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Erratic tacrolimus levels at 6 to 12 months post-lung transplant predicts poor outcomes

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BACKGROUND: It has previously been described that erratic tacrolimus blood levels are associated with graft failure in kidney and liver transplantation. Using a small cohort, we previously described that a higher tacrolimus standard deviation (SD) 6 to 12 months after lung transplantation increased the risk of chronic lung allograft dysfunction (CLAD) and death. We aimed to assess this in a larger cohort using the coefficient of variation (CoV) and identify potential risk factors for higher CoV.

METHODS: We retrospectively reviewed 351 lung transplant recipients who received tacrolimus-based immunosuppression therapy. Cox proportional hazard modeling was used to investigate the effects of mean tacrolimus and CoV levels on survival and CLAD.

RESULTS: Tacrolimus CoV from 6 to 12 months was independently associated with both CLAD (hazard ratio [HR], 19.99; 95% CI, 7.55-52.91; $p < 0.001$) and death (HR, 14.57; 95% (confidence interval) CI, 6.08-34.90; $p < 0.001$). Conversely, the mean trough tacrolimus blood concentration between 6 to 12 months was not associated with an increased risk of CLAD (HR, 0.94; 95% CI, 0.84-1.06; $p = 0.34$) or death (HR, 0.91; 95% CI, 0.82-1.01; $p = 0.07$). In a multivariable model, erratic tacrolimus levels were associated with antifungal use (β 0.10 95% CI 0.54-1.51, $p < 0.001$) and younger age ($\hat{I}^2$ –0.0015, 95% CI –0.17 to –0.03, $p = 0.005$ per 5 years).

CONCLUSIONS: Erratic tacrolimus levels at 6 to 12 months post-lung transplant were associated with poor lung transplant outcomes. Future studies are required to determine whether interventions designed to optimize tacrolimus CoV could improve lung transplant outcomes.

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Background

Among lung transplant patients, chronic lung allograft dysfunction (CLAD) is a major complication leading to substantial morbidity and mortality, and its prevention is one of the primary focuses of post-transplant care.[1,2] CLAD is characterized by a permanent decline in forced expiratory volume in 1 second by 20% after transplantation.[3] The use of calcineurin inhibitor immunosuppressants has revolutionized the practice of solid organ transplantation, including lung transplantation; therefore, transplantation is an effective therapeutic option for a range of life-threatening respiratory diseases. Tacrolimus has recently become the preferred calcineurin inhibitor for preventing rejection.[4-10] However, despite very effective immunosuppressive medications in the

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current era, CLAD continues to be the primary cause of death in lung transplant patients.[11] According to the latest International Society for Heart and Lung Transplant registry report, the 5-year CLAD-free survival rate is only 47%.[12]

The recommended oral dosing interval for tacrolimus is 12 hours (for immediate-release tablets) or 24 hours (for extended-release tablets). The dose was adjusted to achieve the target therapeutic trough tacrolimus level, with mandated therapeutic drug monitoring of tacrolimus trough levels. Although the trough tacrolimus levels required to prevent rejection have been extensively studied, the role of erratic tacrolimus levels in lung transplant survival has been less studied. For kidney and liver transplant patients, previous studies have indicated a relationship between high tacrolimus intrapatient trough-level variability and late allograft failure.[13-15] We have previously published a small study outlining that high tacrolimus variability, as measured by standard deviation (SD), between 6 and 12 months postlung transplant, independently increased the risk of CLAD and death.[16] Interestingly, we were able to show in this study, tacrolimus variability in the first 6 months after lung transplant did not increase the risk of CLAD or death indicating the critical time period was 6 to 12 month post-transplant. The current study aimed to assess these findings using a larger cohort and measuring variability by coefficient of variance (CoV) rather than SD 6 to 12 months after lung transplant and to further identify factors associated with tacrolimus trough level variability.

