

Erratic Tacrolimus Exposure, Assessed using Standard Deviation of Trough Blood Levels, Predicts Chronic Lung Allograft Dysfunction and Survival

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ERRATIC TACROLIMUS EXPOSURE, ASSESSED USING STANDARD DEVIATION OF TROUGH BLOOD LEVELS,
PREDICTS CHRONIC LUNG ALLOGRAFT DYSFUNCTION AND SURVIVAL

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Abstract**ACCEPTED MANUSCRIPT**

Background: Erratic tacrolimus blood levels are associated with liver and kidney graft failure. We hypothesized that erratic tacrolimus exposure would similarly compromise lung transplant outcomes. This study assessed the effect of tacrolimus mean and standard deviation (SD) on the risk of chronic lung allograft dysfunction (CLAD) and death after lung transplantation.

Methods: We retrospectively reviewed all lung transplant recipients who received tacrolimus based immunosuppression (n=110). Cox proportional hazard modeling was used to investigate the effect of tacrolimus mean and SD on survival and CLAD. At census 48 (44%) patients had developed CLAD and 37 (34%) were deceased.

Results: Tacrolimus SD was highest for the first six post-transplant months (median (IQR) 4.01 (3.04-4.98)) before stabilising at 2.84 (2.16-4.13) between 6-12 months. The SD then remained the same (2.85 (2.00-3.77) between 12-24 months. A high mean tacrolimus level 6-12 months post-transplant independently reduced the risk of CLAD (HR 0.74 (0.63-0.86), $p < 0.001$) but not death (HR 0.96 (0.83-1.12), $p = 0.65$). In contrast, a high tacrolimus SD between 6-12 months independently increased the risk of both CLAD (HR 1.46 (1.23-1.73), $p < 0.001$) and death (HR 1.27 (1.08-1.51), $p = 0.005$).

Conclusions: Erratic tacrolimus levels are a risk factor for poor lung transplant outcomes. Identifying and modifying factors which contribute to this variability may significantly improve outcomes.

Chronic loss of allograft function is the major cause of morbidity and mortality after transplantation and its prevention rightly remains one of the main focuses of post-transplant care. In the lung, this process is now termed chronic lung allograft dysfunction (CLAD) and is characterised by a permanent 20% decline in FEV₁ over the best achieved post-transplant. CLAD is associated with reduced quality of life and increased mortality following lung transplantation (1-6). Acute rejection remains the most important risk factor (7).

The currently available immunosuppressive agents (typically a corticosteroid, a cell cycle inhibitor and a calcineurin inhibitor) are generally very effective at preventing the development of acute rejection if therapeutic drug levels can be reached (6, 8-10). Of these agents, cyclosporine led to the biggest step-change in the success of solid organ transplantation, with more recent evidence supporting a slight advantage for tacrolimus over cyclosporine in lung transplantation (11-16).

Therapeutic drug monitoring of tacrolimus trough levels is essential to achieve adequate immunosuppression while minimising toxicity. While the mean drug level required to prevent rejection has been well studied, little attention has been paid to the effect of erratic tacrolimus exposure, but with a satisfactory mean, in lung transplantation (15, 17-22). There are good reasons to believe that erratic exposure may be just as deleterious as sub-therapeutic tacrolimus exposure, but this possibility has been little explored. High tacrolimus trough level variability (assessed using standard deviation (SD)) increases the risk of acute rejection early post adult lung transplant but its affect on CLAD and survival are unknown (23). In the liver and kidney transplant literature, an association has been found between high intra-patient tacrolimus level variability and late graft failure (24-26). This has not been assessed in adult lung transplant recipients. We aimed to assess the effect of mean tacrolimus trough level as well as variation in tacrolimus trough levels, assessed using SD, on the development of CLAD and survival following adult lung transplantation. We also aimed to identify factors which influence tacrolimus trough level variability.

