring post-transplant could guide maintenance immunosuppression and improve graft outcomes.

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Key words
calcineurins antagonists, histocompatibility, HLA-antibody post-transplantation, immunogenetics, immunosuppression clinical, immunosuppression kidney clinical

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Introduction

The association between the appearance of donor-specific anti-HLA antibodies (DSA) after kidney transplantation, referred to as *de novo* DSA (dnDSA), and poor graft outcome is clearly established [1,2]. However, the optimal management for patients who develop dnDSA has yet to be determined. Different protocols have been described for the treatment of acute antibody-mediated rejection (ABMR), but very few studies have reported on the treatment of patients with dnDSA in the context of chronic ABMR, and there is no clear therapeutic strategy for patients who develop dnDSA in the absence of rejection [2,3]. Given the lack of such data, there is reluctance to use plasma exchange, IVIG and rituximab in the absence

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of acute ABMR, due to the toxicity associated with these treatments.

It is also unclear if escalation of maintenance immunosuppression, in particular increased target levels of calcineurin inhibitors (CNIs), would improve the outcomes of these patients. We recently reported that patients with transplant glomerulopathy were more likely to have been prescribed reduction or withdrawal of immunosuppression, most frequently characterized by reduced CNIs [4]. Indeed, many CNI-sparing clinical trials have resulted in higher rejection rates [5–9]. From an immunological standpoint, the detection of dnDSA indicates plasma cell secretion of high-affinity alloantibodies, a process not reversible by CNIs. However, it is has long been known that CNIs can inhibit B-cell activation both directly, predominantly by blocking cell cycle progression through late G1 [10], and indirectly, by inhibiting T-cell activation and consequent B-cell activity. It is not yet established if either mechanism is sufficient to reduce ongoing immunologic damage to the graft and improve outcomes in patients with dnDSA.

We hypothesized that, in patients with dnDSA, higher blood CNI levels would be associated with better clinical outcomes. The aim of this study was to examine the relationship between CNI blood levels and graft loss in a consecutive cohort of kidney transplant recipients in whom dnDSA were detected prospectively and longitudinally by routine clinical protocol. We show that there is a strongly positive, independent association between higher CNI blood levels and graft survival, suggesting that DSA monitoring is clinically beneficial and can direct optimal patient management to improve graft outcome.

Materials and methods

Study design and population

This is a single-center, observational cohort study with prospective detection of circulating anti-HLA alloantibodies and prospective collection of follow-up clinical data. The study population consisted of consecutive patients in whom alloantibodies were detected during the screening period from January 2005 to April 2016, which corresponds to the time during which alloantibody detection was carried out using sensitive techniques for screening and identification, as detailed below. All incident kidney transplant recipients were routinely monitored for anti-HLA alloantibody development at 1, 3, 6 and 12 months in the first year post-transplant and yearly thereafter. Antibody monitoring was also conducted at the time of any protocol or indication biopsy, and 2–4 weeks following any significant sensitizing event. Any patient demonstrating at least one dnDSA was included. No patient was sensitized at the time of transplantation, and no patients were lost to follow-up. The study was approved by the institutional ethics committee. The clinical and research activities reported are consistent with the Principles of the Declaration of Istanbul.

Anti-HLA antibody assessment

Serum samples were screened for anti-HLA antibodies by flow cytometry using FlowPRA beads (One Lambda, Canoga Park, CA, USA). Whenever antibody screening was positive, samples were tested for anti-HLA antibody identification by flow cytometry using flow single antigen beads from 2005 to 2012. Starting in 2012, HLA antibody identification was performed using LABScreen single antigen beads (One Lambda) on a Luminex platform. Antibody specificities were identified based on normalized mean fluorescence intensity (nMFI) $\geq$1500. However, HLA specificities falling below the established nMFI cutoff were also considered positive if a reactivity pattern consistent with a commonly shared expressed epitope was seen. Such was the case in two patients. Analysis of epitope reactivity is verified using the HLA epitope registry (http://epregistry.ufpi.br).