Methods

This study was approved by the Human Research and Ethics Committee of Prince Charles Hospital. We retrospectively reviewed the medical records of patients who underwent lung transplantation at Prince Charles Hospital (TPCH) between 1996 and 2020. Patients were included if they received tacrolimus immunosuppression between 6 and 12 months post-transplant. Most patients (99%) were prescribed a twice-daily dose of tacrolimus. Since April 2011, TPCH has routinely used basiliximab as an induction therapy and tacrolimus as the preferred calcineurin inhibitor. Vaccination, antibiotic, and antifungal prophylaxis practices have not changed since lung transplant inception, with universal prophylaxis using nebulized amphotericin, voriconazole, or posaconazole. Corticosteroid prescription, cell cycle inhibitors (mycophenolate), and the timing of surveillance biopsies (weeks 3, 6, and 12 and months 6 and 12) also remained the same throughout the study period.

No changes in tacrolimus treatment were observed during the study period. The oral tacrolimus dose was administered (commencing dose of 0.15 mg/kg/day) in 2 divided doses, 1 hour before or 2 hours after food intake. Target trough levels were 10 to 15 μg/liter for the first 6 months, 8 to 10 μg/liter at 6 to 12 months, and 6 to 8 μg/liter from 12 months onward. A history of rejection, infection, kidney function, and other adverse events influenced individual target level (i.e., between 6 and 12 months, patients with severe kidney dysfunction had tacrolimus target around 8 μg/liter, patients with recurrent rejection had tacrolimus target around 10 μg/liter); however, indications for transplant did not. Oral pancreatic enzyme replacement therapy was administered to patients with cystic fibrosis and pancreatic insufficiency. Strict tacrolimus monitoring was routinely performed post-transplant. Minimum tacrolimus measurements were measured as follows: in weeks 1 to 2 post-transplant, monitoring was performed 3 times a week, twice weekly in weeks 3 to 6 followed by fortnightly in weeks 7 to 10 post-transplant. For patients with stable levels, further tacrolimus monitoring was performed monthly until 6 months and then 6 to 8 weekly from 6 months to 1 year post-transplant. From 1 year onward, routine monitoring was performed every 3 months. Blood concentrations were checked 3 to 4 days after tacrolimus dose adjustments. Tacrolimus trough levels were measured by 2 pathology providers using liquid chromatography-tandem mass spectrometry or immunoassay (Abbott Architect, EMIT 2000), with good reproducibility and correlation between methods.[17,18] As mentioned earlier, our previous study revealed that the critical time period for tacrolimus variability to predict poor outcomes was 6 to 12 months.[16] For this reason, tacrolimus trough-level variability between 6 and 12 months post-lung transplant was calculated and compared using the CoV. CoV is best described as a statistical measure of the dispersion of data points in a data series around the mean, indicating relative dispersion rather than absolute dispersion (defined by SD).[19] CoV corrects for the case where the standard deviation is numerically large (or small) because the mean is numerically large (or small), facilitating better comparison between disparate datasets and populations. CoV is now widely accepted as the preferred measure of intrapatient tacrolimus variability.[20] Patient demographics, disease indications for transplantation, and transplant type were collected. All patients were censored on December 1, 2021. Tacrolimus blood concentration trough levels were recorded between 6 and 12 months post-transplant. According to the International Society of Heart and Lung Transplant guidelines, CLAD is defined as at least a 20% fall from the baseline forced expiratory volume in 1 second post-transplant.[3] Data analysis was performed using Stata 17 software (StataCorp LP). Differences were considered statistically significant at a p-value of 0.05. The results are presented as mean and SD. The time from transplant to CLAD or death was modeled using Cox proportional hazard regression. Potential predictors of CLAD or death included demographic factors, such as sex, age at transplant, donor type (circulatory or brain-dead donors), transplant type, and indication for transplant, and clinical factors, including use of induction therapy, cytomegalovirus (CMV) status, and antifungal use. Finally, the mean tacrolimus CoV blood concentration trough levels were compared—6 to 12 months postlung transplant as a predictor of CLAD and death.