A retrospective review was performed of the medical records of patients who underwent lung transplantation at our centre between 1996 and 2013 and had received tacrolimus for at least the first year post-transplant. Those patients prescribed cyclosporine after the first 6 months were excluded. Almost all (97%) patients were prescribed twice-daily tacrolimus. Our centre has been using basiliximab routinely for induction therapy and tacrolimus as the preferred calcineurin inhibitor since April 2011. Prior to this cyclosporine was the preferred calcineurin inhibitor with tacrolimus reserved for patients experiencing refractory acute rejection while receiving cyclosporine, or (in females) excessive hair growth. Azithromycin has been used for prevention of BOS since April 2009. The practice of vaccination, antibiotic and antifungal use did not alter. No changes in prescription of corticosteroids, cell cycle inhibitors or timing of surveillance biopsies (week 3, 6, 12 and months 6 and 12) were made during the study period. There were no changes in tacrolimus management over the study period. Tacrolimus oral dose was given (commencing dose of 0.15 mg/kg/day, target trough level 5-15 µg/l) in two divided doses, one hour before or two hours after food. Target trough levels were 5-15 µg/l (10-15µg/l for 0-6 months followed by 5-10µg/l from 6 months). Individual target ranges varied, taking into account history of rejection, infection and other side-effects, but not pre-transplant diagnosis. Tacrolimus dose adjustments for CF patients were made as for the non-CF cohort. Oral pancreatic enzyme replacement was provided to those CF patients with pancreatic insufficiency. Tacrolimus monitoring was performed 3 times/week for the first 2 weeks post transplant, then twice weekly for 4 weeks, weekly for 1 month and then fortnightly for 1 month. For patients with stable levels further blood draws were performed monthly up until 6 months and then 3 monthly thereafter. Levels were checked 3-4 days following tacrolimus dose adjustments and twice weekly for inpatients.

Collected data included patient demographics, date of transplant, time post-transplant or date of death, disease indication for transplant, type of transplant and tacrolimus blood trough levels. Acute rejection burden was assessed by summing the A grades in the first 12 months. CLAD was defined as a 20% fall from best post-transplant FEV1 as per the evolving International Society of Heart and Lung Transplant (ISHLT)

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guidelines (27). Respiratory function tests were performed at 2 weeks post transplant and then twice weekly for 4 weeks, weekly for 1 month, fortnightly for 1 month and then 3 monthly thereafter. Patients performed daily home spirometry and were instructed to present to clinic if they had a reduction of $\geq 10\%$. Tacrolimus trough levels were measured as part of routine post transplant care in both inpatients and outpatients by two pathology providers using liquid chromatography-tandem mass spectrometry (LC-MS) or immunoassay (Abbott ARCHITECT, EMIT 2000), with good correlation shown between the methods (28). The mean and SD tacrolimus trough level was determined for each subject in each of the 0-6 month, 6-12 month and 12-24 month post-transplant epochs. The study was approved by the Prince Charles Hospital Human Research and Ethics Committee.

Data was analysed using Stata v11 (StataCorp, TX, USA) and a p-value < 0.05 was considered statistically significant. The results are presented as median (interquartile range). The time from transplant to CLAD or death was modeled using Cox proportional hazard regression. Potential predictors included demographic factors (sex, age at transplant, donor type (cardiac or brain death), transplant type, indication for transplant), clinical factors (use of induction therapy, sum of A grades, PGD grades) and tacrolimus mean trough levels and tacrolimus SD over the time periods as stated. The final model was obtained by forwards and backwards selection, retaining covariates where inclusion or exclusion changed the coefficients of other predictors by more than 10% or where predictors were statistically significant at $\alpha = 0.05$. Simple and multivariate linear regressions were performed with tacrolimus SD as the dependent variable. Simple linear regression analysis was initially used to evaluate the relationship between variables and SD and those variables in which $p < 0.1$ were then subjected to multiple linear regression analysis.