Donor HLA-DQ typing data were not available for three of seven patients with anti-DQ antibodies. Unfortunately, typing could not be repeated retrospectively as no stored donor DNA or cell sample was available. As HLA-DR typing was available for all donors, HLA-DQ typing was assigned based on frequency associations within the donor ethnic group. This was performed using NMDP data (Haplostats). Whenever initial patient HLA-DQ typing was unavailable, it was performed retrospectively to rule out any nonspecific reactivity or autoreactivity.

Pathologic classification

Biopsies were prospectively graded by the local attending pathologists (J.R. and E.L.) according to the Banff 1997 criteria, which were updated in 2003, 2008 and 2013 [11–13]. Pathologists were blinded to the results of antibody monitoring.

Measurement of CNI exposure

Exposure to tacrolimus was defined as the mean of blood levels measured at 1, 3, 6, 12 and 24 months

following dnDSA detection. If a measurement was not available at one of these precise time points, a mean of the values obtained within 2 weeks of the time point was used. To account for variability in blood level measurements at these defined time points, a mean of these values was computed when more than one value was available within 5 days of the time point. In sensitivity analyses, the mean of all tacrolimus blood levels available within the first 24 months post-dnDSA detection was used as the exposure variable for each patient. Nonadherence was defined as described previously [14]. Four patients received cyclosporine instead of tacrolimus. In these cases, C_2 blood levels were converted to C_0 tacrolimus equivalents using an empiric 1/115 correction factor, based on respective targets of 800 ng/ml for cyclosporine and 7 ng/ml for tacrolimus [6,15].

Statistical analyses

Comparisons of baseline clinical characteristics between patients biopsied for clinical indication and those biopsied solely due to detection of dnDSA were conducted using Mann–Whitney test, Fisher's exact test or Chi-squared test. Comparison of tacrolimus blood levels at the time of and after detection of dnDSA was performed with a paired *t*-test. The association between mean tacrolimus levels, used as a continuous variable, and graft loss was assessed by Cox proportional hazards models. Violations of the proportional hazards assumption were examined by plotting the negative logarithm of the estimated survivor function versus log time. Additional analyses were performed using tertiles of mean tacrolimus levels in Kaplan–Meier curves and log-rank testing. In the sensitivity analysis, correlation between the mean of tacrolimus levels at defined time points versus the mean of all tacrolimus levels available was conducted with Pearson's test. Statistical analyses were performed using STATA version 11.0 (StataCorp, College Station, TX, USA) and SPSS STATISTICS version 23 (IBM, Armonk, NY, USA) All tests were two tailed, and a $P < 0.05$ was considered statistically significant.

Results

Study population

Anti-HLA antibodies were monitored routinely in the first year post-transplant at months 1, 3, 6 and 12, and then on an annual basis for every patient. In addition, screening was performed on the day of any protocol or indication biopsy and within 2–4 weeks of a sensitizing event. We included all patients in whom dnDSA were first detected between January 2005 and April 2016. Forty-two patients met this criterion. No patient was excluded. These patients were transplanted between February 2001 and April 2014, a period during which 734 transplants were performed. The incidence of dnDSA within the total cohort was 5.7%. Patients were mostly male recipients of a first transplant (62%, Table 1). The proportion of males was similar in patients without DSA 67%, $P = 0.50$). In thirty patients (71%), dnDSA were detected by surveillance monitoring in the context of stable graft function. Median time to dnDSA detection was 52 months (25–75th percentiles, 25, 90) post-transplant. Twelve patients (29%) were diagnosed before 2012.

Biopsy results

Twenty-three (55%) patients had a biopsy performed, either because clinically indicated or triggered by positive anti-HLA antibody screening performed as part of the routine immunosurveillance. Five patients declined a biopsy. In three patients, dnDSA to HLA-DQ were identified only upon retrospective data analysis. In these cases, dnDSA to HLA-DQ were identified based on high frequency association with donor mismatched HLA-DR antigens, as HLA-DQB1 typing was not routinely performed in the earlier transplant patients and donors. In the 11 remaining cases, the attending physician did not order a biopsy.

