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Article type : TI on Transplantation

Tacrolimus Intrapatient Variability in Solid Organ Transplantation: A Multiorgan Perspective

Running Title: Tacrolimus IPV in SOT

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Disclosure: The authors declare no conflicts of interest.

Keywords: tacrolimus, solid organ transplantation, outcomes, therapeutic drug monitoring, intrapatient variability, coefficient of variation, time in therapeutic range, standard deviation

Abstract

Background: Tacrolimus therapy in solid organ transplant (SOT) recipients is challenging due to its narrow therapeutic window and pharmacokinetic variability both between patients and within a single patient. Intrapatient variability (IPV) of tacrolimus trough concentrations has become a novel marker of interest for predicting transplant outcomes. The purpose of this review is to evaluate the association of tacrolimus IPV with graft and patient outcomes and identify interventions to improve IPV in SOT recipients.

Methods: A systematic review of the literature was performed using PubMed and Embase from database inception to September 20, 2020. Studies were eligible only if they evaluated an association between tacrolimus IPV and transplant outcomes. Both pediatric and adult studies were included. Measures of variability were limited to standard deviation, coefficient of variation, and time in therapeutic range.

Results: Forty-four studies met the inclusion criteria. Studies were published between 2008 and 2020 and were observational in nature. Majority of data were published in adult kidney transplant recipients and identified an association with rejection, *de novo* donor specific antibody (dnDSA) formation, graft loss, and patient survival. Evaluation of IPV-directed interventions was limited to small preliminary studies.

Conclusions: High tacrolimus IPV has been associated with poor outcomes including acute rejection, dnDSA formation, graft loss, and patient mortality in SOT recipients. Future research should prospectively explore IPV-directed interventions to improve transplant outcomes.

INTRODUCTION

Tacrolimus remains the primary immunosuppressive agent used in solid organ transplantation as it is highly effective at preventing rejection and graft loss compared to

other agents.[1] Due to its narrow therapeutic index and extensive pharmacokinetic variability, individualized and frequent dose adjustments are necessary to minimize therapeutic failures such as rejection and debilitating adverse effects.[2] Although trough concentration is most frequently utilized for therapeutic drug monitoring (TDM) of tacrolimus therapy, a snapshot of tacrolimus exposure at a single time point has limited performance as a surrogate for drug exposure over time and therapeutic responses.[3] Therefore, it remains a critical need to identify more reliable TDM tools to optimize personalized tacrolimus therapy and improve long-term outcomes in solid organ transplant recipients.

In recent years, intrapatient variability (IPV) in trough concentrations has become recognized as a novel marker to identify transplant recipients at risk for poor outcomes such as rejection and graft loss.[4-6] IPV describes the extent of variation in tacrolimus trough concentrations over time for a single patient and is frequently expressed using standard deviation (SD), coefficient of variation (CV), and time in therapeutic range (TTR). These IPV metrics seem particularly attractive as all three can be calculated by utilizing trough concentrations from routine TDM. It is hypothesized that IPV, as a composite measure of drug exposure over time, may better capture the overall degree to which an individual patient is at risk for complications from over or under exposure to tacrolimus. Although the understanding of sources of variability continues to evolve, causes of IPV are thought to include non-adherence, drug-drug interactions, drug-food interactions, and drug-disease interactions such as diarrhea.[4]

Previous published reviews on tacrolimus IPV provided a focused examination of available literature, with conclusions generally derived from data in adult kidney transplant recipients.[4-6] In this systematic review, we critically evaluate the literature for the relationship between tacrolimus IPV and outcomes in solid organ transplantation, and discuss the strategies that have been utilized to reduce IPV. The aim was to expand upon previous reviews and comprehensively appraise literature across all organ types and age groups to understand how IPV may begin to be integrated into clinical care.

METHODS

A search for relevant articles published from inception to September 20, 2020 was conducted using the databases PubMed and Embase. Search terms included: “tacrolimus”, “variability”, (“intrapatient variability” OR “IPV”), “transplant”, (“coefficient of variation” OR “CV”), (“standard deviation” OR “SD”), and (“time in therapeutic range” OR “TTR”). Boolean operators were used to produce the final search algorithm: ("Tacrolimus" OR "Tacrolimus"[Mesh]) AND "variability" AND ("Organ Transplantation"[Mesh] OR "Transplantation"[Mesh] OR "transplant") AND ("coefficient of variation" OR "CV" OR "standard deviation" OR "SD" OR "time in therapeutic range" OR "TTR" OR “IPV”). References of relevant articles were reviewed for additional studies.

Articles were excluded because of overlap, irrelevance (did not evaluate tacrolimus IPV using SD, CV, or TTR), or study design (did not relate IPV to objective outcomes). Both pediatric and adult studies were included. As there are a sufficiently large number of full articles describing IPV and transplant outcomes, abstracts or conference papers were excluded for this portion of the review to capture the best available evidence. For the developing topic of interventions to address IPV, abstracts were included to capture expanding areas of research.

RESULTS

Using the search strategy described 127 unique references were identified. After applying exclusion criteria, 44 studies were included in this review (Figure 1). There were no randomized or interventional studies; data supporting the association of IPV and outcomes were limited to prospective observational and retrospective cohort studies. The results of the included studies are summarized in Tables 1-3.