Cohort Characteristics

110 subjects, median age 41.3 (IQR 27.6 – 51.6) years were included. Table 1 displays the baseline characteristics. 56% of subjects were female, 87% had bilateral transplants, and the indications for transplant were cystic fibrosis (CF, 47%), chronic obstructive pulmonary disease (COPD, 26%), pulmonary fibrosis (8%) and other (21%). Median follow up was 60 (30.6-95.7) months. At census 37 patients (34%) were deceased and 48 (44%) had developed CLAD.

Tacrolimus Mean and SD

Tacrolimus SD was 4.01 (3.04 – 4.98), 2.84 (2.16 – 4.13) and 2.85 (2.00-3.77) for the 0-6, 6-12 and 12-24 month epochs, respectively (Figure 1). Since the tacrolimus SD was similar for the 6-12 and 12-24 month epochs ($p=0.73$), we chose to use the 6-12 month epoch for all further analyses. During this time, there were 33 patients who had tacrolimus levels measured while an inpatient, however there was no difference in the standard deviation of inpatient versus outpatient tacrolimus levels (data not shown). The mean tacrolimus level for the 6-12 month epoch was 9.8 (8.56 – 10.75) $\mu\text{g/L}$ and showed a moderate correlation with SD over the same period ($\rho=0.360$, $p<0.001$, Figure 2). There was no relationship found between the sum of A grade rejection (1 month, 6 month or 12 month cumulative) and either the 0-6 month or 6-12 month SD. As there was a long study period, we analysed the mean and standard deviation between those transplanted before and including 2005 ($n=48$) and those transplanted from 2006 onwards ($n=62$). We found no difference between either the tacrolimus mean or standard deviation between the two groups (data not shown).

The median number of tacrolimus measurements per patient for the 0-6, 6-12 and 12-24 month periods were 15 (IQR 8 – 24), 11 (7-17, range 2-30 measurements) and 13 (7-20) respectively. There was a positive correlation between the number of tacrolimus measurements and trough level SD ($r = 0.489$ $p<0.001$, Figure 3) but not with the mean ($p=0.698$).

Neither the 6-12mth mean tacrolimus level nor the standard deviation were associated with demographic factors, the sum of A grade rejection, transplant type or disease indication (including a diagnosis of cystic fibrosis, Supplementary Table 1).

Predictors of CLAD and death

No demographic factors were associated with CLAD or death, however a history of acute rejection increased the risk of both CLAD and death (Table 2 and 3). Mean tacrolimus levels from 6-12 months were negatively associated with CLAD on both univariate (Hazard Ratio (HR) 0.85 (95% Confidence Interval (CI) 0.74 – 0.99), $P=0.032$) and multivariate analysis (HR 0.74 (CI 0.63 – 0.86), $P<0.001$, Table 2), but not with survival (Table 3). Tacrolimus SD from 0-6 months was not a predictor of CLAD (HR=1.07 CI 0.96-1.21 $p=0.227$), but tacrolimus SD from 6-12 months was independently associated with time to CLAD (HR 1.46, CI 1.23 – 1.73, $P<0.001$, Table 2) and death (HR 1.27, CI 1.08 – 1.51, $P=0.005$, Table 3). For each 1 unit increase in the tacrolimus standard deviation, the risk of CLAD and death was increased by 46% and 27% respectively. In order to exclude the possibility that inpatients, who may be sicker and also have more tacrolimus blood draws perhaps thus affecting the SD, may confound our findings, we repeated the analysis after excluding tacrolimus levels taken from inpatients. Even after excluding these levels, tacrolimus SD from 6-12 months remained a strong independent predictor of time to CLAD (HR 1.57, CI 1.29 – 1.93, $P<0.001$) and death (HR 1.36, CI 1.09 – 1.70, $P=0.006$).

In this retrospective, single-centre cohort study we found that both lower mean and higher standard deviation of tacrolimus levels, assessed between 6 and 12 months post-transplant, independently predicted time to the development of CLAD, with the standard deviation also predicting mortality.