Results

Cohort characteristics

A total of 351 patients (49.9% male) who received a lung transplant at our center between 1997 and 2020 were included (Table 1). The average age of the patients was 45.8 years with a mean follow-up period of 5.9 years. The majority of patients had bilateral lung transplants (90.9%), with a lesser fraction having a single lung transplant (4.0%), heart and lung transplants (3.7%), or heart, liver, and lung transplants (1.4%). The main indication for transplant was cystic fibrosis (CF, 35%), followed by chronic obstructive pulmonary disease (COPD, 29.9%), interstitial lung disease (ILD, 23.6%), and other indications (11.4%). In the census, 29.3% of patients had CLAD (Table 1) and 33.9% died or

Table 1 Baseline Characteristics of the Study Participants and the CLAD-Free and CLAD Groups

Variables	Total Cohort (n = 351)	CLAD-free –248	CLAD –103	p-value
Male sex, n (%)	176 (49.9)	123 (49.6)	53 (51.5)	0.81
Transplant type				
Bilateral, n (%)	319 (90.9)	226 (91.1)	93 (90.3)	0.86
Single lung, n (%)	14 (4.0)	10 (4.0)	4 (3.9)	
Heart/lung, n (%)	13 (3.7)	8 (3.2)	5 (4.9)	
Heart/lung/liver, n (%)	5 (1.4)	4 (1.6)	1 (1.0)	
Transplant indication				
CF, n (%)	123 (35.0)	83 (33.5)	40 (38.8)	0.66
COPD, n (%)	105 (29.9)	76 (30.6)	29 (28.2)	
ILD, n (%)	83 (23.6)	62 (25.0)	21 (20.4)	
Other, n (%)	40 (11.4)	27 (10.9)	13 (12.6)	
Transplant age, years (SD)	45.81 (14.9)	47.44 (14.45)	41.9 (15.3)	0.001
CLAD at census, n (%)	103 (29.3)	0	103 (100)	
Death or re-Tx, n (%)	119 (33.9)	43 (1.7)	76 (73.8)	< 0.001
Time to CLAD, years (SD)	3.80 (2.53)	…	3.85 (2.52)	0.11
Time to death, years (SD)	5.84 (3.86)	4.71 (3.50)	6.48 (3.93)	0.015
Total follow-up time, years (SD)	5.90 (3.99)	5.52 (3.92)	6.79 (4.03)	0.006
CMV mismatch				
No, n (%)	272 (81.4)	197 (81.7)	75 (80.6)	0.82
Yes, n (%)	62 (18.6)	44 (18.3)	18 (19.4)	
6-12 months tacrolimus, CoV (SD)	0.33 (0.16)	0.31 (0.15)	0.39 (0.17)	< 0.001
Number tacrolimus values, n (SD)	14.46 (7.08)	14.4 (6.98)	14.62 (7.36)	0.79
6-12 months tacrolimus, mean (SD)	9.35 (1.77)	9.35 (1.56)	9.38 (2.21)	0.88

Abbreviations: CF, cystic fibrosis; CLAD, chronic lung allograft dysfunction; CMV, cytomegalovirus; COPD, chronic obstructive pulmonary disease; CoV, coefficient of variation; ILD, interstitial lung disease; Tx, Transplantation.

required a retransplant (Table 2, n = 9, 2.5%). The Median CLAD-free survival for the cohort was 9.7 years while the median overall survival was 9.65 years.

Tacrolimus mean and CoV trough levels

The mean CoV for tacrolimus trough-levels between 6 and 12 months postlung transplant was 0.33 (SD = 0.16) across the cohort, with an average of 14.46 (SD = 7.08) blood samples measured in the same time period. The overall mean tacrolimus trough level in the cohort was 9.36 (SD = 1.78). Those who developed CLAD (Table 1) or died (Table 2) had a higher tacrolimus-CoV (both $p < 0.001$), but there was no difference in the mean trough level or number of tacrolimus measurements in those who developed CLAD or died. To exclude an era effect, we calculated tacrolimus CoV for the pre-2011 (n = 85) and 2011 and later subgroups (n = 266) in our cohort. Both eras had the same mean CoV of 0.33 (p = 0.91).