Intrapatient Variability and Transplant Outcomes

Standard Deviation

Investigations of SD as a tacrolimus IPV tool preceded CV and TTR in both adult and pediatric transplant recipients. Early reports evaluated tacrolimus SD as a measure of medication adherence in pediatric liver transplant recipients.[7] Later, Venkat et al studied SD as a predictor of outcomes in pediatric liver transplant recipients.[8]

Pediatrics

The Medication Adherence in children who had Liver Transplant study evaluated the association of tacrolimus SD and late biopsy-proven acute rejection (BPAR) in adolescent liver transplant recipients. The odds of late BPAR were 2.5 times greater when SD>2.5. Further sensitivity analysis suggested SD>2 as the optimal threshold for predicting rejection.[9] A second analysis was completed to associate duration of variability with outcomes. SD<2 for 2 years of follow-up had the lowest rate of rejection (4.4%). Late acute rejection frequency was significantly higher for those with SD>2 for 1 year (22.9%) and 2 years (34.9%, *P*<0.001).[10]

Two other studies in pediatric recipients reported similar associations with rejection, one also identifying an increased risk of graft loss.[8,11] In a study of adolescent heart, kidney, liver, and lung transplant recipients, those who experienced rejection had a significantly higher SD compared with those who were rejection-free (2.7 vs 1.5, *P*=0.005), respectively. Additionally, SD>2 after 6 months post-transplant was predictive of graft loss.[11]

Not all studies in the pediatric population have identified an association between SD and rejection.[12,13] Higher rates of alanine aminotransferase (ALT) elevation without increased acute rejection was observed in pediatric liver transplant recipients.[12] Results may have been influenced by the younger age of this cohort compared with other studies and small sample size. A study in adolescent kidney transplant recipients indicated numerically higher SD in those with BPAR (5.3 BPAR vs 3.5 no BPAR, *P*=0.031) but was not significant per study protocol (prespecified α=0.01). Authors attributed the higher overall SD in their cohort to be related to inclusion of all levels, as other groups excluded undetectable levels or outliers due to acute illness or drug interactions.[13] However, in a similar age group, inclusion of outliers did not result in similarly elevated SD.[11] At this time, no studies have evaluated tacrolimus level selection on IPV calculations or the impact on predictive value.

Liver

High SD in adult liver transplant recipients has been associated with increased risk of rejection and graft failure.[14,15] SD was significantly higher in patients with BPAR

compared with those who were rejection-free (3.2 vs 1.5, *P*<0.01), respectively.[14] Another study in this population determined SD>2.1 to be predictive of graft failure.[15]

Other Organs

Elevated SD has also been related to poor outcomes in adult kidney and lung transplant recipients.[16,17] Time-varying SD in kidney transplant recipients ≥1 year post-transplant was predictive of worse long-term outcomes.[16] In lung transplant recipients, elevated SD after 6 months post-transplant was independently associated with time to chronic lung allograft dysfunction (CLAD) and patient death. For each one-unit increase in SD, the risk of CLAD increased by 46% and the risk of death increased by 27%. However, elevated SD between 0-6 months post-transplant was not associated with increased risk of CLAD or mortality.[17] These important findings suggest that elevated SD alone in the early post-transplant period is likely a poor predictor for transplant outcomes.

Coefficient of Variation

Borra et al were the first to report tacrolimus mean absolute deviation, a measure of IPV similar to CV, and the association with long-term outcomes in adult kidney transplant recipients.[18] Since then, tacrolimus CV has become the most common predictor of patient and graft outcomes.

Kidney

Three studies have evaluated tacrolimus CV early in the post-transplant course, here defined as within 6 months of transplant.[19-21] Of the two studies that evaluated acute rejection at 6 months post-transplant, an association with CV was not observed.[19,20] This may be explained by the use of induction immunosuppression. Long-lasting lymphocyte depleting agents, such as antithymocyte globulin or alemtuzumab, likely offers protection against the potentially harmful effects of tacrolimus variability in the early post-transplant period. Contrary to these reports, high CV in the early post-transplant period has been associated with graft loss in the long-term setting.[19,21] Interpretation of these results should be taken in the context of other findings, including absence of association with acute rejection and limitations of early IPV. As tacrolimus variability is expected early post-transplant due to acute changes in patient status and

medication regimens, early measurement of IPV may not be a good predictor of outcomes.[4,22] An observation of interest within these studies is subtherapeutic tacrolimus troughs as an independent predictor of acute rejection.[20,21] The importance of early goal trough attainment has been previously established and may provide more meaning than IPV alone in the early post-transplant period.[23,24]

As opposed to early CV, evaluation of tacrolimus CV after the acute post-transplant period, here defined as at least 6 months of measurement beginning after 3 months post-transplant, has been associated with increased rates of rejection.[25-31] The definition of high CV varied among studies. Most frequently, high CV was defined as CV greater than the cohort median or highest quartile. Acute rejection has been associated with CV ranging from >15% to >35%. In most of the studies, a CV of 25% and above was associated with acute rejection ≥1 year post-transplant. Although the particular cutoff selected by the investigator varied, the sample medians of CV were generally comparable but exceed the inherent variability in a controlled environment of a clinical trial (median CV range of 13.7-16.4%).[32-34]

High CV after the acute post-transplant period has also been associated with graft dysfunction, graft loss, *de novo* donor specific antibody (dnDSA) formation, and patient mortality in adult kidney transplant recipients.[25,27-30,35-41] Again, the numerical definition of high CV varied among studies but CV cutoffs associated with graft loss mirrored those associated with acute rejection (>15% to >35%).[25,27-29,35,37-41] Additionally, a trend for subtherapeutic tacrolimus troughs (<4 ng/mL to <5 ng/mL) as a risk factor for graft survival was observed in several studies.[19,25,26,37] Patients with high CV due to subtherapeutic troughs and overall low tacrolimus exposure appear to be at highest risk for graft loss. Taken together, combining average tacrolimus trough and CV may provide better risk factor stratification for transplant recipients at risk of poor outcomes rather than either measure alone, but this must be evaluated in context of center-specific practices.

