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High Calcineurin Inhibitor Intrapatient Variability Is Associated With Renal Allograft Inflammation, Chronicity, and Graft Loss

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Background. High calcineurin inhibitor (CNI) intrapatient variability (IPV) has been associated with poor kidney allograft outcomes. However, the relationship between early allograft histological changes, their progression, and CNI-IPV is less well studied. Hence, we evaluated effect of CNI-IPV defined by the degree of fluctuation of CNI levels in all kidney transplant patients over 2 to 12 months posttransplant on early allograft inflammation, subsequent chronicity, and later clinical outcomes. **Methods.** Two hundred eighty-six patients transplanted from January 2013 to November 2014 were enrolled with protocol and indication biopsies. The mean CNI-IPV was 28.5% and a quarter of our cohort had IPV of 35% or greater (high CNI IPV). Baseline demographic differences were similar between high and low CNI IPV groups. **Results.** High CNI-IPV was associated with a higher incidence of acute rejection (AR) within 1 year (52% vs 31% $P < 0.001$), more persistent/recurrent AR by 1 year (18.2% vs 6.2%, $P = 0.002$), higher-grade AR (≥Banff 1B, 27.5% vs 7.3%, $P < 0.001$), and worse interstitial fibrosis/tubular atrophy ($P = 0.005$). High CNI-IPV was associated with increased graft loss (GL) and impending graft loss (iGL, defined as eGFR<30 ml/min and >30% decline in eGFR from baseline), regardless of donor-specific antibody, delayed graft function, rejection, or race. In a multivariate Cox Proportional Hazards Model, high CNI-IPV was independently associated with GL + iGL (hazard ratio, 3.1; 95% confidence interval, 1.6–5.9, $P < 0.001$). **Conclusions.** High CNI-IPV within 1 year posttransplant is associated with higher incidence of AR, severe AR, allograft chronicity, GL, and iGL. This represents a subset of patients who are at risk for poor kidney transplant outcomes and potentially a modifiable risk factor for late allograft loss.

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Calcineurin inhibitors (CNI), specifically tacrolimus (TAC), have been a cornerstone in the immunosuppressive management of kidney transplant (KT) recipients.[1-4] Despite the improvements in short-term outcomes, long-term KT survival rates remain suboptimal.[5] Late KT failure can be due to many causes, most commonly derived from alloimmune mechanisms leading to acute and chronic T cell–mediated rejection (TCMR) and antibody-mediated rejection (AMR).[6] Early immunological events, including unrecognized and untreated early subclinical inflammation, may lead to progressive graft damage and can impact long-term KT survival.[7-13] Further, Sellarés et al[14] in their prospective cohort study identified nonadherence to therapy as an important variable. They identified that 64% of late renal allograft loss was due to rejection, with elements of AMR, and 47% of these patients with late graft loss due to rejection were nonadherent to therapy.

Importantly, nonadherence likely starts early and persists after transplantation.[15,16] Unfortunately, nonadherence has been difficult to objectively quantify and measure. CNI intrapatient variability (IPV) was initially identified as a potential objective measure to identify nonadherence in pediatric solid organ transplant recipients, which has been

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associated with late rejection and graft loss.[17-20] Subsequently, high CNI-IPV has been associated with poor kidney allograft outcomes.[21-29] However, published series are limited due to insufficient CNI assessment and lack of prospective longitudinal studies coupled with donor-specific antibody (DSA) and protocol biopsies. We hypothesized that patients with high CNI-IPV within first year posttransplant will have heightened early allograft inflammation with subsequent chronicity, playing a role in late allograft dysfunction and loss.

MATERIALS AND METHODS

Study Population

We examined 378 patients who underwent KT during the study period of January 2013 to November 2014 at Thomas E. Starzl Transplantation Institute, University of Pittsburgh. This study period is a prospectively collected database of all KT recipients established in January 2013 with an end date of November 2014. Overall, 92 patients were excluded from the study cohort (details shown below). All study patients were followed up until November 2017.

Inclusion and Exclusion Criteria

Adult ABO-compatible KT recipients (not requiring desensitization before transplant) and those who had at least one documented kidney biopsy in the first posttransplant year were included in this study. Recipients of primary KT, repeat KT, KT after other solid organ transplant, and multiorgan transplants (simultaneous kidney-pancreas or liver-kidney transplant recipients) were included and target CNI trough levels, as well as care team, were the same. All racial and ethnic groups were included in this study. We excluded a total of 92 patients: 84 without documented renal histology within the first year posttransplant (69 due to chronic anticoagulation, 15 with early death/graft loss within 3 months posttransplant), 6 switched to non–CNI-based regimens, and 2 with missing data as demonstrated in **Figure 1, Supplemental Digital Content (SDC) (http://links.lww.com/TXD/A173).**