Of all biopsies performed, 11 (48%) were performed solely due to protocol antibody monitoring, in the absence of any clinical indication; there was no difference in the demographics, the presence of HLA class I- or class II-specific dnDSA, or the histological lesion scores (Table 1) in these patients compared to those who underwent a clinically indicated biopsy. However, there was a nonsignificant trend toward higher HLA-DR mismatch (1.0 ± 0.2 vs. 0.6 ± 0.2, $P = 0.10$) and lower values for the ptc score in the former group (0.6 ± 0.3 vs. 1.4 ± 0.4, $P = 0.17$).

Exposure to CNIs

To study the relationship between tacrolimus levels and outcomes, we first computed the mean of the tacrolimus levels at 1, 3, 6, 12 and 24 months after dnDSA detection for each patient and used it as the exposure variable. This value varied substantially between patients, with an overall mean of 5.9 (SD 1.8) ng/ml (Fig. 1a). Four patients had a CNI-free regimen prior to

dnDSA detection; tacrolimus was reintroduced in each at varying time points: in one patient at 1 month following the appearance of dnDSA, in two patients at 6 months and at 12 months in one patient. We compared the mean tacrolimus levels post-dnDSA detection to the level at the time of detection on a within-patient basis. We found no significant difference ($P = 0.44$ by paired t-test) after exclusion of the four patients in whom tacrolimus was reintroduced (Fig. 1b).

Association between tacrolimus levels and graft outcomes

During follow-up, 12 patients (29%) experienced graft loss at a median time of 16 (25th–75th percentiles, 8–38) months post-dnDSA detection. We first assessed the relationship between tacrolimus levels as a continuous variable and graft survival in Cox proportional hazards models on the full cohort (Table 2). In the unadjusted model, there was a strong positive association between tacrolimus levels and lower risk of the endpoint (HR, 0.49; 95% CI, 0.33–0.75; $P = 0.001$).

Next, we examined whether this association was independent of known risk factors for graft loss in patients who develop dnDSA; Wiebe *et al.* [14] recently identified delayed graft function, nonadherence, tubulitis and cg scores as such factors. In the adjusted Cox model, the association between mean tacrolimus levels post-dnDSA detection and graft loss was persistent when successively adjusted for these predictors (Table 2).

Table 1. Clinical and pathological characteristics of the population.

	All dnDSA positive patients ($n = 42$)	Biopsy for dnDSA as sole indication ($n = 11$)	Biopsy with a clinical indication ($n = 12$)	*P*-value
Age (year)	50 ± 15	43 ± 4	50 ± 4	0.29
Male gender	26 (62)	6 (54)	10 (83)	0.19
First transplant	35 (83)	10 (82)	10 (83)	1.00
Deceased donor	37 (88)	8 (73)	10 (83)	0.64
Time post-transplant (month)	52 [25, 90]	36 [12, 54]	52 [17, 76]	0.49
Warm ischemia time (min)	37 ± 9	38 ± 3	33 ± 2	0.17
Cold ischemia time (h)	18 ± 7	15 ± 2	17 ± 2	0.51
HLA mismatch				
A mismatch	1.2 ± 0.8	1.2 ± 0.2	1.1 ± 0.3	0.76
B mismatch	1.1 ± 0.7	1.2 ± 0.1	1.2 ± 0.2	0.95
DR mismatch	0.8 ± 0.6	1.0 ± 0.2	0.6 ± 0.2	0.10
Protocol dnDSA detection	30 (71)	11 (100)	0 (0)	–
Indication dnDSA detection	12 (29)	0 (0)	12 (100)	–
dnDSA HLA specificity				
Class I only	13 (31)	3 (27)	5 (42)	0.74
Class II only	20 (48)	4 (36)	3 (25)	
Class I and Class II	9 (21)	4 (36)	4 (33)	
Banff scores	($n = 23$)	($n = 11$)	($n = 12$)	
t	1.4 ± 0.8	1.2 ± 0.1	1.6 ± 0.3	0.41
i	1.2 ± 1.0	1.2 ± 0.2	1.2 ± 0.3	0.88
v	0.1 ± 0.2	0.1 ± 0.1	0.0 ± 0.0	0.98
g	1.3 ± 1.1	1.0 ± 0.3	1.4 ± 0.3	0.44
ptc	1.0 ± 1.1	0.6 ± 0.3	1.4 ± 0.4	0.17
cg	1.0 ± 1.3	1.0 ± 0.4	1.0 ± 0.4	0.93
ct	1.9 ± 0.8	2.1 ± 0.7	1.7 ± 0.3	0.27
ci	1.9 ± 0.8	2.0 ± 0.2	1.8 ± 0.3	0.61
cv	1.9 ± 0.8	2.0 ± 0.2	1.9 ± 0.3	1.00
ah	2.2 ± 0.9	2.4 ± 0.1	2.1 ± 0.3	0.53
C4d	0.9 ± 1.2	1.2 ± 0.4	0.7 ± 0.4	0.21