Liver

Three studies have evaluated CV and outcomes in adult liver transplant recipients.[42-44] In a retrospective study utilizing tacrolimus levels collected within the first month post-transplant, CV >40% was associated with 57% greater risk of graft loss at 1

year (*P*=0.002). High CV was also associated with diminished patient and graft survival with up to 12 years follow-up. While the multivariable analysis controlled for the higher MELD and Child-Pugh score at baseline, the high CV group also had more neurologic complications, cardiovascular complications, and acute renal failure requiring dialysis during the initial hospitalization.[42] As proposed previously, a complicated post-operative course would be hypothesized to result in greater tacrolimus variability. Other studies in adult liver transplant recipients, calculating CV over the majority of the first year, have been unable to demonstrate similar associations with high CV and graft loss or patient mortality but support a relationship with acute rejection.[43,44] In one study, CV >35% was associated with a 3-fold increase in odds of BPAR (*P*=0.003) and a 4-fold increase in formation of dnDSA at 2 years post-transplant (*P*=0.001).[43] Another group noted a numerical increase in late acute and chronic rejection in patients with CV >28% compared with CV <28% (24.4% vs 18.5%, *P*=0.068).[44]

Cutoffs to define high CV appear to be higher in liver transplant recipients compared with the kidney transplant population. In liver transplant, poor outcomes may not present until relatively high CV, such as >35 to >40%.[42,43] Potentially, the reduced immunogenicity of the liver may offer protection from poor outcomes related to tacrolimus variability.[45]

Heart

Two studies reported varying effects of CV and outcomes in adult heart transplant recipients.[46,47] One study observed an 8-fold increased risk for rejection (*P*=0.011) in those with CV >28.8%, as well as increased rejection severity. The difference was seen only with rejection episodes occurring after 1 year post-transplant; there was no difference between groups in rejection frequency between 3-12 months post-transplant. Opposingly, Shuker et al did not find a difference in proportion of patients who experienced acute rejection or cardiac allograft vasculopathy at 4 years post-transplant based on CV measurements.[47] However, the high CV group was defined by CV >17.7% (group CV median 22.6%), which may have been too low to determine a difference in outcomes.

Pediatrics

Elevated CV has been associated with increased risk of rejection and dnDSA formation in pediatric kidney transplant recipients.[13,48-52] Median CV in patients with BPAR has ranged from 44%-53%, compared with 24%-33% in non-rejecters. [13,48,51] Authors have attempted to identify a CV cutoff for poor allograft outcomes with results ranging 31%-41%.[13,49,50] There are several differences between studies worth highlighting. One study began CV measurement at 1 month post-transplant and therefore may be subject to an overall increased variability.[13] Others elected to measure CV at 6 or 12 months prior to rejection and generally identified lower CV cutoffs (31%-44%).[48,50] Findings from the studies suggest overall CV may be higher in pediatrics but share the same trend seen in adults.

Tacrolimus CV has also been predictive of rejection in pediatric liver transplant recipients.[53] In young liver recipients, CV was significantly higher in patients with BPAR compared with no BPAR (56.7% vs 40.9%, respectively, *P*=0.04) at 1 year post-transplant. These findings did not persist beyond 1 year post-transplant, possibly related to the overall median CV decrease seen over time from 41.6% at 1 year and 30.9% at 2 years to 28.5% at 3 years.[53] Similar findings were not seen by Riva et al when evaluating BPAR within 2 years post-transplant in pediatric liver recipients, likely due to limiting the CV calculation to tacrolimus troughs 7-10 days prior to BPAR diagnosis.[54]

Time in Therapeutic Range

As TTR was recently introduced as a tool to evaluate tacrolimus IPV, fewer studies are available for TTR. TTR is calculated using the Rosendaal method which assumes a linear relationship between values to calculate the percentage of time in range.[55]

Heart and Lung

In adult heart transplant recipients, early TTR during the first 30 days post-transplant was similar among patients who did and did not experience rejection (31.4% vs 36.2%, *P*=0.512), respectively.[56] Similar to SD and CV, TTR in the early post-transplant period is likely to have limited utility in predicting transplant outcomes. In lung transplant recipients, every 10% increase in TTR was inversely related to rate of rejection, high-grade acute cellular rejection, CLAD, mortality, and infection at 1 year

post-transplant.[57] Recently, a second study in lung transplant recipients failed to find an association between TTR and acute rejection.[58] Comparison of these two studies highlights the number of variables related to the risk and diagnosis of immune-mediated outcomes that will complicate establishing TTR targets across centers such as induction therapy, goal trough concentration range, and frequency of protocol biopsies.

Kidney

A recent study in adult kidney transplant recipients also identified an association between TTR <78% and risk of rejection, graft loss, mortality, and infection.[59] Davis et al conducted two analyses within a single group of adult kidney transplant recipients utilizing TTR and CV.[60,61] The first analysis used a cutoff of TTR <60% to identify high-risk patients based on warfarin literature. TTR <60% was associated with increased risk of dnDSA and acute rejection at 12 months post-transplant. Likewise, increased death-censored graft loss was seen at 5 years in those with a TTR <60%.[60] The second analysis utilized a TTR threshold of 40% based on receiver operating curve analysis and observed similar dnDSA, acute rejection, and death-censored graft loss risk. Authors then compared TTR and CV for the same outcomes in a 2x2 design. Among patients with high CV, those with low TTR had significantly higher risk for dnDSA, acute rejection, and death-censored graft loss compared with high TTR. Among patients with high TTR, outcomes were not significantly different when comparing those with low and high CV.[61] These results suggest combining IPV measures may offer stronger predictive value, however, further studies in this area are necessary.

Interventions to Reduce Tacrolimus Intrapatient Variability

The association between elevated tacrolimus IPV and transplant outcomes has been established in numerous studies. Theoretically, reducing IPV through controlling sources of variation may improve long-term outcomes. Potential sources of tacrolimus variability have been described previously.[4] Briefly, sources of variability are thought to include food effects, drug interactions, diarrheal illness, laboratory assay, iatrogenic variability, and non-adherence. Several small, prospective trials have evaluated interventions to reduce tacrolimus IPV primarily targeting adherence through educational or technological programs.