Protocol Biopsies

Protocol biopsies were performed at 3 and 12 months posttransplant as an outpatient procedure. All biopsies required at least a minimum of 7 glomeruli and 1 artery to meet the adequacy requirement for biopsy specimen. All biopsies were graded and scored by our experienced transplant pathologists according to Banff classification 2013.[30] Acute rejection (AR) was predominantly TCMR but included AMR as a combination of TCMR + AMR. There were no AMR-alone cases in our cohort. Acute rejection was diagnosed as clinical AR on indication biopsies for renal dysfunction defined as serum creatinine (SCr) greater than 25% from baseline (and/or proteinuria >1.5 g/d or >1 g/g creatinine) and subclinical AR on protocol biopsies without evidence of renal dysfunction. Overall, 71% (n = 203) of patients had paired biopsies during the first year posttransplant.

Posttransplant DSA Monitoring

Blood samples were collected for DSA testing at 1, 3, 6, 9, and 12 months posttransplantation per our center protocol and also at the time of any biopsy. Donor-specific antibodies were detected by One Lambda LAB Screen single antigen bead assay per manufacturer protocol. An adjusted mean fluorescence intensity of 1000 units or greater was considered as significant for the detection of HLA-specific antibodies.

Immunosuppression

Induction antibody thymoglobulin (6 mg/kg, divided over 4 doses over the first 4 days posttransplant) was administered for majority of patients, and basiliximab was used in minority of the patients, which were typically low immunologic risk (6 antigen match, 0% calculated panel reactive antibody [cPRA]) living donor KT recipients. All patients received a rapid steroid taper over 7 days. Mycophenolate mofetil was initiated immediately posttransplant and TAC was initiated within 72 hours posttransplant. For maintenance immunosuppression, the majority of patients were maintained on dual therapy with mycophenolate mofetil and TAC (all patients on BID dosing). Target trough TAC levels were aimed between 8 and 12 ng/mL for the first 3 months and then 6 to 10 ng/mL after 3 months TAC was measured using "Waters MassTrak Immunsuppressants Kit," which is cleared by FDA for monitoring TAC levels in the whole blood of kidney transplant recipients with reported high sensitivity (reliable measurement to 0.5 ng/mL level) and low coefficient of variation (CV) for the test itself (<7%).[31,32] For highly sensitized patients (cPRA >90%) or patients on prednisone before transplant, patients were maintained on oral prednisone (5 mg/d or their previous dose). For a very small group of patients (1%, n = 4), Cyclosporine was used as CNI for maintenance regimen, typically continued from regimen before transplant due to previous KT or other solid organ transplant. Target cyclosporine trough levels were 250 to 350 ng/mL for the first 3 months and then 200 to 300 ng/mL after 3 months. Both clinical and subclinical TCMR (Banff AR 1A and 1B) were treated with 3 doses of intravenous methylprednisolone (250 mg) and optimization of maintenance immunosuppression, including addition of maintenance oral prednisone (5 mg/d). For Banff AR ≥ 2A, patients were treated with thymoglobulin (4–6 mg/kg divided over 3–4 doses) and optimization of maintenance immunosuppression, including addition of maintenance oral prednisone (5 mg/d). Patients with acute and active AMR were treated with 4 to 6 treatments of plasmapheresis/IVIG, in addition to treatment for any coinciding TCMR component they may have had.

CNI Levels and Variability Measurement

The mean CNI values tested per patient was 37 ± 15 (median, 35; range, 8–123; high CNI IPV vs low CNI IPV, 46.3 ± 20.9 vs 34.3 ± 11.1; $P < 0.001$) and trough level less than 6 ng/mL was considered as subtherapeutic. Our primary clinical variable of interest was CNI-IPV, which was defined as the patient-specific mean CV (%CV = $\sigma/\mu \times 100$). For our study, we included all CNI levels recorded from 2 to 12 months posttransplant for calculation of subtherapeutic trough levels. We calculated CNI IPV based on the highest and lowest CNI trough value for each month from 2 to 12 months (number of readings used to calculate CNI IPV, high vs low, 18.2 ± 4.5 vs 18.1 ± 3.5; $P = 0.9$). We extracted CNI levels from the electronic medical record. For any outlier reading, we extensively reviewed the electronic medical record manually to confirm the accuracy of the level as a true trough (between 11 and 13 hours postdose) and subsequently excluded any reading that was not a

reflective of a true 12-hour trough level. We chose to exclude the first months posttransplant due to varying bioavailability during early posttransplant period. We reviewed the distribution of CNI-IPV in our study cohort (**Figure 2, SDC, http://links.lww.com/TXD/A174**) and chose a cutoff point of IPV ≥ 35% as defining "high CNI-IPV" (highest quartile) group and less than 35% as "low CNI-IPV" group, similar to other published in other studies.[23,33]