Data are provided as mean ± SD for the first column, mean ± SEM for the last two columns, *n* (%) or median [25th, 75th percentiles]. Comparisons were performed using Mann–Whitney test, Fisher's exact test or Chi-squared test. Biopsy scores are provided according to the Banff classification (0–3): t, tubulitis; i, interstitial infiltration; v, intimal arteritis; g, glomerulitis; ptc, peritubular capillarities; cg, transplant glomerulopathy; ct, tubular atrophy; ci, interstitial fibrosis; cv, fibrous intimal thickening; ah, arteriolar hyaline thickening; C4d, deposition of the C4d fragment of complement component C4.

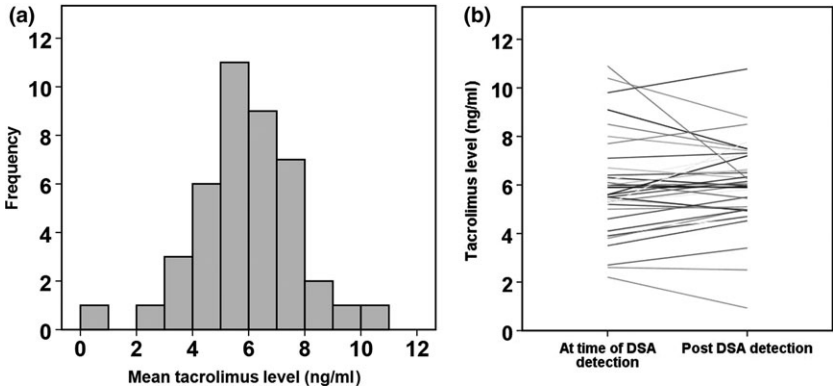

Figure 1 Tacrolimus levels over time. (a) Histogram showing the distribution of individual mean tacrolimus levels post-dnDSA development. (b) Tacrolimus levels at the time of dnDSA, and mean tacrolimus levels post-dnDSA development. Each line represents a single patient.

Table 2. Univariate and multivariate risk estimates for graft loss associated with tacrolimus levels post-dnDSA detection

	Hazard ratio (95% CI)	*P*-value
TAC levels as continuous variable (ng/ml)		
Unadjusted (n = 42)	0.49 (0.33–0.75)	0.001
Adjusted model 1 (n = 42)*	0.45 (0.27–0.76)	0.003
Adjusted model 2 (n = 23)†,‡	0.52 (0.30–0.89)	0.019
Adjusted model 3 (n = 23)‡,§	0.26 (0.07–0.99)	0.049

*Adjusted for delayed graft function and nonadherence.

