Original Article

Low early posttransplant serum tacrolimus levels are associated with poor patient survival in lung transplant patients

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Abstract:

BACKGROUND: Low-dose tacrolimus-based immunosuppression is a standard therapy in kidney and liver transplantation; however, the optimal therapeutic level of tacrolimus has not been established in lung transplantation. We aimed to identify the tacrolimus level associated with better outcomes in lung transplant patients.

METHODS: This retrospective study included patients who underwent lung transplantation at Seoul National University Hospital between 2006 and 2016. Kaplan–Meier survival analysis and Cox regression were performed according to tacrolimus levels at several time-points within 1-year posttransplantation.

RESULTS: A total of 43 patients received bilateral lung transplantation. The median age was 53 years and the median follow-up was 20.5 months. Overall and 1-year patient survival rates were 55.8% and 74.4%, respectively. Infection was the most common cause of death (78.9%). Chronic lung allograft dysfunction was observed in 16.3%. A tacrolimus level <9 ng/ml at 1 month was associated with lower rejection-free survival ($P = 0.009$). A time-averaged tacrolimus level <10 ng/ml within 1 month posttransplantation was an independent risk factor for poor patient survival (hazard ratio: 4.904; 95% confidence interval: 1.930–12.459; $P = 0.001$). Furthermore, higher tacrolimus levels did not increase infectious complications.

CONCLUSIONS: These finding suggest that tacrolimus levels ≥10 ng/ml within 1 month after lung transplantation appear to be associated with better patient survival.

Keywords:

Chronic lung allograft dysfunction, lung transplantation, rejection, survival, tacrolimus

Lung transplantation is the life-saving treatment option for end-stage lung disease.[1-3] The annual number of lung transplantations has markedly increased, as this has emerged as the standard care for advanced lung diseases; however, long-term outcomes following lung transplantation remain poor. Lung transplantation has the lowest 10-year survival rate (27%) compared to other solid organ transplantations, such as the liver (70%), kidney (58%), heart (56%), and intestine (44%).[4] The most important obstacle to long-term survival following lung transplantation is chronic lung allograft dysfunction (CLAD), which is associated with bronchiolitis obliterans syndrome (BOS) and infection. Infection is the primary cause of death within 1 year after transplantation, whereas BOS and related graft failures are the most common causes of death >1 year after transplantation.[3] Because immunosuppressive therapy is a main therapeutic agent in posttransplantation

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management, fine tuning of the immunosuppressive regimen is important for minimizing infection and maintaining graft function.

A calcineurin inhibitor (CNI)-based triple immunosuppressive regimen is the standard maintenance immunosuppressive therapy following lung transplantation.[5,6] A recent systematic review revealed that tacrolimus-based immunosuppressive therapy is superior to cyclosporine-based therapy with respect to the prevalence of obstructive CLAD, lymphocytic bronchitis, therapeutic withdrawal, and arterial hypertension following lung transplantation. Tacrolimus, therefore, is the primary CNI-based treatment for lung transplant patients.[7] Tacrolimus reduces acute allograft rejection and graft loss, but is associated with serious side effects, including nephrotoxicity, hyperlipidemia, hypertension, and development of diabetes.[8,9] Several trials have been conducted to reduce tacrolimus levels following solid organ transplantation.[10-12] Although low-dose tacrolimus-based immunosuppression is the standard of care for kidney and liver transplantation,[13,14] the optimal therapeutic level of tacrolimus has not been established in lung transplantation. Many lung transplantation centers proposed the target level of tacrolimus kept in 10–20 ng/mL within 2 weeks and 10–15 ng/mL thereafter[15,16] or 12–15 ng/mL within 1 year after posttransplant.[17] However, these recommendations were not based on concrete evidences or references to validate these target level in lung transplantation. Here, we aimed to identify the level of tacrolimus associated with better outcomes in lung transplant patients.