Outcome Measures

We evaluated the differences in recipient/donor demographic characteristics and transplant/posttransplant variables between high CNI-IPV and low CNI-IPV patients, including age, sex, race, cause of end-stage kidney disease, dialysis vintage (in days), body mass index (BMI) (last BMI before transplant), previous transplant status, donor age, donor type, Kidney Donor Profile Index (KDPI) score (taken at time of transplant, >20% vs < 20%), cold ischemia time (CIT), number of HLA mismatches (HLA m/m), PRA class I/II status (≥70% vs < 70%), cPRA (at the time of transplant), delayed graft function (DGF) (dialysis requirement within first 7 days posttransplant), DSA (transient or persistent, present anytime within first posttransplant year), CMV viremia (any time during the first year, CMV testing done by PCR when clinically indicated), BK viremia (anytime during the first year, BK testing done in plasma at 1, 3, 6, 9, and 12 months posttransplantation per our center protocol and also at the time of any biopsy) and other variables noted in Table 1. For our primary outcomes, we evaluated AR events within the first posttransplant year, chronic histological changes on late biopsies (6–12 months posttransplant), including interstitial fibrosis and tubular atrophy and interstitial fibrosis + inflammation (IF"+"i"), actual graft loss (GL, defined as requiring permanent dialysis or repeat transplantation), and a composite endpoint (CEP), including GL and/or impending graft loss (iGL, defined as eGFR<30 mL/min and > 30% decline from 3-month eGFR at last follow-up point). We also evaluated subclinical versus clinical AR, severity of AR defined by Banff grade, and early versus late AR among patients with high versus low CNI-IPV groups.

Ethical Guidelines and Patient Privacy

Patient information used for this analysis was obtained from transplant registry through institutionally designated individuals at our center and the Thomas E. Starzl Transplantation Institute as regulated by the institutional review board guidelines at the University of Pittsburgh. This institution maintains a prospectively collected electronic database of all kidney transplant patients. We collected data under the IRB number PRO-13060220 approved by the University of Pittsburgh. The clinical and research activities being reported are consistent with both the Principles of the Declaration of Istanbul as outlined in the "Declaration of Istanbul on Organ Trafficking and Transplant Tourism" and Declaration of Helsinki.

Statistical Methods

All continuous variables were presented as mean ± standard deviation and categorical variables as a percent of study population. Continuous variables were compared using independent sample *t* test or Mann-Whitney *U* test based on distribution of variable. Multiple group comparison was done by ANOVA with Dunnet post hoc correction for multiple comparisons or by Kruskal Wallis test. Categorical variables were compared by χ^2 test. Univariate and multivariate logistic regression analysis was used to derive risk factors for the development of CEP (GL + iGL) with variables that were significant for *P* less than 0.05 subsequently included in multivariate analysis. Graft survival and CEP were analyzed by the Kaplan Meier method with survival curves compared by Log rank test. The independent effect of CNI-IPV on the CEP (GL + iGL) was examined by univariate and multivariate Cox proportional hazards analysis. A *P* value less than 0.05 was considered statistically significant.

RESULTS

Study Population

A total of 378 adult patients who underwent kidney transplantation at the Thomas E. Starzl Transplantation Institute, University of Pittsburgh from January 2013 to November 2014 were considered for this study. After excluding 92 patients (see method section), a total of 286 patients were included for this study.

Definition of High CNI-IPV Patients

The mean CNI-IPV was 28.5% (±12%) for the entire study population. When patients were divided into quartiles based on their CNI-IPV, the highest quartile had IPV ≥ 35% (**Figure 2, SDC, http://links.lww.com/TXD/A174**). We used this cutoff to define high variability in our population (High CNI-IPV). The low CNI-IPV was defined as those with less than 35%.

Patient Demographics

The clinical characteristics of the study population stratified by high versus low CNI-IPV are detailed in Table 1. There were 68 (24%) patients with high CNI-IPV and 218 (76%) patients with low CNI-IPV. The demographics and transplant variables between the groups were similar, except for a trend toward more nonwhite patients in the high CNI-IPV group (28% vs 18.3%, *P* = 0.09). The remaining donor and recipient demographics, including KDPI scores and immunological variables, were similar between high and low CNI-IPV groups.

CNI-IPV and Posttransplant Variables

Patients in the high CNI-IPV group had a significantly greater incidence of DGF (30.4% vs 15.6%, *P* = 0.006). Although patients had similar renal function as measured by SCr at 1 month posttransplant (1.8 ± 0.9 vs 1.9 ± 0.7, *P* = 0.4), SCr was elevated in patients with high CNI-IPV group at 3 months (1.73 ± 0.7 vs 1.5 ± 0.5, *P* = 0.001) and at 12 months (2.0 ± 1.2 vs 1.4 ± 0.4, *P* < 0.001) posttransplant. Additionally, though the mean CNI trough levels were similar between CNI-IPV groups (8.9 ± 1.3 vs 8.8 ± 1.5, *P* = 0.8), patients with high CNI-IPV had a greater proportion of subtherapeutic trough levels than the low CNI-IPV group (29% vs 11%, *P* < 0.001, subtherapeutic trough defined as % of trough levels <6 ng/mL per patient). Further, those patients with more frequent CNI measurements (highest quartile) had a higher percentage with CEP at end of follow-up (35.6% vs 11.1%, *P* < 0.001), although as noted previously in the Materials and Methods section, similar number of

TABLE 1.