†Adjusted for delayed graft function and nonadherence, tubulitis score and transplant glomerulopathy score.

‡Analyzed restricted to the 23 patients who had a biopsy.

§Adjusted for delayed graft function and nonadherence, tubulitis, transplant glomerulopathy, interstitial fibrosis, tubular atrophy and arteriolar hyaline thickening scores.

Importantly, the hazard ratio was similar when the analysis was restricted to the patients who were biopsied (Table 2, adjusted model 2), and the analysis was robust to the adjustment for arteriolar hyalinosis (ah score) and interstitial fibrosis/tubular atrophy (IFTA score). Overall, these results indicate that the mean tacrolimus level in the first 2 years post-dnDSA detection is a strong, independent predictor of graft survival, and this association persists when only biopsied patients are analyzed.

To further assess the relevance of the association between tacrolimus levels and graft survival in the real clinical setting, we next categorized tacrolimus levels by tertiles, which generated cutoffs at 5.3 and 6.3 ng/ml. There was a significant difference between the three groups, with eight events in the lowest tertile, three in the middle tertile and one in the highest tertile (P = 0.005 by log-rank; Fig. 2). An analysis restricted to the biopsied patients showed similar results (Fig. S1). Categorizing the cohort into quartiles, with cutoffs at 5.0, 6.0 and 7.2 did not improve the identification of an optimal level (Fig. S2a). A receiver operating curve (ROC) analysis built to identify graft survival as the event concurred with the Kaplan–Meier plot shown in Fig. 2 to indicate that a tacrolimus level of 5.3 had the best predicting accuracy for graft survival (area under

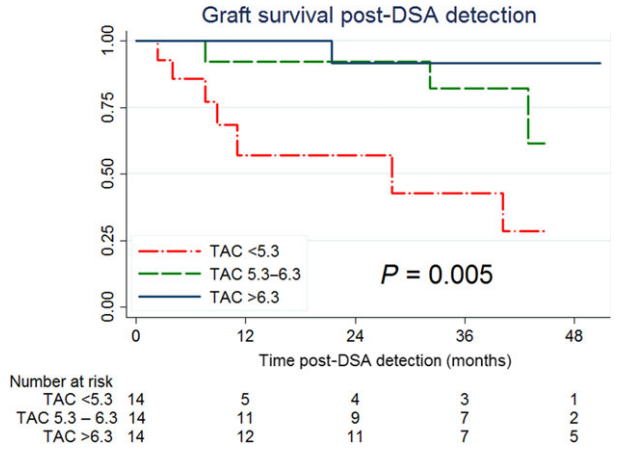

Figure 2 Kaplan–Meier plots for graft loss by tertile of mean tacrolimus levels post-dnDSA development. Comparison was assessed using log-rank test.

the curve 0.75, $P = 0.01$; sensitivity 80%, specificity 67%; Fig. S2b).

Sensitivity analyses

First, because tacrolimus levels vary over time, the arbitrary time points used above may not capture the true ongoing exposure to the drug. To examine this possibility, we repeated the analysis, using for each patient the mean of all outpatient tacrolimus levels available between 1 and 24 months post-dnDSA detection. The correlation between the mean of these levels and the mean tacrolimus level used above was high ($\sigma = 0.87$, $P < 0.001$; Fig. S3). Hazard ratios using this variable were consistent across the models and showed a slightly stronger positive effect for the fully adjusted model in the sensitivity analysis than in the primary analysis (HR, 0.40; 95% CI, 0.20–0.83 versus HR, 0.52, 95% CI, 0.30–0.89 respectively; Table S1).

Second, because the conversion method used to analyze cyclosporine-treated patients was empiric, we tested the robustness of the findings after removing these patients from the analysis. Of note, only one of these four patients had a biopsy, which means that the sample size became 38 for the full unbiopsied and biopsied cohort, and 22 for the biopsied-only cohort. As displayed in Table S2, results were similar using this restricted cohort.