Predictors of CLAD and death

Tacrolimus CoV from 6 to 12 months was independently associated with both CLAD (HR, 19.99; 95% CI, 7.55-52.91; $p < 0.001$) and death (HR, 14.57; 95% CI, 6.08-34.90; $p < 0.001$). The mean trough tacrolimus blood concentration between 6 and 12 months was not associated with increased CLAD (HR, 0.94; 95% CI, 0.84-1.06; p = 0.34) or death (HR, 0.91; 95% CI, 0.82-1.01; p = 0.07). No association was observed between the number of measurements or other clinical and demographic factors and the risk of CLAD or death. As no other factors were identified, only univariate models were presented.

Predictors of tacrolimus variability

We found that 2 factors were associated with erratic tacrolimus levels: antifungal use (β 1.02, 95% (confidence interval) CI 0.54-1.51, $p < 0.001$, Table 3) and younger age at transplant (β –0.10, 95% CI –0.17 to –0.03 p = 0.029 per 5 years, Table 3). While a univariable analysis showed that heart and lung transplant recipients and those transplanted for ILD were significant predictors of tacrolimus-CoV, when a multivariable analysis was performed, these 2 factors were no longer significant; thus, it can be concluded that these are not predictors of tacrolimus-CoV. Other factors that did not influence erratic tacrolimus levels included sex, transplant type, and CMV mismatch status (Table 3).

Discussion

Our findings from this retrospective single-center cohort study indicate that erratic tacrolimus levels, as measured by CoV, 6 to 12 months after lung transplantation, are independently associated with CLAD and mortality. These findings are broadly consistent with those of our previous study involving 110 patients.[16] However, in contrast to our previous study, the mean tacrolimus level was not associated with the risk of CLAD.[16] In

Table 2 Baseline Characteristics of the Total Study Participants and the Deceased and Nondeceased Participants

Variables	Not deceased −132	Deceased −119	*p*-value
Male sex, *n* (%)	117 (50.4)	58 (48.7%)	0.82
Transplant type			
Bilateral, *n* (%)	211 (90.9%)	108 (90.8%)	0.98
Single lung, *n* (%)	9 (3.9%)	5 (4.2%)	
Heart/lung, *n* (%)	9 (3.9%)	4 (3.4%)	
Heart/lung/liver, *n* (%)	4 (1.6%)	1 (1.0%)	
Transplant indication		47 (39.5%)	
CF	76 (32.8%)	29 (28.2%)	0.38
COPD	76 (30.6%)	22 (18.5%)	
ILD	61 (26.3%)	14 (11.8%)	
Other	26 (11.2%		
Transplant age, years (SD)	46.94 (14.25)	43.61 (15.9)	0.001
CLAD at census, *n* (%)	27 (11.6%)	76 (63.9%)	< 0.001
Time to CLAD years, mean (SD)	4.46 (3.05)	3.57 (2.3)	0.12
Time to death, years (SD)	…	5.84 (2.3)	
Total follow-up time, years (SD)	5.52 (3.92)	6.79 (4.03)	0.015
CMV mismatch, *n* (%)	36 (15.9%)	26 (24.3%)	0.06
6-12 months tacrolimus, CoV (SD)	0.30 (0.15)	0.39 (0.17)	< 0.001
Number tacrolimus values, *n* (SD)	14.17 (6.54)	15.03 (8.04)	0.28
6-12 months tacrolimus, mean (SD)	9.42 (1.5)	9.24 (2.24)	0.38

Abbreviations: CF, cystic fibrosis; CLAD, chronic lung allograft dysfunction; CMV, cytomegalovirus; COPD, chronic obstructive pulmonary disease; CoV, coefficient of variation; ILD, interstitial lung disease.