The study population (n = 286) divided into those with low calcineurin inhibitor intrapatient variability (CNI-IPV) (N = 218) and high CNI-IPV (n = 68)

Characteristics	All patients	Low CNI IPV (<35%)	High CNI IPV (≥35%)	*P*
Number	286	218	68	
Recipient				
Age, y	52 ± 15	52 ± 14	51 ± 16	0.5
Male sex	58.3%	60.7%	50.7%	0.1
Nonwhite (%)	20.5%	18.3%	28%	0.09
BMI	27.9 ± 5.4	28.1 ± 5.5	27.3 ± 5.0	0.3
Dialysis vintage, d	1180 ± 1131	1211 ± 1201	1090 ± 815	0.4
Cause of ESKD				0.5
HTN	13.8%	13.5	14.6%	
DM	25.3%	23.3%	31.7%	
Glomerular	21.8%	22.6%	19.5%	
Congenital/inherited	12.1%	14.3%	4.9%	
Others	27%	26.3%	29.3%	
Donor				
Donor age in years	39 ± 13	39 ± 13	41 ± 12	0.3
Donor type				0.9
Live	42%	41.6%	43.5%	
DBD	43.8%	44.3%	42%	
DCD	14.2%	14.1%	14.5%	
KDPI score >20%	40.6%	40.2%	42%	0.8
Transplant				
Previous transplant	18.4%	18.7%	17.4%	0.8
HLA m/m	4.3 ± 2.0	4.2 ± 1.9	4.5 ± 2.2	0.3
PRA-I ≥ 70%	4.9%	5.5%	2.9%	0.4
PRA-II ≥70%	10.1%	10.5%	8.7%	0.7
cPRA	32.0%	31.8%	32.5%	0.9
CIT	434 ± 357	420 ± 342	480 ± 400	0.3
Posttransplant				
DGF	19.2%	15.6%	30.4%	0.006
AR	36.1%	31.1%	52.2%	0.001
DSA	22.9%	20.5%	30.4%	0.09
Creatinine, 1 mo	1.8 ± 0.9	1.8 ± 0.9	1.9 ± 0.7	0.4
Creatinine, 3 mo	1.6 ± 0.6	1.5 ± 0.5	1.7 ± 0.7	0.001
Creatinine, 12 mo	1.5 ± 0.7	1.4 ± 0.4	2.0 ± 1.2	<0.001
Any CMV viremia	13.6%	13.8%	13.2%	0.9
CMV viremia >1000 IU	9.4%	10.6%	5.8%	0.2
Any BK viremia	15.7%	16.5%	13.2%	0.5
BK viremia >1000 copies/mL	9.8%	11.0%	5.8%	0.2
Immunosuppression				
%Thymoglobulin induction	94.8%	95%	94.2%	0.8
Mean CNI trough level	8.9 ± 1.4	8.9 ± 1.3	8.8 ± 1.5	0.8
Subtherapeutic CNI trough levels (% per patient)	15.4 ± 14.5	11 ± 11	29 ± 15	<0.001
CNI-IPV	28.5 ± 12	23 ± 6	45 ± 11.6	<0.001

Recipient and donor demographics as well as transplant and posttransplant variables of patients with low CNI-IPV and high CNI-IPV are illustrated.

BMI, body mass index; CIT, cold ischemia time; cPRA, calculated panel reactive antibody; DSA, donor-specific antibody; ESKD, end-stage kidney disease; KDPI, Kidney Donor Profile Index; PRA, panel reactive antibody.

readings were used to calculate CNI IPV. Lastly, there were no differences among high and low CNI-IPV groups with respect to opportunistic viral infections, such as CMV and BK, during the first posttransplant year.