The conversion of immediate-release tacrolimus (Tac-IR) to once-daily extended-release formulations of tacrolimus (Astagraf; Tac-ER or Envarsus; LCP-Tac) has had varying effects on IPV. Several authors have demonstrated a significantly lower IPV with Tac-ER compared to Tac-IR in kidney transplant recipients.[33,62,63] The effect of formulation change on tacrolimus IPV may depend on baseline variability of patients. Shuker et al did not find an overall improvement in CV with conversion from Tac-IR to Tac-ER (17.3% vs 16.4%, *P*=0.31), respectively. When only patients with high baseline variability (CV>17.9%) were considered, this subgroup demonstrated a significant improvement in CV after conversion from Tac-IR to Tac-ER (25.6% vs 17.1%, *P*=0.01), respectively.[34] Potentially, the high CV group was reflective of those with medication non-adherence that benefitted from once-daily dosing. This hypothesis aligns with another study where formulation change did not reduce CV in a population with a low baseline CV (15.3% on Tac-IR to 13.7% on Tac-ER, *P*=0.2).[33] There has also been a report of increased CV after conversion from Tac-IR to LCP-Tac.[64] The observed variability of LCP-Tac is counterintuitive but likely reflects iatrogenic variability due to provider unfamiliarity with new products.

The impact of pharmacist education on CV in 126 adult kidney transplant recipients was investigated by Bessa et al. Participants were randomized to receive standard instructions by nursing staff only or pharmacist education in addition to standard nursing instructions. At 90 days post-transplant there was no difference in mean CV between groups (32.5% control vs 31.4% pharmacist education, *P*=0.673). Likewise, mean tacrolimus troughs and clinical outcomes were similar between groups.[65] Although early pharmacist education did not appear to influence CV in adult kidney transplant recipients, the long-term effect of this intervention remains unknown. Since medication adherence is expected to be high during the time frame of this study but drift over time, educational interventions to improve IPV through adherence may be better employed later in the post-transplant course.[66]

Two groups reported implementation of tacrolimus CV reports in ambulatory care settings as a patient monitoring and risk assessment tool.[67,68] Cheng et al instituted an online CV reporting system at an outpatient clinic. Based on tacrolimus CV 183 adult kidney transplant recipients were stratified into two risk groups: high-risk group (CV >

30%) and alert group (CV 22-30%). Six months after implementation of the online reporting tool, significant decreases in CV were observed in both the high-risk group (median 41% to 25%, *P*<0.001) and the alert group (median 26% to 20%, *P*=0.003). Unfortunately, the authors did not describe the actions taken by the transplant team in the setting of an elevated CV.[68] Kaiser et al also describe implementation of an automated tacrolimus IPV report as a longitudinal monitoring tool.[67] Instant online reporting of tacrolimus IPV appears to be a simple way to identify high-risk patients that may allow targeted interventions to improve tacrolimus IPV.

A pilot program utilizing cognitive behavioral therapy and motivational interviewing was implemented in adult kidney transplant recipients with <98% adherence as determined by pill counts. Thirty-three adults were randomized to receive the intervention or standard of care. Mean tacrolimus troughs were similar between groups at study completion; however, there was a decrease in SD in the intervention arm (2.8% to 1.8%, *P*<0.05) but not in the control arm (3.5% to 3.5%, *P*>0.05).[69]

Finally, mobile technology been investigated to improve adherence and IPV. Levine et al utilized Transplant Hero®, a transplant mobile app, as an interactive alarm and educational tool in kidney, pancreas, and/or liver transplant recipients. Participants were randomized to receive the mobile app, both the app and a smart watch, or neither. Tacrolimus CV was not different between groups at 1 month (30.4% mobile app vs 35.5% both vs 31.7% neither, *P*=0.96) or 3 months post-transplant (33.0% mobile app vs 33.8% both vs 32.8% neither, *P*=0.81).[70] These results may be due to the close proximity to time of transplant. A similar study showed a significant reduction in tacrolimus CV among Transplant Hero® users compared to nonusers at 1 month (27.7% vs 37.0%, *P*=0.014) but not at 3 months (33.6% vs 35.4%, *P*=0.63) suggesting the need to investigate the impact of attrition.[71] Jung et al evaluated the use of text message and pill box alarms to improve unintentional forgetfulness among kidney transplant recipients. No difference in tacrolimus CV was observed between those randomized to receive the intervention compared to control (23.9% vs 25.1; *P*=0.645), respectively.[72] Notably, outcomes should be interpreted in the context of high adherence (>98%) observed within both arms throughout the 6 month study period. Finally, McGillicuddy et al evaluated use of an mHealth app and electronic pillbox in adult kidney transplant

recipients with poor medication adherence. Eighty participants were randomized to the mobile health intervention or control at mean 2 years post-transplant. Tacrolimus CV after intervention was significantly lower in the intervention group compared with the control group (*P*=0.046). There was also a significant reduction in tacrolimus CV in patients with CV <40% (*P*=0.001) in conjunction with an improvement in medication adherence as determined by electronic pillbox use (*P*<0.001).[73] Mobile health techniques to reduce IPV through improved adherence have had mixed results, but application of such interventions may benefit tech savvy patients or patients with low baseline adherence but confirmation from additional studies is necessary. Further, additional studies should investigate the impact of mobile health technology later into the post-transplant course when medication nonadherence is often a larger concern.

DISCUSSION

Available data support positive associations between tacrolimus IPV and worse outcomes in transplant recipients, although results were not consistent across all organs and age groups evaluated. Currently, there are no randomized controlled trials evaluating tacrolimus IPV-directed interventions to improve patient and graft outcomes. In the absence of such data, we provide the following recommendations when considering how to utilize IPV in solid organ transplant recipients.