Discussion

In this brief report, we studied a cohort of kidney transplant recipients who underwent prospective anti-HLA antibody monitoring post-transplant. We assessed the relationship between mean tacrolimus levels following dnDSA detection and graft loss. We observed a strong protective effect of higher tacrolimus levels, persistent after adjustment for established predictors of outcome in this population. Unbiased survival analysis by tertiles of tacrolimus levels as well as ROC curve analysis further suggested that a level of 5.3 ng/ml could be clinically relevant in these patients.

These data are relevant, because they suggest that anti-HLA antibody monitoring and identification of dnDSA post-transplant facilitate optimization of maintenance immunosuppression and improvement in graft outcomes. Although it is widely accepted that development of dnDSA is a major determinant of graft outcomes, the management of dnDSA-positive patients remains one of the most controversial areas in kidney transplantation [2,16]. Indeed, the lack of clear evidence pertaining to modulation of immunosuppression upon dnDSA detection renders anti-HLA antibody screening itself controversial, reflected by significantly heterogeneous practice amongst, and even within, centers [16]. Historically, large multicenter clinical trials focusing on management of *de novo* anti-HLA antibodies, such as the Clinical Trials in Organ Transplantation (CTOT)-02 trial, were unsuccessful in the enrollment of patients in their intervention phases, largely because of concerns about the toxicity of therapies proposed to patients with otherwise stable graft function [2]. However, the widespread practice of reducing immunosuppression, particularly CNIs, in the early 2000s, has now largely fallen of favor: trials of CNI withdrawal, reduction or avoidance produced mixed results, with some reporting high incidences of acute rejection and dnDSA development even in very low-risk patients [5–9,17,18]. For instance, following fifteen years of follow-up, a multicenter trial recently reported that a CNI-free regimen was not associated with a lower incidence of death by malignancy or cardiovascular disease, but led to a worse death-censored graft survival [8].

The introduction or augmentation of CNI therapy in patients previously managed on CNI-free or CNI-low regimens has received little attention to date. This is first due to the nephrotoxicity of the drug, which makes such a strategy unappealing during the maintenance phase of therapy. Second, the immunological rationale to increasing CNI blood levels to prevent or dampen B-cell activation may seem counterintuitive. However, it is increasingly appreciated that dnDSA are not only causative of immune-mediated graft injury, but are also a consequence of ongoing, uncontrolled alloreactive T-cell activation [19]. Inhibition of calcineurin, a phosphatase found in T and B cells, by cyclosporin and tacrolimus has been shown to reduce the induction of cytokine transcription in activated B cells [20], and to directly inhibit naive B cells [21]. Clinically, T-cell-mediated rejection (TCMR) concurrent with ABMR is an independent predictor of kidney graft outcome [22]. Very recently, within-patient variability in tacrolimus levels has been shown to predict dnDSA development [23]. Overall, these data and the ones reported here suggest that the effect of CNIs on B cells may be clinically beneficial, even following the detection of dnDSA.

The lack of difference in the pre- and post-DSA mean tacrolimus levels indicates that, in this cohort, the management of CNI levels was not strictly based on the presence of dnDSA. This is in contrast to other studies examining the outcomes of patients with dnDSA. For instance, Wiebe *et al.* [14] reported on a strategy to optimize the tacrolimus dose targeting blood levels of 8 ng/ml in patients with dnDSA. Indeed, the aim of the

prospective anti-HLA antibody monitoring undertaken here was primarily to better understand the natural history of patients with dnDSA. The heterogeneous practice observed, as demonstrated by decisions to undertake biopsy or to adjust the immunosuppression following dnDSA detection, reflects the equipoise at play within the clinical team during that period. Although these data are preliminary, we noted a trend toward more microcirculation inflammation in the patients who had an indication biopsy. If this is confirmed in an independent, larger cohort, it would support enhancing CNI immunosuppression to prevent the long-term graft damage associated with dnDSA.