High CNI-IPV Was Associated With Increased Renal Allograft Rejection and Chronic Histology

The overall incidence of AR within 1 year posttransplant among all patients was 36%, with 16% clinical and 20% subclinical; all predominantly TCMR (97% TCMR alone, 3% mixed AMR/TCMR, 0% AMR alone). As noted in Figure 1, high CNI-IPV was associated with overall higher incidence of AR within the first posttransplant year (52% vs 31%, $P = 0.001$), and more specifically a greater incidence of clinical AR (clinical AR, 30% vs 12%, $P = 0.001$; subclinical AR, 22% vs 19%, P = Ns), as well as an increased incidence of high-grade AR (≥Banff 1B, 27.5% vs 7.3%, $P < 0.001$). Further, as shown in Table 2, patients with high CNI-IPV have increased incidence of early AR, late AR, and recurrent/persistent AR when compared with patients with Low CNI-IPV. Reflecting these findings, high CNI-IPV patients had worse acute allograft histological scores nearly across all categories when compared to low CNI-IPV patients (**Figure 3, SDC, http://links.lww.com/TXD/A175**). Furthermore, patients with high CNI-IPV had increased chronicity scores measured by Banff interstitial fibrosis and tubular atrophy ($P = 0.005$) and higher percentage of IF+"i" ($P < 0.001$) on their late biopsies (6–12 months) than their low CNI-IPV counterparts (**Figure 2**). Thus, high CNI-IPV was associated with greater incidence of early and late AR, high-grade AR, recurrent/persistent AR, and increased allograft chronicity within 1 year.

CNI-IPV and DSA

Overall, DSA presence was 22.9% for the entire study cohort, of which 36% was detected within the first month posttransplant and 40% of which was transient. There was a trend for more DSA presence within the high CNI-IPV group (30.4% vs 20.5%, $P = 0.09$, Table 1). Importantly, the combination of DSA and high CNI-IPV was associated with a much higher rate of AR within the first posttransplant year (24.6% vs 9.1%, $P = 0.001$, **Figure 4, SDC, http://links.lww.com/TXD/A176**).

High CNI-IPV Was Associated With Poor Kidney Allograft Outcomes

Results of actuarial graft survival and freedom from iGL are shown in **Figure 3**. High CNI-IPV was associated with increased GL as demonstrated in **Figure 3A** ($P < 0.001$). Additionally, patients with high CNI-IPV had a strong association with the CEP (GL + iGL) as demonstrated in Figure 3B ($P < 0.001$). Nearly all patients in the low CNI-IPV group had stable grafts over the follow-up period in comparison to nearly half the patients within the high IPV group with graft decline. Additionally, after censoring first year posttransplant events, we found that high CNI-IPV was still strongly associated with CEP as shown in **Figure 5, SDC, http://links.lww.com/TXD/A177**.

As shown in Table 3, High CNI-IPV, HLA mismatches, CIT, DSA, AR, and SCr at 12 months were associated with graft loss and impending graft loss in a univariate Cox survival model. In a multivariate Cox proportional hazards model, only high CNI-IPV (HR, 3.1; 95% CI, 1.6–5.9;

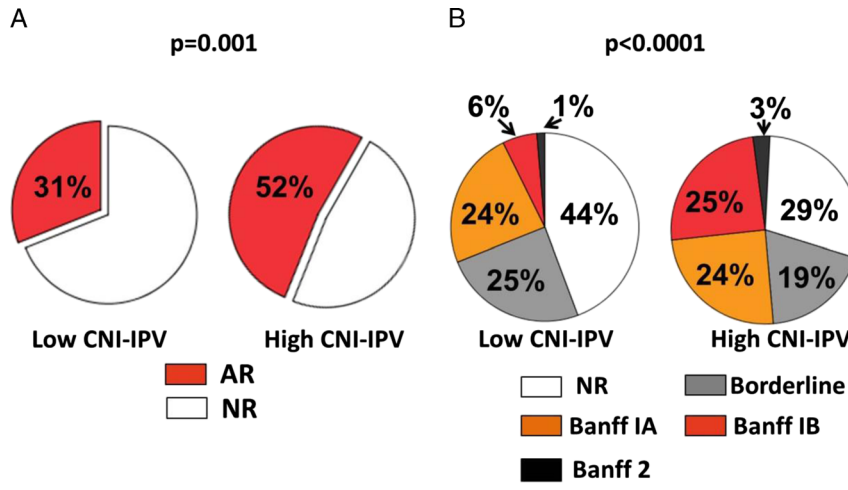


FIGURE 1. Incidence of Acute Rejection (A) and various Banff grades (B) among patients with high and low calcineurin inhibitor intrapatient variability (CNI-IPV) during the first posttransplant year for all patients. AR, acute rejection. NR, no rejection.

$P < 0.001$), SCr at 12 months, and longer CIT were independently associated with the CEP (GL + iGL). Next, in a stratified Kaplan Meier analysis, high CNI-IPV was associated with graft loss and impending graft loss (CEP) regardless of race and events within the first year posttransplant such as DGF, DSA, or AR (Figure 4A-D). Importantly, high CNI-IPV was associated with much worse graft loss and impending graft loss (CEP) in patients with graft dysfunction (SCr above the median) at 1 year ($P < 0.001$, Figure 4E). Thus, high CNI-IPV was independently associated with poor graft outcomes.