Based on the extent of literature evaluating CV, ease of calculation, and standardization for the scale of the dataset, we agree with previous recommendations that CV is the best supported IPV metric for clinical use.[5,6] Considering CV within a highly adherent population approximated 15%; CV >15% indicates a potential risk for poor outcomes. To identify high-risk patients, clinicians could consider a CV cutoff of 30% based on available data in the adult kidney transplant population. Regarding other measures of variability, identifying a definitive cutoff is challenging due to center differences in tacrolimus therapeutic windows. Future research should evaluate CV cutoffs among non-adult and non-kidney transplant recipients. It is possible that the extent of variability may be larger before becoming clinically significant among certain populations (e.g., liver transplant recipients or pediatric recipients), but current data are not yet strong enough to support differentiation. Another area for future research is the

opportunity to improve predictive value through combination of CV with TTR or tacrolimus trough concentration. IPV measures are unable to discriminate variability due to subtherapeutic or supratherapeutic levels. TTR offers the promising advantage of evaluating variability relative to the therapeutic target and merits further research. However, establishing TTR goals will require appreciation of the therapeutic tacrolimus window and a universal TTR goal will be unlikely. Alternatively, IPV may be evaluated in the context of tacrolimus exposure, measured by tacrolimus trough concentration. This method has been previously proposed to identify kidney transplant recipients at high risk of developing dnDSA.[74]

Measures of IPV appear to be of greatest predictive potential when applied at least 3 to 6 months post-transplant. After this time period, IPV likely better reflects patient behaviors and clinical conditions of interest. Others have recommended measuring IPV between 6-12 months post-transplant due to the limited data outside this time period.[5,6] Prior to this period, achieving therapeutic tacrolimus troughs should be the focus to optimize outcomes. Similarly, the clinical utility of IPV after 1-2 years post-transplant is not well established.

There are other logistical aspects to consider for IPV measurements. No data exists to suggest a minimum number of levels for best predictability. A common approach in studies has been to require at least 3 levels but final calculations have generally consisted of a median of 5-15 levels over a 6-12 month time period. We recommend no less than 3 levels when calculating IPV and ideally at least 1 level per month, to best replicate available literature. Additionally, we recommend the use of only outpatient levels due to the added variability anticipated within the inpatient setting.[5,6] We also urge clinicians to be cautious when calculating and interpreting IPV. Several scenarios may introduce unintentional variability to the IPV calculation, such as tacrolimus concentrations not representative of a “true” trough, alterations in a patient’s goal trough concentration, and changes in laboratory assay.

Finally, utilizing IPV as a direct surrogate for medication non-adherence (MNA) should be avoided and investigations to reduce IPV should incorporate measures to confirm MNA. Previous reviews have concluded that MNA is a primary determinant of elevated IPV and correspondingly IPV is capable of serving as a proxy to identify

tacrolimus nonadherence.[5,6] Although IPV is theoretically an attractive strategy to objectively evaluate MNA, this claim is made without proper prospective validation. Arguments rely heavily on the findings of Leino et al which demonstrated that median CV was lower in an adherent population than in observational cohorts. However, such arguments often fail to recognize other differences in the study population including the clinical stability of the patients, prohibition of dose changes including changes to potentially interacting medications, and calculation of CV on a weekly basis using daily troughs.[32] All of these factors could reasonably reduce IPV by altering sources of variability particularly those which are time dependent. For example, the number of dose changes has previously been associated with increased IPV.[18,21] Increased frequency of dose changes could be a manifestation of multiple issues; most interesting is the role of iatrogenic variability arising from the limited ability to forecast the impact of dose changes on trough concentration. Several enhanced dosing models have demonstrated improvements in IPV and suggest computer-assisted dosing, capable of accounting for higher levels of clinical complexity, is another area to target for intervention.[75-77] Further, the understanding of additional sources of tacrolimus variability continues to grow with recent evidence supporting a role for the microbiome and inflammation.[78,79]

Retrospective studies utilizing physician records, patient report, or clinic nonattendance to define MNA have not consistently supported a relationship between MNA and IPV.[15,28,50,53] Conflicting results have also been observed among prospective studies assessing MNA using electronic monitoring or rigorous multimodal approaches.[65,73,80-82] Although some discordance may be related to data quality pertaining to the method(s) of defining MNA or small patient population, clinicians must evaluate IPV in a patient-specific context including all other possible sources of IPV. Further, most of the evidence supporting an association between MNA and high IPV is on a population level relying on differences in mean or median IPV values. Little evidence exists applying IPV to the individual. Evaluation of the studies directly evaluating MNA and IPV reveals a wide, overlapping range of IPV values among both adherent and nonadherent patents. This data suggests IPV possesses low sensitivity and specificity for identifying nonadherence in a particular patient. Clinicians should be aware that while an increased

IPV may be associated with MNA, MNA is not the sole cause of IPV and elevated IPV will not capture all nonadherent patients.

CONCLUSION

High tacrolimus IPV has been associated with poor outcomes in various organ transplant recipients. Variation in tacrolimus troughs can be related to a number of modifiable sources. Several novel interventions to reduce tacrolimus IPV have been piloted including pharmacist education, cognitive behavioral therapy, online CV reporting, and technology to support medication use. A direct relationship between interventions that improve IPV and outcomes has yet to be established. At this time, in clinical practice IPV should be limited to an additional screening tool to identify patients at increased risk for negative outcomes. The cause of IPV should be carefully evaluated and not assumed to be related to MNA without further investigation. Moving forward, this area of research would benefit from standardization of IPV metrics as a predictor of transplant outcomes and potential area for intervention.