Methods

Study population and treatment protocols

A total of 48 patients underwent lung transplantation at Seoul National University Hospital between January 2006 and December 2016. All lungs were obtained from deceased donors. Patients who underwent bilateral lung transplantation and received tacrolimus-based immunosuppressive regimens were included in this study; patients who received a unilateral lung transplant or heart-lung transplant were excluded from the study. All recipients of a bilateral lung transplantation received extracorporeal membrane oxygenation (n = 15) or cardiopulmonary bypass (n = 28) during the transplant surgery. Basiliximab was used as induction therapy in most patients (n = 41); however, one patient received anti-thymoglobulin and another patient received no induction therapy due to severe leukopenia. All patients received triple-maintenance immunosuppressive therapy, which included prednisolone, tacrolimus, and mycophenolate mofetil or its equivalents. Daily tacrolimus dose was adjusted according to trough level. Mycophenolate mofetil was started at the time of available oral diet with 500 mg every 12 h/day and adjusted according to leukocyte count. After initial intravenous administration of methylprednisolone (500 mg on day 0, 250 mg on day 1, and 125 mg on day 2), oral corticosteroid dose was tapered according to the following scheduled; 30 mg on day 15, 15 mg on day 29, 10 mg on day 36, and 5 mg on day 50. Valganciclovir and itraconazole were used for the prevention of cytomegalovirus (CMV) and fungus, respectively. Trimethoprim/sulfamethoxazole was used for prophylaxis against pneumocystis jiroveci. We did not perform routine protocol biopsy after lung transplantation. The surveillance of graft function was done mainly through the office spirometry every 1–2 months during the 1st year and thereafter every 3–6 months. Bronchoscopy is performed every 1–2 years during the first 5 years. BAL was performed when the allograft showed decreased function at spirometry or need to be differentiated between rejection and infection. This study was approved by the Institutional Review Board.

Data collection and definition of outcomes

Data were obtained from the electronic medical records of the hospital and reviewed, retrospectively. Demographic characteristics, medical history, allograft rejection, mortality, and posttransplant complications were evaluated. Weekly tacrolimus levels were collected until 1 month after transplantation, and monthly levels were collected thereafter until 1 year after transplantation. Diagnosis of acute rejection was made by for-cause transbronchial biopsy or percutaneous needle biopsy or by physicians' clinical decision when the patient' lung function is abruptly aggravated, is not improved by antibiotic treatment, and the bronchoscopic biopsy is not available. CLAD was defined as a sustained decrease of at least 20% in the first second of forced expiratory volume or forced vital capacity from the posttransplant baseline, which was the average of the two best posttransplant measures taken at least 3 weeks apart. Deterioration of allograft function by infectious causes was excluded by pathogen culture for bronchoalveolar lavage. Time to development of acute rejection, CLAD, and mortality were measured as outcomes. Acute rejection-free survival, CLAD-free survival, and overall mortality were analyzed according to serum tacrolimus levels after lung transplantation. Posttransplant complications such as infection (nonCMV and CMV), new-onset diabetes, and malignancy were reviewed.

Statistical analysis

Recipients were categorized according to their serum tacrolimus levels at each time point. Data were expressed in terms of median and interquartile range. Continuous variables were compared using the Mann–Whitney U-test. The Wilcoxon rank test was used to compare paired values between two groups. Pearson's

Chi-squared test was used to compare categorical variables. Patient survival and other complication-free survival according to the serum tacrolimus level were estimated using the Kaplan-Meier survival analysis and compared using the log-rank test. Multivariate stepwise Cox regression analysis was performed to define independent risk factors for each outcome. All analyses were performed using SPSS software, version 24 (IBM, Armonk, NY, USA). A two-tailed $P < 0.05$ was considered statistically significant.

Results

Patient characteristics

A total of 43 patients who received bilateral lung transplantation were included in this analysis. The baseline characteristics of these patients are summarized in Table 1. The median age of patients was 53 years. The median follow-up duration was 616 days (range, 357–1476 days). The most common indication for lung transplantation was idiopathic pulmonary fibrosis (32.5%). The mean serum tacrolimus level was 10.9 ng/ml within 1 month of transplantation, and progressively decreased to 7.6 ng/ml at 1 year posttransplant [Figure 1].

Outcomes

Acute rejection occurred in 20 patients (46.5%), all of which received steroid pulse therapy (500 mg/day for 3 days). CLAD was reported in 6 patients (14.0%), new-onset diabetes after transplantation developed in 12 patients (27.9%), new-onset hypertension occurred in 5 patients (11.6%), posttransplant acute renal dysfunction was observed in 2 patients (4.7%), malignancy was observed in 6 patients (14.0%), and posttransplant lymphoproliferative disease were in 3 patients (7.0%).