DISCUSSION

Long-term kidney transplant outcomes remain suboptimal. There are many factors impeding improvement in long-term kidney transplant survival. Among them, immunosuppression nonadherence is increasingly recognized as a key factor that influences long-term graft survival in kidney transplantation. Although many studies have examined the relationship between nonadherence and graft outcomes later in the course of transplantation, the relationship between early allograft histological changes, their progression, and nonadherence is less well studied. Hence, we embarked on our comprehensive longitudinal prospective study that evaluated the effects of nonadherence, as assessed by high CNI-IPV, on early allograft inflammation, subsequent chronic histological changes, and long term graft outcomes.

We confirmed that high CNI-IPV is associated with increased AR and worse graft outcomes similar to other series.[24,26-29,33-37] First and foremost, we clearly demonstrated that patients with high CNI-IPV not only have more AR within the first year posttransplant, but expand on previous literature by demonstrating the AR is more severe (≥Banff 1B) and that patients with high CNI-IPV have a less proportion of "normal biopsies" without any inflammation. Second, through paired biopsies, we showed that patients with high CNI-IPV not only have more early AR (0–4 months posttransplant), but have more persistent and recurrent late AR (6–12 months posttransplant) when compared with patients with low CNI-IPV. Third, although there was no strong association between DSA and high CNI-IPV within our cohort, which was previously noted by Rodrigo et al,[23] our data clearly demonstrated that the combination of high CNI-IPV and DSA was associated with more AR. Importantly, the combination of DSA + AR has been recently identified as an indicator for poor kidney transplant outcomes.[23,38] Next, similar to Vanhove et al,[25] we also displayed that high CNI-IPV was associated with increased allograft chronic changes. We expand on their findings by

TABLE 2.

The incidence of early, late, and persistent rejection among low calcineurin inhibitor intrapatient variability (CNI-IPV) and high CNI-IPV groups

	Low CNI IPV (<35%)	High CNI IPV (≥35%)	*P*
Early rejection (0–4 mo)	13.2%	22.7%	0.01
Late rejection (6–12 mo)	15.7%	25%	0.01
Recurrent/persistent rejection	6.3%	18.2%	<0.001

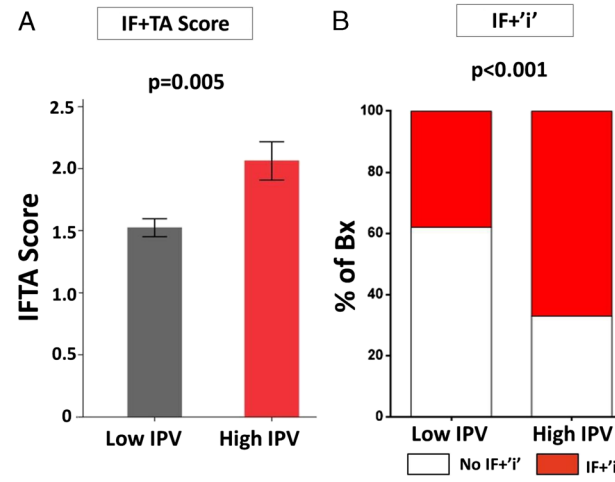
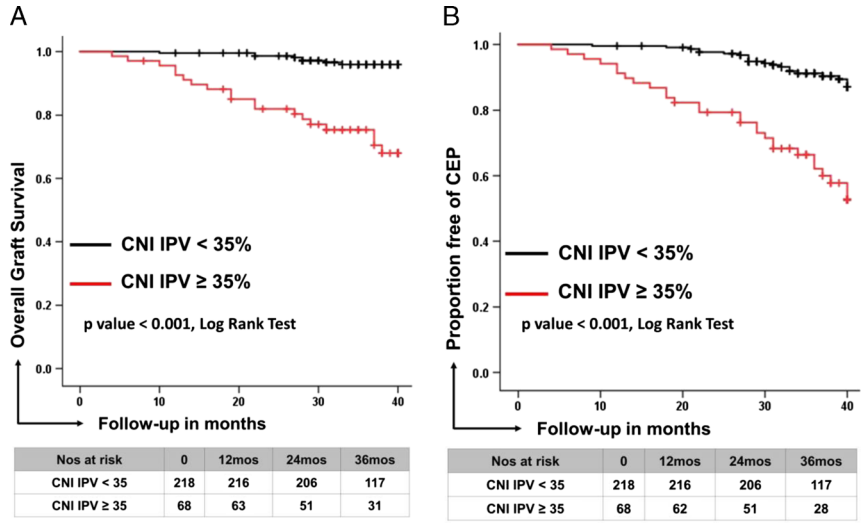


FIGURE 2. Renal allograft chronicity scores among patients with high and low calcineurin inhibitor intrapatient variability (CNI-IPV) groups on late biopsies (6–12 months). Interstitial fibrosis + tubular atrophy (IF + TA) with mean IF + TA scores in panel A, IF + i as percentage of biopsies with IF + i present in panel B.