Table 3 Predictors of Tacrolimus CoV at 6 to 12 Months Postlung Transplant

Predictors of tacrolimus CoV	Beta coefficient value (CI)	*p*-value
Univariable		
Sex	0.026 (−0.0076 to 0.6)	0.129
Age at Tx (per 5 years)	−0.006 (−.012 to −0.0007)	0.029
Transplant type (compared to bilateral lung Tx)		
Sing lung Tx	−0.07 (−0.012 to 0.015)	0.108
Heart and lung Tx	−0.113 (−0.2 to −0.025)	0.012
Heart, lung, and liver Tx	−0.06 (−0.21 to 0.078)	0.378
Indication for Tx (compared to CF)		
COPD	0.015 (−0.057 to 0.026)	0.482
ILD	−0.062 (−0.107 to −0.018)	0.006
Other	−0.036 (−0.094 to 0.021)	0.02
Antifungal use	0.103 (0.058 to 0.148)	0.0001
CMV mismatch	< 0.001 (−0.04 to 0.04)	0.988

Abbreviations: CF, cystic fibrosis; CI, confidence interval; CMV, cytomegalovirus; COPD, chronic obstructive pulmonary disease; CoV, coefficient of variation; ILD, interstitial lung disease; Tx, Transplantation.

our cohort, 29.3% of patients had CLAD and 33.9% had died or required retransplantation. The median CLAD-free survival for the cohort was 9.7 years while the median overall survival was 9.65 years. These outcomes compare favorably to international data from the International Society for Heart and Lung Transplantation registry, with a median CLAD-free survival of 8.2 years (2000-2017) and an overall survival (2009-2016) of 6.5 years.[11]

CLAD remains the major cause of long-term morbidity and mortality postlung transplant; thus, a large part of post-transplant care is dedicated to avoiding its development.[3] Our findings suggest that the mean tacrolimus trough levels are not associated with CLAD development and that tacrolimus variability is highly associated with CLAD development. It is speculated that tacrolimus levels outside the therapeutic range increase the risk of cellular and humoral rejection, drug toxicity, and infection,[21] which in turn increases the risk of CLAD and death.

Previous studies in other solid organ transplant contexts have reported similar results. Five separate studies examined the relationship between intrapatient variability of tacrolimus concentrations at 6 to 12 months after kidney transplant and subsequent allograft outcomes, with all studies indicating that high intrapatient variability was associated with inferior outcomes.[13,22-25] Borra et al were the first to show that high tacrolimus variability is associated with graft failure in solid

kidney transplants, which was later confirmed by Shuker et al.[13,22] In liver transplant patients, the evidence is less convincing, with one study showing no difference between high and low tacrolimus variability and overall survival.[26] However, this study examined variability over a longer time period with fewer trough measurements than our study. Other liver transplant studies have shown that high tacrolimus variability is associated with an increase in acute rejection, poor graft survival, or dnDSA levels with varying degrees of certainty.[27-29] The literature on cardiothoracic organ transplants is even sparser. Gueta et al examined the relationship between tacrolimus levels 3 to 12 months postheart transplant and subsequent outcomes by dividing recipients into either high or low tacrolimus variability. Those with high variability had higher rates of late rejection (>1 year post-transplant).[30] One cross-sectional study assessed the time-in-therapeutic range and outcomes of adult lung allograft recipients. These investigators found that an increased time-in-therapeutic range was associated with a lower likelihood of rejection at 1 year while also reducing CLAD and mortality.[31]

We previously performed a small study investigating tacrolimus variability, as defined by the standard deviation, and found that SD was associated with an increased risk of developing CLAD and mortality. Importantly, we found that the critical period was between 6 and 12 months, with tacrolimus variability prior to this period playing no role in predicting poor outcomes. In the current study, we included a much larger cohort and focused on the CoV to predict intrapatient variability tacrolimus levels as CoV is more accepted in transplant patients. We found that erratic tacrolimus levels, as defined by the CoV of trough levels, were associated with both CLAD and death. Given the potential importance of this observation, we investigated the factors associated with erratic tacrolimus levels.

Our study identified antifungal use as an independent risk factor for higher tacrolimus CoV levels. It is well documented that tacrolimus and other calcineurin inhibitors are CYP3A4 substrates, and therefore, are frequently involved in drug-drug interactions, including antifungal medication.[32] Given this observation, it may be prudent to factor in this additional risk of prescription of antifungal medication when prescription is being considered. If antifungal medication is administered, to pay particular attention to tacrolimus dosing and the risk of worsening of CoV should be considered.