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Table 1. Summary of Studies Evaluating Tacrolimus Intrapatient Variability defined by SD on Outcomes

Author Year n	Population	SD measurement	Tacrolimus measurement	Follow up	Study Groups	High SD and Outcomes				
						Rejection	dnDSA	Graft Loss	Mortality	Other
Venkat et al (2008)[8] n=117	Pediatric liver (median age 9.1 years)	≥12 months post-transplant	≥4 outpatient levels MEIA Selective: excluded levels >2 SD from mean in association with explainable causes	5.3 years	High SD defined as >2 (cohort median 1.6)	↑ (Late BPAR)	--	--	--	--
Shemesh et al (2017)[9] n=379	Pediatric liver transplant (mean age at baseline 9.6 years)	Rolling SD over 12 months between 0 and 24 months post-transplant	≥3 levels (mean 11.8) Selective: discarded undetectable readings or assigned local laboratory's lower limit of detection	2 years	Rejection (SD 2.4) vs rejection-free (SD 2.6)	↑ (Late BPAR)	--	--	--	--
Shemesh et al (2018)[10] n=400	Pediatric liver	Rolling SD over 12 months between 0 and 24 months post-transplant	≥3 levels Selective: discarded undetectable readings or assigned local laboratory's lower limit of detection	2 years	High SD defined as SD >2	↑ (Late BPAR)	--	--	--	--
Pollock-Barziv et al (2010)[11] n=144	Pediatric heart, kidney, liver, and lung (median age at transplant	3 – 6 months preceding rejection or last 6 months follow up	All levels (median 12 levels in rejecters and 7 levels in non-rejecters)	Up to 10 years	High SD defined as SD >2	↑ (Late AR)	--	↑	--	--

	approx. 13.0 years)									
de Oliveria et al (2017)[12] n=50	Pediatric liver (mean age at transplant 4 years)	13 – 35 months post-transplant	≥5 outpatient levels CMIA Selective: levels during concomitant fluconazole, anticonvulsant, and/or diarrhea were excluded	--	High SD defined as ≥2	↔	--	--	--	↑ ALT >60 IU/L
Hsiau et al (2011)[13] n=46	Pediatric kidney (median age 14.7 years BPAR vs 13.8 years no BPAR)	1 – 12 months post-transplant	Levels at least monthly (median 17.2 levels) Selective: excluded levels with concomitant diarrhea	4.3 years	BPAR (SD 5.3) vs no BPAR (SD 3.5)	↔ (BPAR)	--	--	--	--
Christina et al (2014)[14] n=150	Adult liver	≥6 months post-transplant	≥3 levels at 3 month intervals over 3 years Selective: discarded undetectable readings	Approx. 4 years	Rejection (SD 3.8) vs rejection-free (SD 2.3)	↑ (BPAR)	--	--	--	--
Lieber et al (2013)[15] n=359	Adult liver	6 – 18 months post-transplant	≥3 levels	At least 5 years	Median as cut point, >2.1	--	--	↑	--	--
Sapir-Pichhadze et al (2014)[16] n=356	Adult kidney	Time-varying SD ≥ 1 year post-transplant	Median 15 levels	3.72 years	SD thresholds (>1.5, >2.0, >2.5, and >3.0)	↑†	--	↑†	--	--
Gallagher et al (2015)[17] n=110	Adult lung	0 to 6 months, 6 to 12 months, and 12 to 24 months	Median 11 – 15 levels per time period EMIT	60 months	Outcome vs outcome-free (median 4.01	↑ (CLAD)	--	--	↑	--

		(combined 6 – 12 and 12 – 24 for analyses)			months 0 – 6; 2.84 months 6 – 12; 2.85 months 12 – 24)					

↑ = increased risk; ↔ = no difference between groups; -- = not studied

Abbreviations: AR, acute rejection; BPAR, biopsy proven acute rejection; CLAD, chronic lung allograft dysfunction; CMIA, chemiluminescent immunoassay; dnDSA, *de novo* donor specific antibodies; EMIT, enzyme multiplied immunoassay technique; MEIA, microparticle enzyme immunoassay; SD, standard deviation

†Composite end point of late acute rejection, transplant glomerulopathy, or total graft loss (graft failure or death with function)

Table 2. Summary of Studies Evaluating Tacrolimus Intrapatient Variability defined by CV on Outcomes

Author Year n	Population	CV measurement	Tacrolimus measurement	Follow up	Study Groups	High CV and Outcomes				
						Rejection	dnDSA	Graft Loss	Mortality	Other
Seibert et al (2018)[19] n=1472	Adult kidney	0 – 6 months post-transplant	≥5 levels (mean 17.7) All levels included LC/MS	Up to 10 years	Highest quartile (CV >49% AA; >38% in EA) vs all other quartiles combined	↔	--	↑	--	--
Israni et al (2013)[20] n=1930	Adult kidney	Day 8 to 6 months post-transplant	Mean 16.3 levels per patient Two measurements each from weeks 1 – 8 and one from months 3, 4, 5, and 6 post-transplant	6 months	AR (31%) vs no AR (33%)	↔	--	--	--	--
Rozen-Zvi et al (2017)[21] n=803	Adult kidney	Time-weighted CV 0 – 6 months post-transplant	Mean 20.7 levels MEIA, CMIA	3.7 years	Highest tertile vs all others (mean CV 34.8%)	--	--	↑	--	--