A total of 19 patients died during the follow-up period (44.1%). Median survival was 242 (58–659) days. The overall 1-, 2-, 3-, and 5-year survival in our study was 74.4%, 55.8%, 53.8%, and 41.6%, respectively. The causes of death included infection (n = 9, 47.4%), malignancy (n = 4, 21.1%), CLAD (n = 2, 10.5%), postsurgery complication (n = 2, 10.5%), and other causes (n = 2, 10.5%), which included asphyxia and multi-organ failure due to thrombotic microangiopathy. All fatal infections were identified as non-CMV infections. Malignancies included posttransplant lymphoproliferative disease (n = 2), lung cancer (n = 1), and leukemia (n = 1).

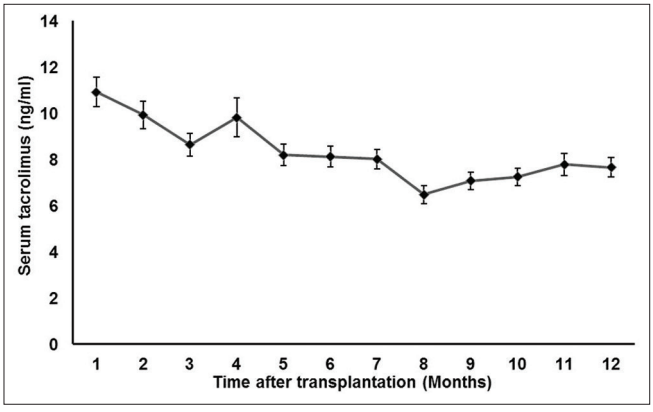

Figure 1: Serum tacrolimus concentration within 1 year after lung transplantation. Each monthly value was displayed as mean ± standard error of mean

Impact of 1-year tacrolimus levels on each outcome

Patients were categorized into two groups according to their monthly serum tacrolimus level at each time point or time-averaged concentration during a specific period following transplantation. A tacrolimus level <9 ng/ml at 1-month posttransplant was associated

Table 1: Baseline characteristics of study population (n=43)

Variables	n=43
Male gender, *n* (%)	28 (65.1)
Age, years	53 (45-60)
Follow-up duration (days)	616 (357-1476)
BMI (kg/m^2)	19.4±3.4
Diabetes, *n* (%)	5 (11.6)
Hypertension, *n* (%)	3 (7.0)
Cardiovascular disease, *n* (%)	2 (4.7)
Chronic kidney disease, *n* (%)	3 (7.0)
Causes of lung transplant, *n* (%)	
COPD	2 (4.7)
IPF	14 (32.5)
ILD related with connective disease or LAM	12 (27.9)
TB destroyed lung	2 (4.7)
BO after SCT	4 (9.2)
Primary pulmonary hypertension	1 (2.3)
Lung cancer	2 (4.7)
Others	6 (14.0)
Laboratory data	
Hemoglobin (g/dL)	11.1 (9.6-12.0)
Blood urea nitrogen (mg/dL)	15.0 (10.0-19.3)
Creatinine (mg/dL)	0.64 (0.52-0.84)
AST (IU/L)	23.0 (17.8-29.3)
ALT (IU/L)	22.0 (15.0-29.3)
PRA, *n* (%)	
Negative	31 (73.8)
MFI <1000	5 (11.9)
1000< MFI <3000	2 (4.8)
MFI >3000	4 (9.5)
DSA, *n* (%)	3 (7.0)
PFT, percentage to predicted value	
FEV_1	41 (29-68)
FVC	44 (34-71)

Data is represented as median (IQR) or *n* (%). BMI=Body mass index, COPD=Chronic obstructive pulmonary disease, IPF=Idiopathic pulmonary fibrosis, ILD=Interstitial lung disease, LAM=Lymphangioleiomyomatosis, TB=Tuberculosis, BO=Bronchiolitis obliterans, SCT=Stem cell transplantation, AST=Aspartate transaminase, ALT=Alanine transaminase, PRA=Panel reactive assay, MFI=Mean fluorescence intensity, DSA=Donor specific antibody, PFT=Pulmonary function test, FEV_1=Forced expiratory volume in 1 s, FVC=Forced vital capacity, IQR=Interquartile range