Identifying those patients most at risk of developing chronic rejection and premature death post transplant is one of the key challenges facing treating physicians. This study is important as it is the first to assess the effect of tacrolimus trough level variability on the incidence of CLAD or survival in the setting of adult lung transplantation. While much attention is paid to the attainment of satisfactory tacrolimus levels after transplant, little attention is paid to the variability of those levels. We postulate that patients with fluctuating tacrolimus trough levels are more likely to have levels outside of the therapeutic range predisposing them to immune mediated rejection, toxicity (including nephrotoxicity) and infection. These findings are clinically relevant since tacrolimus SD is simple to calculate and predicts subsequent graft loss and mortality, providing an opportunity for this new knowledge to rapidly translate into better graft and patient outcomes in a highly cost-effective manner. Our results suggest that at the end of the first post-transplant year, assessing the tacrolimus SD may identify patients with erratic levels who are at high risk for subsequent CLAD and/or death.

The first 6 months post transplant is often complicated by more frequent use of high dose steroids (for acute rejection), triazole antifungal prophylaxis and gastrointestinal factors (including gastroparesis) which may affect absorption or metabolism of tacrolimus. These difficulties are reflected in the high tacrolimus level SD during this period. However our data suggest that the erratic tacrolimus exposure during this period does not impact on long term outcomes. In this respect, the 6-12 month period was found to be particularly important as a high tacrolimus SD during this period both predicted a persistently high SD in the second post-transplant year and poor outcome. Collectively our data suggest that there are a subgroup of individuals who have persistently high variability in tacrolimus levels across the life of their transplant and that these individuals could be readily identified before their outcome is compromised.

Our work expands on previous studies that have looked for an association between tacrolimus level SD and acute rejection in adult lung transplant recipients as well as chronic rejection and graft failure in other solid organ transplants. We found no association between acute rejection and tacrolimus SD. Chiang *et al*, on the other hand, found a 23% increase risk of acute rejection for each 1 unit rise in tacrolimus trough level SD in the first 8-90 days post transplantation (23). In adult kidney transplant recipients, high within patient variability in tacrolimus clearance (estimated by correcting the blood trough level for the oral dose) in the 6-12 month period was found to increase the risk of graft failure and rejection after 12 months (24). Further, Pollock-BarZiv's group assessed tacrolimus level variability using SD in paediatric solid organ (including 15 lung) transplant recipients finding a threshold of 2 SD, above which there was increased risk of late rejection and graft loss (26). Venkat *et al* also found a 2 SD threshold above which there was an increased risk of late rejection in their paediatric liver transplant cohort (29). We chose to assess our standard deviation data as a continuous variable and found that for each one unit increase in SD, the time to CLAD or death increased by 46% and 27% respectively, implying that even small increases in SD can adversely affect outcome.

There are a multitude of factors which may cause highly variable tacrolimus trough levels beyond the early post-transplant period. For a given patient, the oral bioavailability of tacrolimus can vary greatly from dose to dose (30). One particular group of interest are those with CF who have a unique gastrointestinal pathology with significant risk of variability in absorption. Chiang *et al* proposed that tacrolimus absorption may affect tacrolimus level SD in those with CF. In their cohort of 99 lung transplant recipients (<3 months post transplant), those that developed acute rejection had higher pre-rejection tacrolimus level SD and were made up of a higher proportion of patients with CF (23). However, our findings did not reflect this, with no association between indication for transplant, or indeed any demographic factor, and tacrolimus variability, CLAD or survival. Given that poor adherence to post-transplant care is well known to compromise outcomes, we believe it is likely that poor adherence to the prescribed tacrolimus dosing schedule will explain at least some of the variability in tacrolimus exposure in our cohort. Non-adherence to

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the dose, the dosing interval, the requirement to take tacrolimus without food, or the timing of blood draws could all contribute to increased tacrolimus SD. In this respect, tacrolimus SD may be an excellent surrogate not only for tacrolimus exposure, but also the ability and/or willingness to follow relatively complicated medical advice. Although adherence is generally high in lung transplant recipients, poor adherence has been shown to be associated with a greater tacrolimus level SD in liver transplant recipients (25, 31). The relationships between tacrolimus SD, more conventional measures of adherence, and transplant outcomes will be a key focus of future studies.