Whalen et al (2017)[25] n=432	Adult kidney	6 – 12 months post-transplant	All levels (median 7 – 8 levels) LC/MS Selective: excluded non-troughs	4 years	Median as cut point, >15%	↑ (early and late BPAR)	--	↑	--	↓eGFR
Huang et al (2016)[26] n=161	Adult kidney	6 months prior to BPAR or study end	CMIA	4.3 years	Rejection (CV 39%) vs rejection-free (CV 12.1%)	↑ (BPAR)	--	--	--	--
Shuker et al (2016)[27] n=808	Adult kidney	6 – 12 months post-transplant	≥3 outpatient levels (median 5 levels) EMIT	5.5 years	Median as cut point, >16.2%	↑†	--	↑†	--	--
Goodall et al (2017)[28] n=628	Adult kidney	6 – 12 months post-transplant	Outpatient levels (mean 8.9) LC/MS	Until graft loss or 8 years (mean 4.7 years)	Lowest variability: < 1st quartile (13.45%) Low variability: < median (18.15%) High variability: < 3rd quartile Highest variability: >3rd quartile (25.27%)	↑ (AR)	↑	↑	↔	--
Taber et al (2017)[29] n=1411	Adult kidney	1 month – 387 days post-transplant or until day before rejection	LC/MS Selective: excluded non-12 hour troughs or levels > 30 ng/mL	4.6 years	Median CV (Non-AA): 34.8% Median CV (AA): 39.9%	↑ only in AA	--	--	↑	--
Sharma et al (2019)[30] n=286	Adult kidney	Highest and lowest trough for each month from 2 to 12 months post-transplant	Median 35 levels Selective: excluded non-12 hour trough levels	Up to 40 months	High CV defined as ≥35% (highest quartile)	↑ (early, late, and recurrent/persistent)	↔	↑	--	↑ Chronicity score‡
Scheel et al (2017)[31] n=267	Adult kidney	12 months prior to non-adherence assessment (>6	≥4 outpatient levels (mean 9.6) LC-MS/MS	7.1 years	Rejection vs rejection-free (cohort mean 21.3%)	↑ (BPAR)	--	--	--	--

		months post-transplant)	Standardized by target level							
Mo et al (2019)[35] n=671	Adult kidney	6 – 12 months post-transplant	≥3 outpatient levels (approx. 7 levels) LC/MS Selective: excluded non-trough levels	58.5 months	Median as cut point, >20.5%	↑	--	↑	↔	↑ Chronicity score§
Vanhove et al (2016)[36] n=220	Adult kidney	6 – 12 months post-transplant	≥3 outpatient levels (mean 5.3) MEIA, CMIA Selective: excluded non-trough levels	2 years	3 groups based on median: Low: <14.4% Middle: 14.4 – 22.1% High: >22.1%	--	--	--	--	↑ Chronicity score¶
O'Regan et al (2016)[37] n=394	Adult kidney	3 – 12 months post-transplant	All levels (median of 6 – 10) MEIA	6.94 years	4 groups based on median CV quartile: Q1: 12.5% Q2: 18.17% Q3: 24.63% Q4: 36.91%	↔ (BPAR)	--	↑	↔	--
Rodrigo et al (2016)[38] n=310	Adult kidney	4 – 12 months post-transplant	≥3 outpatient levels (mean 7) MEIA, CMIA	6.6 years	High CV defined >30% (median 29.1%)	↔ (BPAR)	↑	↑	--	↓eGFR
Susal et al (2019)[39] n=6639	Adult kidney	1, 2, and 3 years post-transplant	--	7.4 years	High CV defined as >30% (median 34%)	--	--	↑	↑	--
Rahamimov et al (2019)[40] n=878	Adult kidney	≥6 months post-transplant	Time-weighted CV MEIA	1263 days	Highest CV defined as >25% (mean 26.2%)	--	--	↑	--	--
Sablik et al (2018)[41] n=248	Adult kidney	3 years prior to AMR diagnosis, >6 months post-transplant	≥8 outpatient levels over time period of 2 years	3 years	Case (mean 24.4%) vs control (mean 23.6%)	↔ (Chronic active AMR)	--	↑ (those with AMR)	--	--

Rayar et al (2018)[42] n=812	Adult liver	Day 8 to 30 post-transplant	All levels	Up to 12 years	High CV defined as ≥40% (third quarter limit of 41.8%)	↔	--	↑	↑	--
Del Bello et al (2018)[43] n=116	Adult liver	Months 0 – 24 post-transplant	≥3 levels (median 10) Dose-corrected LC/MS	Up to 2 years	Continuous, >35% and >40% (median 30.5%)	↑ (BPAR)	↑	↔	↔	--
Van Der Veer et al (2019)[44] n=326	Adult liver	6 – 18 months post-transplant	≥5 outpatient levels (median 7) Only levels prior to rejection Dose normalized LC-MS/MS and EMIT	5.2 years (up to 17 years)	Median as cut point, >28%	↔ (composite of acute and chronic rejection)	--	↔	--	↓eGFR (if low baseline)
Gueta et al (2018)[46] n=72	Adult heart	3 – 12 months post-transplant	≥2 levels at steady state (mean 13.5) CMIA	51.1 months	Median as cut point, >28.8%	↑ (total rejection score[‖])	↔	--	↔	--
Shuker et al (2018)[47] n=86	Adult heart	6 – 18 months post-transplant	≥3 outpatient levels EMIT, CMIA	4 years	Median as cut point, >17.7%	↔ (CAV or BPAR)	--	--	--	--
Abu Bakar et al (2019)[48] n=50	Pediatric kidney (mean age of transplant: 10.85 years for cases, 11.69 years for controls)	12 months prior to rejection, or up to 6 months if ACR occurred 12 – 18 months post-transplant	≥3 levels	1 – 8 years	Rejection (CV 44%) vs rejection-free (CV 24%)	↑ (Late AR)	--	--	--	↑ Creatinine
Kaya Aksoy (2019)[49] n=67	Pediatric kidney	0 – 6, 6 – 12 and ≥12 months post-transplant	Mean 22.5 levels 0 - 6 months, 6.24 levels 6 – 12	50.8 months	CV >32% based on ROC analysis	--	↑	--	--	↔ eGFR