with lower acute rejection-free survival [log-rank test, P = 0.009, Figure 2a]. Interestingly, time-averaged tacrolimus levels <10 ng/ml within 1 month after transplantation were associated with poor patient survival [log-rank test, P = 0.022, Figure 2b]. Male gender distribution was not different between two groups (73.3% in lower tacrolimus groups vs. 60.7% in high tacrolimus group). Median age was 51 (36.3–58.8) years and 57 (46–63) years, respectively. The ranges in doses of mycophenolate mofetil were similar between two groups as the dose of 250–750 mg q12 h. Median corticosteroid dose was 12.5[10-15] mg in the lower-tacrolimus group and 10[10-15] mg in the higher-tacrolimus group without significant difference (P = 0.399). There were no differences in disease-free survival between various tacrolimus level groups for other complications, such as infection, malignancy, or new-onset diabetes [Table 2]. Cox regression analysis revealed that a time-averaged tacrolimus level <10 ng/ml was also an independent risk factor for poor patient survival [hazard ratio: 4.094; 95% confidence interval: 1.930–12.459; P = 0.001; Table 3]. The median age and gender distribution were similar between the two groups (data not shown).

Discussion

This study investigated the impact of early tacrolimus levels on clinical outcomes in lung transplant recipients. We found that a tacrolimus level ≥10 ng/ml within 1 month posttransplant was associated with higher patient survival. This range of tacrolimus levels was not associated with an increased risk of immunosuppression-related complications such as infection and malignancy. The findings from this study therefore provide evidence that maintenance of tacrolimus levels >9 ng/ml at 1 month posttransplant may prevent acute rejection.

Although the optimal tacrolimus levels in kidney transplantation have been studied, the results remain controversial. Over the past several decades, several studies have reported that low-dose tacrolimus maintenance regimens are associated with good clinical outcomes in kidney transplant patients.[10-12] Other studies, however, have warned of an increased risk of rejection among kidney transplant patients with low tacrolimus levels.[18-21] A recent systematic review has suggested that CNI withdrawal or avoidance is associated with an increase in acute rejection following kidney transplantation.[22] These findings highlight

Table 2: Clinical outcomes according to time-average concentration of tacrolimus (10 ng/ml) during 1 month after transplantation

Variables	TAC of tacrolimus		*P*
	<10 ng/ml (*n*=15)	≥10 ng/ml (*n*=28)	
Acute rejection	6 (40.0)	14 (50.0)	0.749
Chronic lung allograft dysfunction	2 (25.0)	4 (16.0)	0.616
Non-CMV infection	11 (73.3)	17 (60.7)	0.512
Bacteria	9 (60.0)	16 (57.1)	
Fungus	1 (6.7)	0 (0.0)	
Atypical pathogen	1 (6.7)	0 (0.0)	
Herpes simplex	0 (0.0)	1 (3.5)	
CMV infection	5 (33.3)	5 (17.8)	0.259
NODAT	5 (38.3)	7 (25)	0.714
Posttransplant hypertension	2 (13.3)	3 (10.7)	1.000
Posttransplant renal dysfunction	1 (6.7)	1 (3.6)	1.000
Malignancy			0.781
Lymphoma	1 (6.7)	2 (7.1)	
Nonlymphoma	0	3 (10.7)	
Death	11 (73.3)	8 (28.5)	0.003
Graft failure	2 (13.3)	0 (0.0)	
Infection	6 (40.0)	3 (10.7)	
Malignancy	1 (6.7)	3 (10.7)	
Uncontrolled bleeding	1 (6.7)	1 (3.5)	
Others	1 (6.7)	1 (3.5)	

TAC=Time-average concentration, NODAT=New onset diabetes after transplantation, CMV=Cytomegalovirus