There are some limitations to this study. First, although the results are statistically significant, they are derived from a relatively small, single-center cohort. However, it is important to mention that major studies on the risk factors and outcomes related to dnDSA development in kidney recipients were all reported from unicenter cohorts of similar sizes [1,14,23]. Second, the incidence of dnDSA was low within the population. The reason for this is multifactorial, including the use of triple regimen immunosuppression therapy, the absence of sensitized patients in our program, the points given to the DR match in the organ procurement organization allocation scheme and the relatively high nMFI threshold used. Given that the study population is restricted to patients with dnDSA, this is unlikely to affect the results beyond the fact that it resulted in fewer patients included. Finally, conversion of cyclosporine C_2 levels to tacrolimus C_0 values for the purpose of the analysis was performed using an empiric conversion factor based on target levels for each drug. Given the low number of patients on cyclosporine ($n = 4$), use of a slightly higher or lower correction factor is unlikely to affect the results of the study.

The observations reported here suggest that, beyond known predictors, the CNI level is an independent risk factor for graft loss following development of dnDSA. Confirmation of these results in an independent cohort and specification of potential threshold levels will provide a strong rationale to design a clinical trial investigating if optimization of CNI levels is beneficial in this population. Furthermore, we can postulate that targeting higher than usual maintenance levels may present sufficient equipoise to warrant clinical investigation. Given its ease of use and importantly, the present lack of a proven beneficial alternative, optimization of the current maintenance immunosuppressive strategy in patients with dnDSA may be the best option until an immunological agent able to safely inhibit or reverse generation of DSA becomes available.

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Conflicts of interest

The authors of this manuscript have no conflict of interests to disclose as described by *Transplant International*.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1. (a) Kaplan–Meier plots for graft loss by quartiles of mean tacrolimus levels post-dnDSA development. Comparison was assessed using log-rank test. (b) ROC curve analysis using graft survival as the binary event and mean tacrolimus levels as the exposure.

Figure S2. Dot plot of tacrolimus values used for the sensitivity analysis (mean of all the levels available between month 1 and 24 post-dnDSA detection) versus values used in the main analysis (mean of the levels at month 1, 3, 6, 12 and 24 post-dnDSA detection).

Figure S3. Dot plot of the mean tacrolimus T0 levels (ng/dL) computed using all values available in the database vs. mean tacrolimus T0 levles (ng/dL) computed using the values available at the following defined timepoints: 1, 3, 6, 12 and 24 months after dnDSA detection..

Table S1. Univariate and multivariate risk estimates for graft loss associated with tacrolimus levels post dnDSA detection.

Table S2. Univariate and multivariate risk estimates for graft loss associated with tacrolimus levels post dnDSA detection.