Nos at risk	0	12mos	24mos	36mos
CNI IPV < 35	218	216	206	117
CNI IPV ≥ 35	68	63	51	31

Nos at risk	0	12mos	24mos	36mos
CNI IPV < 35	218	216	206	117
CNI IPV ≥ 35	68	62	51	28

FIGURE 3. Overall actuarial graft survival (A) and composite endpoint (CEP) (B) for patients with high and low calcineurin inhibitor intrapatient variability (CNI IPV). CEP defined as graft loss and/or impending graft loss (defined as eGFR<30 mL/min and > 30% decline from baseline).

demonstrating these chronic changes can occur as early as 1 year and can occur in a much different cohort. Subsequently, we displayed that High CNI-IPV was associated with the CEP of graft loss and impending graft loss including in individual groups of patients with DGF, DSA, AR, and graft dysfunction at 1 year. Importantly, even in patients without AR or DGF during the first year posttransplant, high CNI-IPV was still associated with CEP. This highlights that high CNI-IPV was not merely resulting due to changes in immunosuppression from treatment of AR but rather from other mechanisms, including possible nonadherence, and also the increased incidence of DGF in high CNI-IPV cohort was not responsible for the CEP association. It also raises the possibility that alloimmune injury beyond the first posttransplant year may be more prevalent in High CNI-IPV group, although this was not confirmed in our study cohort. Finally, we demonstrated high CNI-IPV was independently associated with the CEP (GL + iGL).

The strengths of our study lie in the large cohort of patients followed longitudinally with histological information through paired protocol biopsies and with detailed clinical follow up information, including serial DSA monitoring. With this combination, we are able to comprehensively display the relationship between CNI-IPV and early immunologic events along with the possible subsequent effects on later histology and hard clinical outcomes. Additionally, given our extensive review of CNI levels, we had a much higher number of CNI values for analysis limiting the effect of a CNI trough level sampling error on the assessment of the variability (eg, 18.1 ± 3.7 in our cohort vs 5.3 ± 1.9 in Vanhove et al cohort).[25]

We do acknowledge that there are limitations to our study. First, we hypothesize that the graft loss seen in high CNI-IPV patients is alloimmune in nature, possibly the result from both increased early severe AR and late recurrent/persistent AR within the first year and possibly alloimmune injury beyond the 1-year posttransplant. However, specific cause of allograft loss could not be determined. Second, we acknowledge the high overall AR incidence within our cohort during the first year posttransplant, but again emphasize this includes both clinical and subclinical AR from paired (protocol and indication) biopsies in a diverse cohort and actually as a result, we believe adds to the existing literature. Third, again, although we suspect that nonadherence plays a key role in high CNI-IPV, we acknowledge other causes, such as incurrent illness or medication interaction, likely contribute to CNI variability. We accounted for common opportunistic posttransplant infections, such as CMV and BK viremia, which were similar among both groups, but could not exclude other incurrent illness or medication interactions. Given the cause of CNI-IPV is likely multifactorial, we included both inpatient and outpatient CNI levels,

TABLE 3.

Results of univariate and multivariate analyses for composite endpoint (CEP) defined as graft loss and impending graft loss among patients with high and low calcineurin inhibitor intrapatient variability (CNI IPV)

Variables	HR (95% CI)	P	HR (95% CI)	P
	Univariate analysis		Multivariate analysis	
CNI IPV ≥ 35% vs < 35%	5.0 (2.8–8.7)	<0.001	3.1 (1.6–5.9)	<0.001
Recipient age	1.0 (0.98–1.02)	0.8		
Male sex	0.6 (0.3–1.0)	0.06		
Race (white vs Nonwhite)	1.2 (0.7–2.4)	0.6		
Retransplant	1.3 (0.7–2.5)	0.5		
Donor age	1.0 (1.0–1.1)	0.1		
HLA mm (A, B, DR)	1.2 (1.0–1.3)	0.04	1.1 (0.9–1.3)	0.2
CIT: middle vs lowest tertile	1.0 (0.5–2.2)	0.8	1.1 (0.5–2.8)	0.9
CIT: highest vs lowest tertile	2.3 (1.2–2.4)	0.02	2.6 (1.3–5.2)	<0.001
KDPI > 20%	1.3 (0.7–2.2)	0.5		
Donor type				
DBD vs live	1.7 (0.9–3.1)	0.2		
DCD vs live	2.0 (0.9–4.4)	0.09		
DGF	1.7 (0.9–3.2)	0.09		
DSA presence	2.0 (1.1–3.5)	0.03	1.3 (0.6–2.6)	0.8
AR	1.9 (1.1–3.4)	0.02	0.9 (0.5–1.8)	0.8
Creatinine at 12 mo	2.6 (2.0–3.3)	<0.001	2.2 (1.6–2.9)	<0.001

AR, acute rejection; CIT, cold ischemia time; DGF, delayed graft function; DSA, donor-specific antibody; HR, hazard ratio; KDPI, Kidney Donor Profile Index; 95% CI, 95% confidence interval.