	(mean age 15.16 years)		months, 24.3 levels ≥12 months EMIT Selective: discarded levels > 30 ng/mL							
Hsiau et al (2011)[13] n=46	Pediatric kidney (median age 14.7 years BPAR vs 13.8 years no BPAR)	1 – 12 months post-transplant	Levels at least monthly (median 17.2 levels) Selective: excluded levels with concomitant diarrhea	4.3 years	Rejection (CV 53.4%) vs rejection-free (CV 30%)	↑ (BPAR)	--	--	--	--
Pizzo et al (2016)[50] n=23	Pediatric kidney (median age at transplant: 8 years adherent vs 14.1 years non-adherent)	6 months prior to biopsy	≥4 levels (median 5 levels per biopsy) CMIA	6.3 years	Adherent (CV 41.1%) vs non-adherent (CV 48.5%)	↔ (BPAR)	↔	--	--	--
Prytula et al (2012)[51] n=69	Pediatric kidney (median age at transplant: 10 years)	Over 12 months (at least 3 months after tacrolimus initiation)	Median 7 levels MEIA	Up to 4 years	Rejection (CV 48.7%) vs rejection-free (CV 32.9%)	↑ (Late AR)	--	↔	--	↔ eGFR
Solomon et al (2020)[52] n=38	Pediatric kidney (median age 12.1 years at transplant)	3 months post-transplant	Median 40 levels	3.4 years	Cut-points of 30%, 40%, and 50% (median 43.1%)	↔	↑	↔	--	--

Defrancq et al (2019)[53] n=41	Pediatric liver (median age at transplant 30 months)	3 months – 5 years post-transplant	Dose- and weight-normalized CMIA Selective: excluded levels during first 3 months of each year for years 2 – 5	5 years	Rejection or for-cause biopsy vs no outcome	↑ (BPAR)	↔	--	--	↔ CMV viremia
Riva et al (2018)[54] n=71	Pediatric liver (mean age 5.3 years)	7 – 10 days before outcome or median value during last month of follow-up	Dose-normalized CMIA	2 years	BPAR or adverse drug reaction vs no outcome	↔	--	--	--	--

↑ = increased risk; ↔ = no difference between groups; -- = not studied

Abbreviations: AA, African Americans; AMR, antibody mediated rejection; AR, acute rejection; BPAR, biopsy proven acute rejection; CAV, cardiac allograft vasculopathy; CLAD, chronic lung allograft dysfunction; CMIA, chemiluminescent immunoassay; CMV, cytomegalovirus; CV, coefficient of variation; dnDSA, *de novo* donor specific antibodies; EA, European Americans; eGFR, estimated glomerular filtration rate; EMIT, Enzyme multiplied immunoassay technique; LC/MS, liquid chromatography with tandem mass spectrometry; MEIA, microparticle enzyme immunoassay

[†]Composite end point consisting of graft loss, late biopsy-proven rejection, transplant glomerulopathy, or doubling of serum creatinine

[‡]Defined as interstitial fibrosis and tubular atrophy score

[§]Defined as the sum of ci, ct, cg, cv, ah, and mm

[¶]Defined as the sum of ci, ct, ah, cv and cg

[‖]Total rejection score calculated according to ISHLT classification system as 0R=0, 1R=1, 2R=2, and 3R=3, dividing the summed scores by the total number of biopsy specimens during the study period

Table 3. Summary of Studies Evaluating Tacrolimus Intrapatient Variability defined by TTR on Outcomes

Author Year n	Population	TTR measurement[†]	Tacrolimus measurement	Follow up	Study Groups	Low TTR and Outcomes				
						Rejection	dnDSA	Graft Loss	Mortality	Other
Baker et al (2019)[56] n=67	Adult heart	0 – 30 days post-transplant	Levels daily during hospitalization, twice weekly at discharge	30 days	Rejection (TTR 31.4%) vs	↔	--	--	↔	--

			Therapeutic range for TTR: 10-15 ng/mL		rejection-free (TTR 36.2%)					
Ensor et al (2018)[57] n=292	Adult lung	0 – 12 months post-transplant	Median 60 – 75 levels Therapeutic range for TTR: 12 – 15 ng/mL months 0-6, 10-12 ng/mL months 6-12	1 year	Low TTR defined as <30% (median 20.7%)	↑ (any rejection, ACR, CLAD)	--	--	↑	↑pulmonary infection
Kao et al (2020)[58] n=157	Adult lung	0 – 6 months post-transplant	Median 40 levels CMIA Therapeutic range for TTR: 10 – 15 ng/mL	165 days	Rejection vs rejection-free (cohort mean TTR 46.8%)	↔	--	--	--	--
Song et al (2019)[59] n=1241	Adult kidney	0 – 12 months post-transplant	EMIT Exclusive – excluded levels <2 ng/mL or >15 ng/mL if not valid Therapeutic range for TTR: 5-10 ng/mL months 0-3, 4-8 ng/mL months 4-12	41 months	Low TTR defined as <78%	↑	--	↑	↑	↑ infection
Davis et al (2018)[60] n=538	Adult kidney or simultaneous kidney-pancreas	0 – 12 months post-transplant	All levels (median 19) CMIA Therapeutic range for TTR: 5-10 ng/mL	4.1 years	TTR <60% or <75%	↑	↑	↑	--	--
Davis et al (2020)[61] n=538	Adult kidney or simultaneous kidney-pancreas *Additional analysis to above study*	0 – 12 months post-transplant	All levels (median 19) CMIA Therapeutic range for TTR: 6-10 ng/mL	4.1 years	Low TTR defined as <40% High CV defined as >44.2% (highest quartile)	↑	↑	↑	--	--

↑ = increased risk; ↔ = no difference between groups; -- = not studied

Abbreviations: ACR, acute cellular rejection; BPAR, biopsy proven acute rejection; CLAD, chronic lung allograft dysfunction; CMIA, chemiluminescent immunoassay; CV, coefficient of variation; dnDSA, *de novo* donor specific antibodies; EMIT, Enzyme multiplied immunoassay technique; TTR, time in therapeutic range

†Measured using Rosendaal method

Figure 1. PRISMA Flow Diagram for Systematic Review