Pharmacogenomic factors may play a role in determining tacrolimus level mean but little consideration has been paid to its effect on intra-patient variability. Previous studies have found that gene polymorphisms, particularly in CYP3A5, affect tacrolimus concentration-dose relationships, so predicting initial dose requirements in lung transplant recipients (32-34). In kidney transplant recipients, a polymorphism of CYP3A5 was found to be a risk factor for acute rejection and nephrotoxicity (35). Single nucleotide polymorphism analysis of 240 Chinese kidney transplant recipients has revealed a number of other polymorphisms affecting tacrolimus metabolism (36). Another future direction in lung transplantation may involve SNP analysis to identify patients more susceptible to variable tacrolimus levels or low mean, possibly improving preoperative risk stratification and outcomes.

Variable or reduced exposure to other medications may provide an alternate explanation for the association between tacrolimus SD, CLAD and survival. Other medications (including concurrently prescribed immunosuppressants) are often affected by similar factors including adherence, absorption and metabolism. While it is possible, indeed likely, that high tacrolimus SD acts as a surrogate for variable exposure to other medication, most of the other commonly prescribed drugs have a wider therapeutic window so that variability in drug exposure may be expected to have less of an impact on long-term outcome. Indeed in the study by Borra *et al* there was no association between variability in mycophenolate clearance and graft failure (24).

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We believe it is likely that our findings will be able to be generalized to other lung transplant cohorts since our cohort's characteristics are similar to those in most transplant centres, except for a slightly higher proportion of patients transplanted for CF. However we do acknowledge some limitations. Given the retrospective design of the study, the timing of tacrolimus levels post dose may have varied, thus potentially affecting both SD and mean. However, the 'real-world' nature of a retrospective study will capture this kind of variability, which in itself may be an important measure of the patient's ability to ensure accurate timing of blood draws, and to follow other relatively complicated, but vital, components of post-transplant care. Although there were some changes in transplant protocols during the 17-year study period (specifically the introduction of basiliximab induction and azithromycin prophylaxis) these agents are not known to significantly alter tacrolimus metabolism (37). Furthermore we saw no era effect when we specifically tested the possibility that historical changes in transplant practice may systematically alter tacrolimus SD or mean. The correlation between number of tacrolimus measurements and trough level SD is an uncontrolled potential confounder. It is possible that more frequent assessment of levels in sicker patients resulted in more frequent dose adjustment with increased variability. However when inpatients (often sicker, having more frequent levels) were removed from the analysis, the SD and mean were not substantially different, and tacrolimus SD still predicted CLAD and death. In fact the relationship between medical monitoring and scheduling of blood draws on adherence to the prescribed tacrolimus schedule is likely complex and potentially unpredictable. For instance it is possible that less adherent, sicker patients may take medications reliably only immediately prior to blood draws thus increasing variability. Conversely one could envisage a different outcome, with adherence and variability improving with more frequent levels as individuals improve behaviour in response to their awareness of being observed (the Hawthorne Effect). However, perhaps the most obvious mode of confounding – that a non-adherent patient at risk of poor outcome may undergo fewer blood tests and therefore have a higher SD - can be excluded since tacrolimus SD was a positive function of the number of observations. In summary, although we believe the observed association between high tacrolimus SD and poor outcome is robust, our study does not provide insight into potential mechanisms.

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In conclusion, patients with highly variable trough tacrolimus levels in the second half of the first post-transplant year will likely have similar variability in the second year and are at high risk for subsequent CLAD and death. Calculating the tacrolimus SD, identifying those with a high SD, and modifying factors contributing to it would seem pertinent objectives at the one year post-transplant anniversary. Specifically targeting these individuals to identify and correct any factors which may be contributing to erratic levels may significantly improve their outcome and survival.

Disclosure

The authors of this manuscript have no conflicts of interest to disclose.