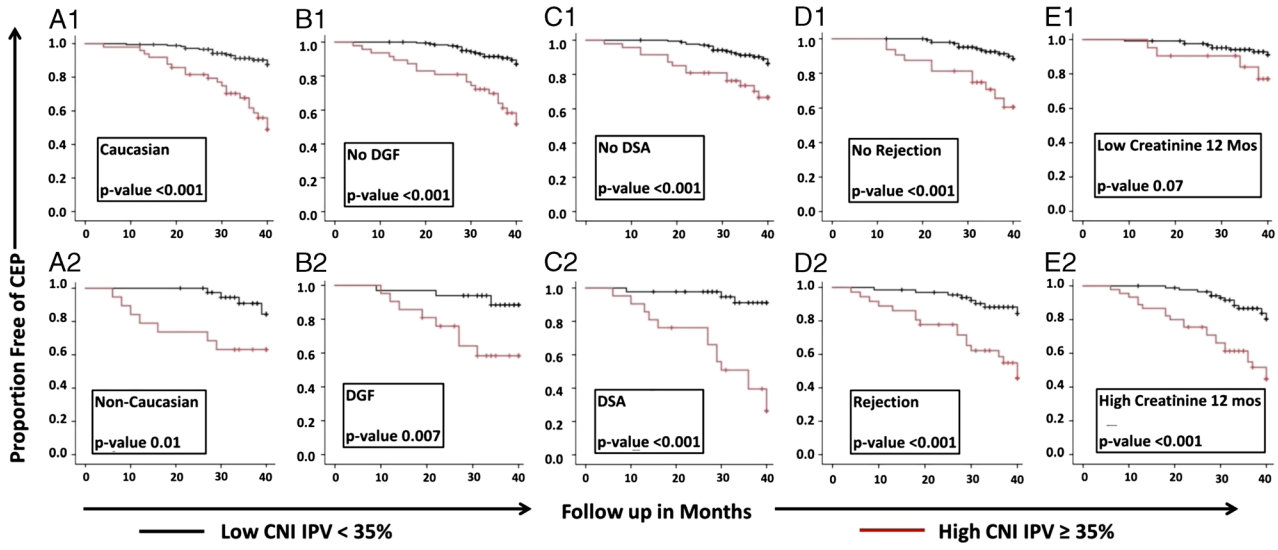


FIGURE 4. Composite endpoint (CEP) (graft loss and/or impending graft loss) among various subgroups are shown in various panels: A, Caucasian vs Non-Caucasian; B, delayed graft function (DGF) (no or yes); C, donor-specific antibody (DSA) (no or yes); D, rejection (no or yes); E, creatinine (low vs high defined as above and below the median serum creatinine [SCr] at 1 year) among patients with high and low calcineurin inhibitor intrapatient variability (CNI IPV).

which we acknowledge has limitations, but we believe that ultimately the CNI variation, regardless of etiology, highlights patients at risk for inappropriate immunosuppression and that all events that occur during the first year posttransplant are vital determinants of long-term renal allograft outcomes. Thus, measuring CNI IPV between 2 and 12 months provides a "real-life" approach that mimics actual clinical practice where we cannot ignore important clinical events. Fourth, for our univariate and multivariate analyses, we acknowledge that use of graft loss alone would be ideal, but our follow-up period did not allow for a sufficient number of events for this type of analysis. However, we do believe that our CEP that includes graft loss and/or impending graft loss (defined as eGFR<30 mL/min and > 30% decline from 3-month eGFR at last follow-up point) captures a clinically relevant group that are doing poorly and at high risk for graft loss. Lastly, given the nature of this study, we were not able to fully capture and account for changes in IS for each individual patient and how this may have affected their overall outcomes.

Nonetheless, we report several key findings that support the use of CNI-IPV within the first posttransplant year as an important factor leading to allograft rejection, chronicity, and subsequently poor late renal allograft outcomes. There are a variety of different approaches that have been proposed to address this CNI-IPV issue including changes to immunosuppression to improve adherence, including once daily TAC administration for better adherence.[39-47] However, further studies are necessary to better define CNI-IPV (outpatient vs inpatient, timing for samples to be collected, optimal cutoff value) to allow for future meaningful interventional trials that will target this high-risk population.[21] Ultimately, we believe that high CNI-IPV patients need to be identified early to allow for multidimensional approach, including changes in IS regimen and increased personnel dedication for oversight to eventually improve long-term renal allograft outcomes in this very high-risk group.

In summary, high CNI-IPV within 1 year posttransplant was associated with increased incidence of AR, specifically clinical AR, more severe AR, both early and late persistent/recurrent ARs, significant allograft chronicity by 1 year, and eventually more long-term graft loss and impending graft loss.

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