REFERENCES

1. Wiebe C, Gibson IW, Blydt-Hansen TD, *et al.* Evolution and clinical pathologic correlations of de novo donor-specific HLA antibody post kidney transplant. *Am J Transplant* 2012; **12**: 1157.
2. Djamali A, Kaufman DB, Ellis TM, Zhong W, Matas A, Samaniego M. Diagnosis and management of antibody-mediated rejection: current status and novel approaches. *Am J Transplant* 2014; **14**: 255.
3. Lefaucheur C, Nochy D, Andrade J, *et al.* Comparison of combination plasmapheresis/IVIg/anti-CD20 versus high-dose IVIg in the treatment of antibody-mediated rejection. *Am J Transplant* 2009; **9**: 1099.
4. Lesage J, Noël R, Lapointe I, *et al.* Donor-specific antibodies, C4d and their relationship with the prognosis of transplant glomerulopathy. *Transplantation* 2015; **99**: 69.
5. Ekberg H, Grinyo J, Nashan B, *et al.* Cyclosporine sparing with mycophenolate mofetil, daclizumab and corticosteroids in renal allograft recipients: the CAESAR Study. *Am J Transplant* 2007; **7**: 560.
6. Ekberg H, Tedesco-Silva H, Demirbas A, *et al.* Reduced exposure to calcineurin inhibitors in renal transplantation. *N Engl J Med* 2007; **357**: 2562.
7. Abramowicz D, Del Carmen Rial M, Vitko S, *et al.* Cyclosporine withdrawal from a mycophenolate mofetil-containing immunosuppressive regimen: results of a five-year, prospective, randomized study. *J Am Soc Nephrol* 2005; **16**: 2234.
8. Roodnat JI, Hilbrands LB, Hene RJ, *et al.* 15-year follow-up of a multicenter, randomized, calcineurin inhibitor withdrawal study in kidney transplantation. *Transplantation* 2014; **98**: 47.
9. Vincenti F, Ramos E, Brattstrom C, *et al.* Multicenter trial exploring calcineurin inhibitors avoidance in renal transplantation. *Transplantation* 2001; **71**: 1282.
10. Sigal NH, Dumont FJ. Cyclosporin A, FK-506, and rapamycin: pharmacologic probes of lymphocyte signal transduction. *Annu Rev Immunol* 1992; **10**: 519.
11. Racusen LC, Solez K, Colvin RB, *et al.* The Banff 97 working classification of renal allograft pathology. *Kidney Int* 1999; **55**: 713.
12. Solez K, Colvin RB, Racusen LC, *et al.* Banff 07 classification of renal allograft pathology: updates and future directions. *Am J Transplant* 2008; **8**: 753.
13. Haas M, Sis B, Racusen LC, *et al.* Banff 2013 meeting report: inclusion of C4d-negative antibody-mediated rejection and antibody-associated arterial lesions. *Am J Transplant* 2014; **14**: 272.
14. Wiebe C, Gibson IW, Blydt-Hansen TD, *et al.* Rates and determinants of progression to graft failure in kidney allograft recipients with de novo donor-specific antibody. *Am J Transplant* 2015; **15**: 2921.
15. Schiff J, Cole E, Cantarovich M. Therapeutic monitoring of calcineurin inhibitors for the nephrologist. *Clin J Am Soc Nephrol* 2007; **2**: 374.
16. Kiberd BA, Miller A, Martin S, Tennankore KK. De novo donor-specific human leukocyte antigen antibody screening in kidney transplant recipients after the first year posttransplantation: a medical decision analysis. *Am J Transplant* 2016; **16**: 3212.
17. Dugast E, Soulillou JP, Foucher Y, *et al.* Failure of calcineurin inhibitor (tacrolimus) weaning randomized trial in long-term stable kidney transplant recipients. *Am J Transplant* 2016; **16**: 3255.
18. Tan HP, Donaldson J, Basu A, *et al.* Two hundred living donor kidney transplantations under alemtuzumab induction and tacrolimus monotherapy: 3-year follow-up. *Am J Transplant* 2009; **9**: 355.
19. Moreso F, Carrera M, Goma M, *et al.* Early subclinical rejection as a risk factor for late chronic humoral rejection. *Transplantation* 2012; **93**: 41.
20. Goldfeld AE, Tsai E, Kincaid R, *et al.* Calcineurin mediates human tumor necrosis factor alpha gene induction in stimulated T and B cells. *J Exp Med* 1994; **180**: 763.
21. De Bruyne R, Bogaert D, De Ruyck N, *et al.* Calcineurin inhibitors dampen humoral immunity by acting directly on naive B cells. *Clin Exp Immunol* 2015; **180**: 542.
22. Matignon M, Muthukumar T, Seshan SV, Suthanthiran M, Hartono C. Concurrent acute cellular rejection is an independent risk factor for renal allograft failure in patients with C4d-positive antibody-mediated rejection. *Transplantation* 2012; **94**: 603.
23. Rodrigo E, Segundo DS, Fernandez-Fresnedo G, *et al.* Within-patient variability in tacrolimus blood levels predicts kidney graft loss and donor-specific antibody development. *Transplantation* 2016; **100**: 2479.