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Figure 1: Standard deviation of tacrolimus trough levels at various times post transplantation. Data are presented as box and whiskers plot

Figure 1

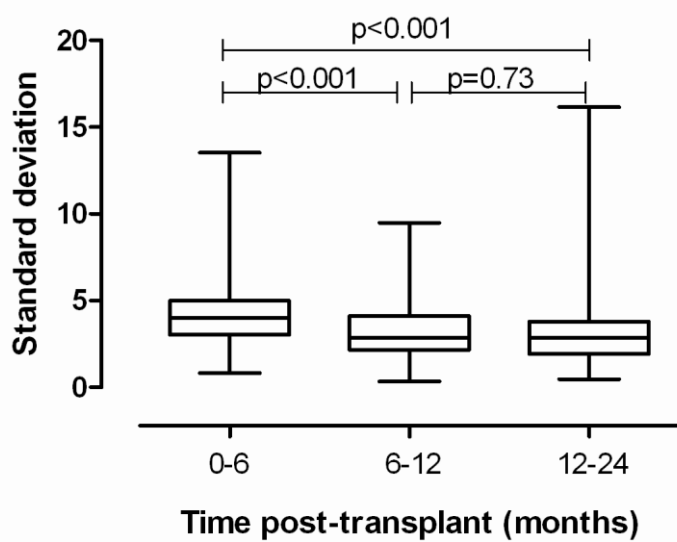


Figure 2: Standard deviation versus mean tacrolimus level 6-12 months post transplant.

$r=0.36$, $p<0.001$

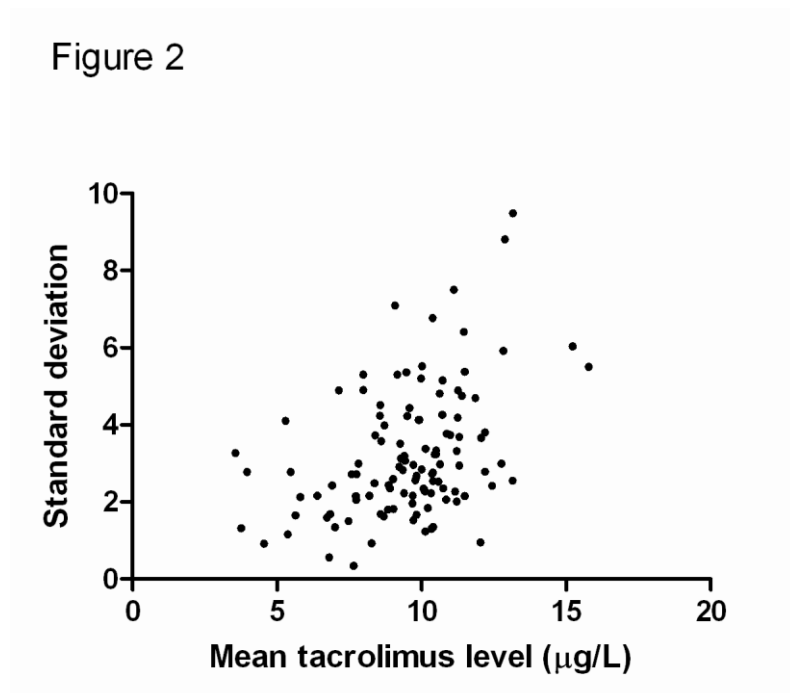


Figure 3: Standard deviation versus number of tacrolimus measurements from 6-12 months post