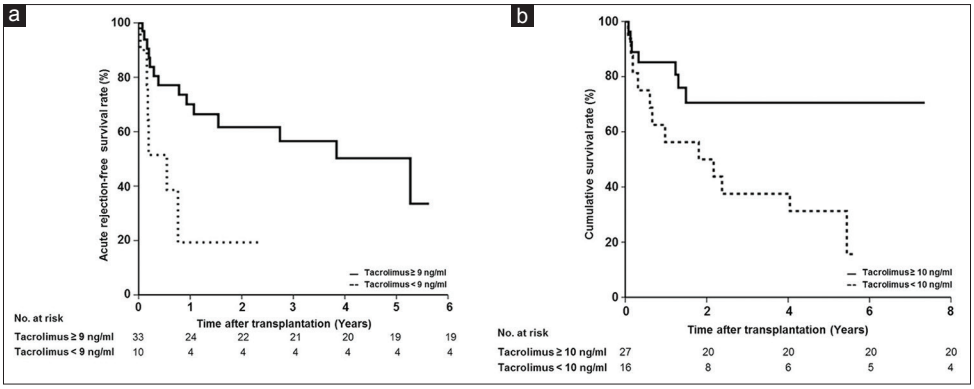

Figure 2: Kaplan–Meir survival analysis according to tacrolimus level. (a) Acute rejection-free survival according to the 9 ng/ml of tacrolimus level at 1 month after transplantation (P = 0.009, log-rank test), (b) Patient survival according to the 10 ng/ml of time-averaged tacrolimus concentration within 1 month after transplantation (P = 0.022, log-rank test)

Table 3: Cox regression analysis about risk factors for overall mortality

Variables	Univariate Cox regression		Multivariate Cox regression	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Age	1.002 (0.927-1.032)	0.920		
Male gender	1.060 (0.422-2.658)	0.902		
NODAT	1.173 (0.451-3.054)	0.744		
BMI	0.885 (0.782-1.001)	0.051		
Acute rejection	1.674 (0.693-4.042)	0.252		
Malignancy	1.843 (0.614-5.531)	0.275		
Non-CMV infection	5.667 (1.306-24.593)	0.021	4.627 (1.057-20.264)	0.042
TAC of tacrolimus <10 ng/ml during 1 month after transplantation	5.693 (2.257-14.358)	0.000	4.904 (1.930-12.459)	0.001

NODAT=New onset diabetes after transplantation, TAC=Time-average concentration, CMV=Cytomegalovirus, BMI=Body mass index, CI=Confidence interval, HR=Hazard ratio

the importance of balancing the risk of rejection with complications such as infection to determine optimal CNI concentrations in kidney transplant patients.

Studies to assess the optimal tacrolimus levels in lung transplant patients are rare. In general, stronger immunosuppression is required for lung transplantation compared to other solid organ transplantation due to a higher rejection risk and higher mortality due to graft failure. Strong CNI-based immunosuppression in lung transplantation frequently causes both nonfatal and fatal side effects, however, including nonfatal nephrotoxicity and new-onset diabetes and fatal non-CMV infections.[3,23] The present study showed that non-CMV infection is an independent risk factor for mortality. In addition, obstructive CLAD may be associated with thymoglobulin use, and restrictive allograft dysfunction can result from alveolar damage associated with various immunosuppressive therapy.[24,25] Taken together, these findings suggest that immunosuppressive treatment should be tailored to both improve lung allograft outcomes and reduce adverse events related to overtreatment.[26] A previous study reported that a low tacrolimus level (mean of 9.8 ng/ml) at 6- to 12-months posttransplant was a significant risk factor for CLAD in lung transplantation.[27] The present study analyzed the impact of tacrolimus levels on posttransplant outcomes at several time points during the 1st year following lung transplantation, and found that a sufficient tacrolimus level (>10 ng/mL) at 1-month posttransplant is associated with the prevention of acute rejection and higher patient survival. Moreover, sufficient tacrolimus levels at 1-month posttransplant did not increase non-CMV infectious complications.

The 5-year patient survival rate in the present study was 41.6%, which seemed to be <54% from the International Society for Heart and Lung Transplantation 2016 report.[28] The high mortality in the early phase of our lung transplantation program and unavailable long-term observation for recent cases might have contributed to the apparently low 5-year survival rate in the present study with median follow-up of about 2 years. Among 6 patients that received lung transplantation in 2013, 5 patients are still alive.

There were a few limitations in the present study. This study was retrospective in nature, analyzed a small sample size from a single center, and included only short-term follow-up. Nevertheless, this is the first study to show the impact of tacrolimus levels at several time points during the 1st year following lung transplantation on both efficacy-and safety-related clinical outcomes. Additional large-scale studies with longer follow-up durations are needed to confirm these findings.

Conclusions

Tacrolimus levels ≥10 ng/ml within 1 month following lung transplantation are associated with better patient survival. Furthermore, this level was not associated with an increase in significant posttransplant complications, such as infection, new-onset diabetes, or malignancy. These results highlight the importance of fine-tuned adjustments in immunosuppressant therapy to prevent both rejection and fatal complications in lung transplantation.

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Conflicts of interest
There are no conflicts of interest.

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