Another independent risk factor was younger age of the recipient at the time of transplantation. While a number of factors could contribute to this, it is possible that this association can be explained by medication nonadherence. Previous studies have identified that the biggest predictor of medication nonadherence is the age of the patient being transplanted. Two studies found that the age groups most at risk include children (which our study did not involve), adolescents, and the very elderly (with dementia playing a role).[33-35] We found that younger patients had the highest tacrolimus-CoV at the critical 6 to 12 month period suggesting that tacrolimus-CoV should be monitored particularly carefully in this group, with measures implemented to reduce medication nonadherence. Several interventions have been trialed to increase medication adherence, including pharmacist education, cognitive behavioral therapy (CBT), motivational interviewing, and the use of mobile apps.[36-38] While the use of mobile apps had variable results, particularly in the acute stages, one study found a significant reduction in tacrolimus variability 12 months postkidney transplant.[39] It can be inferred that younger transplant patients are accustomed to using mobile phone apps, and such technology is well-suited for this age group. CBT showed some encouraging results; thus, its effectiveness in at-risk groups should be studied further. Currently, the best intervention to reduce tacrolimus variability is the introduction of once-daily slow-release tacrolimus instead of immediate release, which requires multiple doses throughout the day.[40,41]

Alternative explanations for the variability in tacrolimus levels observed in our study include the complex tacrolimus pharmacokinetics with variability in both absorption and excretion, causing a wide variety in bioavailability. In a lung transplant cohort, CF patients have an increased risk of variable absorption due to their unique gastrointestinal pathology.[42]

As in many younger patients, the indication for transplant is CF, it is plausible to consider variable absorption as a reason for CoV, since variable drug absorption and pharmacokinetics are characteristic of CF. However, our findings could not be readily explained by CF pharmacokinetics, as a diagnosis of CF was not associated with increased tacrolimus CoV. Polymorphisms in the CYP3A5 gene, which is involved in tacrolimus metabolism, could also explain the differential tacrolimus CoV; however, these data are not available for our cohort. One study found that CYP3A5 polymorphism was a risk factor for acute rejection in kidney transplant recipients.[43] Another study identified several polymorphisms affecting tacrolimus metabolism in kidney transplant patients.[44]

Our study has several limitations. First, our data were extracted retrospectively without knowing the timing of blood draws. The complicated nature of tacrolimus absorption and excretion means that the timing of tacrolimus blood measurement could influence CoV. However, this reflects normal clinical practice and is not easily controlled. Indeed, a poorly timed blood draw may be a manifestation of reduced adherence. Second, the number of blood draws per patient varied between studies. However, our analysis showed that there was no difference in the number of tacrolimus measurements taken in patients who developed CLAD or died. Finally, as this study is retrospective, the study design does not provide insight into the possible mechanisms underlying the association between tacrolimus-CoV and the risk of CLAD and mortality. Despite these limitations, our study has several strengths. Although it is single-center, it includes a large number of transplant recipients who experience uniform post-transplant care. Furthermore, our cohort demographics were similar to those reported by the International Registry,[12] suggesting that our observations are likely to be generalizable to other jurisdictions and transplant programs.

In conclusion, patients with high variability in tacrolimus levels, as measured by tacrolimus CoV 6 to 12 months after lung transplantation, are at increased risk of CLAD and death. Our data suggest that measuring tacrolimus-CoV in lung transplant recipients at 12 months may identify those at higher risk for adverse outcomes, providing an opportunity to intervene to improve outcomes. Further prospective studies are required to test this idea.

CRediT authorship contribution statement

The Authors confirm the paper as follows: the study conception and design: Chandima Divithotawela, Daniel Chambers; Data collection: Samuel Walters, Trish Leisfield, Lesleigh Winks, Chandima Divithotawela, Stephanie Yerkovich; analysis and interpretation of results: Samuel Walters, Chandima Divithotawela, Stephanie Yerkovich, Daniel Chambers, Peter Hopkins; draft manuscript preparation: Samuel Walters. All authors reviewed the results, edited the manuscript as required and approved the final version of the manuscript.

Disclosure statement

The authors declare that they have no affiliations with or involvement in any organization or entity with any financial interest in the subject matter or materials discussed in this manuscript.

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