transplant. $r=0.49$, $p<0.001$

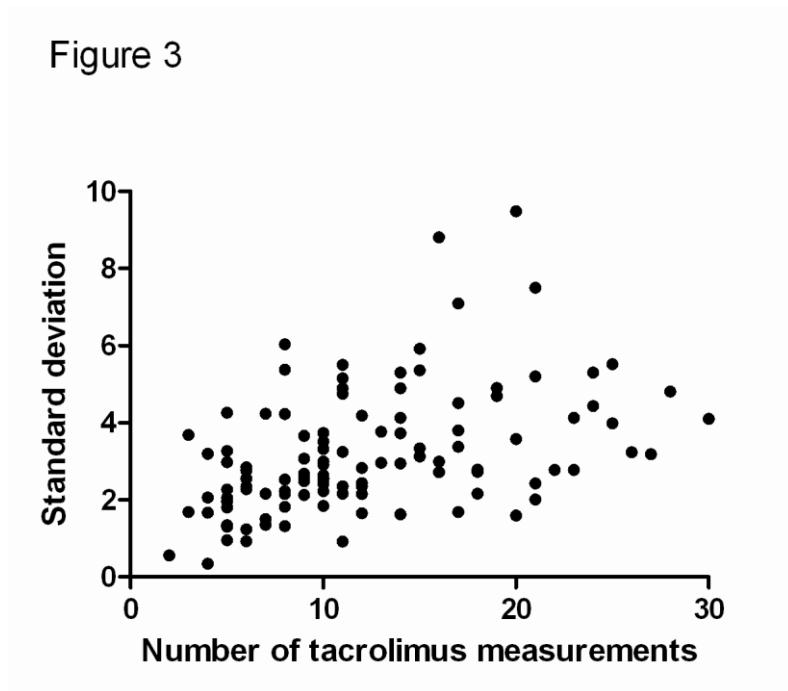


Table 1 Cohort characteristics

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Total cohort	Total cohort (n=110)	CLAD-free (n=62)	CLAD (n=48)	P value
Female sex, n (%)	62 (56%)	37 (60)	25 (52)	0.445
Tx type, n (%)				
Bilateral Sequential Lung Tx	96 (87%)	56 (90)	40 (84)	0.164
Single Lung Tx	3 (3%)	0 (0)	3 (6)	
Heart/Lung Tx	7 (6%)	3 (5)	4 (8)	
Heart/Lung/Liver Tx	4 (4%)	3 (5)	1 (2)	
Tx indication, n (%)				
Cystic fibrosis	52 (47%)	32 (51)	20 (42)	0.529
COPD	28 (26%)	13 (21)	15 (31)	
Idiopathic pulmonary fibrosis	9 (8%)	6 (10)	3 (6)	
Other	21 (19%)	11 (18)	10 (21)	
Tx age, median (IQR) years	41.3 (27.6 – 51.6)	38.9 (27.5-20.5)	44.0 (29.2-52.4)	0.816
CLAD at census, n (%)	48 (44)	0 (0)	48 (100)	
Deceased, n (%)	37 (34)	5 (8)	32 (67)	<0.001
Time to CLAD, median (IQR) months	35.0 (21.3 – 69.4)		35.0 (21.3 – 69.4)	
Time to death, median (IQR) months	54.5 (31.8 – 90.8)	20.3 (18.6-41.6)	59.7 (40.2-95.1)	0.037
Total follow-up time, median (IQR) months	60 (30.6 – 95.7)	45.0 (26.2-79.8)	70.3 (41.9-112.5)	0.004

Table 2: Factors associated with time to CLAD following transplantation

	HR (95% CI)	P value
<i>Univariate</i>		
Mean 6-12mth	0.85 (0.74 – 0.99)	0.032
SD 6-12mth	1.25 (1.08 – 1.45)	0.003
Acute rejection grade (cumulative 0-12mths)	1.14 (1.02 – 1.29)	0.026
<i>Multivariate</i>		
Mean 6-12mth	0.74 (0.63 – 0.86)	<0.001
SD 6-12mth	1.46 (1.23 – 1.73)	<0.001
Acute rejection grade (cumulative 0-12mths)	1.13 (1.00 – 1.28)	0.058

Table 3: Factors associated with time to death following transplantation

	HR (95% CI)	P value
<i>Univariate</i>		
SD 6-12mth	1.29 (1.09 – 1.53)	0.002
Acute rejection grade (cumulative 0-12mths)	1.25 (1.09 – 1.43)	0.001
<i>Multivariate</i>		
SD 6-12mth	1.27 (1.08 – 1.51)	0.005
Acute rejection grade (cumulative 0-12mths)	1.24 (1.08 – 1.43)	0.002