Exploratory associations of tacrolimus exposure and clinical outcomes after lung transplantation: a retrospective, single center experience

Wenwen Du[1], Xiaoxing Wang[1], Dan Zhang[1], WenQian Chen[2], and Xianbo Zuo[1]

[1]China-Japan Friendship Hospital
[2]Affiliation not available

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Abstract

Aims: This study aimed to investigate the potential impact of tacrolimus (TAC) exposure on clinical outcomes after lung transplantation. Methods: This retrospective observational study enrolled a total of 234 lung transplant recipients. TAC trough levels (C_0) were collected for 3 intervals: 0–3 months, 3–12 months, and 12–24 months. The intra-patient variability (IPV) was calculated using coefficient of variation. Genotyping of *CYP3A5*3* (rs776746) was performed. Patients were further divided into groups based on the C_0 cut-off value of 8 ng/mL and IPV cut-off value of 30%. Cox proportional hazards regression models were used to explore the potential impact of C_0 and IPV on outcomes of interests, including donor-specific antibodies (DSA), chronic lung allograft dysfunction (CLAD) and mortality. Results: The influence of *CYP3A5*3* polymorphism was only significant for C_0 and IPV during the first 3 months. Low C_0 (< 8 ng/mL) at 3–12 months increased the risk of DSA (hazard ratio [HR] 2.820, 95% confidence interval [CI] 1.093–7.276) and mortality (HR 2.220, 95% CI 1.162–4.243), while High IPV (>=30%) during this period was associated with an increased risk of mortality (HR 2.100, 95% CI 1.120–3.937). Patients with Low C_0/High IPV combination had significantly higher risks for DSA (HR 4.534, 95% CI 1.326–15.507) and survival (HR 4.205, 95% CI 1.739–10.168), surpassing the predictive power provided by C_0 or IPV alone. Conclusion: A combination of Low C_0/High IPV might be considered in categorizing patients towards risk of adverse clinical outcomes following lung transplantation.

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Running title

Tacrolimus exposure in lung transplantation

Wenwen Du [1], Xiaoxing Wang [1], Dan Zhang [1], Wenqian Chen [1], Xianbo Zuo[1, 2]

[1] Department of Pharmacy, China-Japan Friendship Hospital, Chaoyang District, Beijing, China

[2] Department of Dermatology, China-Japan Friendship Hospital, Chaoyang District, Beijing, China

Correspondence

Xianbo Zuo, Department of Dermatology, Department of Pharmacy, China-Japan Friendship Hospital, Chaoyang District, Beijing, China.

Email: subpharm@163.com

KEYWORDS

Tacrolimus; intra-patient variability; lung transplantation; chronic lung allograft dysfunction; donor-specific antibody

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What is already known about this subject

- There is ongoing controversy regarding the association between tacrolimus C_0 or IPV and clinical outcomes after lung transplantation.
- The impact of *CYP3A5*3* genotype on C_0 is validated. Whether it affects IPV and clinical outcomes remains controversial.
- Furthermore, the impact of C_0/IPV combination on clinical outcomes remains unexplored.

What this study adds

The combination of IPV $>$ 30% and $C_0<$ 8 ng/mL during 3–12 months post-lung transplantation was associated with higher risk of donor-specific antibodies and mortality.

The impact of *CYP3A5*3* polymorphism on C_0 and IPV was limited to the initial three months.

Abstract

Aims: This study aimed to investigate the potential impact of tacrolimus (TAC) exposure on clinical outcomes after lung transplantation.

Methods: This retrospective observational study enrolled a total of 234 lung transplant recipients. TAC trough levels (C_0) were collected for 3 intervals: 0–3 months, 3–12 months, and 12–24 months. The intra-patient variability (IPV) was calculated using coefficient of variation. Genotyping of *CYP3A5*3*(rs776746) was performed. Patients were further divided into groups based on the C_0 cut-off value of 8 ng/mL and IPV cut-off value of 30%. Cox proportional hazards regression models were used to explore the potential impact of C_0 and IPV on outcomes of interests, including donor-specific antibodies (DSA), chronic lung allograft dysfunction (CLAD) and mortality.

Results: The influence of*CYP3A5*3* polymorphism was only significant for $C_0$and IPV during the first 3 months. Low C_0 (<8 ng/mL) at 3–12 months increased the risk of DSA (hazard ratio [HR] 2.820, 95% confidence interval [CI] 1.093–7.276) and mortality (HR 2.220, 95% CI 1.162–4.243), while High IPV (>=30%) during this period was associated with an increased risk of mortality (HR 2.100, 95% CI 1.120–3.937). Patients with Low C_0/High IPV combination had significantly higher risks for DSA (HR 4.534, 95% CI 1.326–15.507) and survival (HR 4.205, 95% CI 1.739–10.168), surpassing the predictive power provided by C_0 or IPV alone.

Conclusion: A combination of Low C_0/High IPV might be considered in categorizing patients towards risk of adverse clinical outcomes following lung transplantation.

KEYWORDS

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1 Introduction

Tacrolimus (TAC) is the primary immunosuppressive agent for lung transplantation recipients.[1]Despite its clinical superiority, prescribing TAC is complicated by its high inter- and intra-individual variability and narrow therapeutic window. The optimal therapeutic range for TAC in lung transplant recipients has not yet been established,[2] and even when the trough level (C_0) falls within predefined range, there is still a risk of sub-therapeutic or supra-therapeutic fluctuations with each individual measurement, leading to under- or over- immunosuppression. Therefore, TAC intra-patient variability (IPV) has been proposed as a potential risk factor for adverse clinical outcomes. Previous studies have confirmed the correlation between high IPV

and rejection, graft failure and mortality in kidney and liver transplantation; [3-6] however, limited research has been conducted in lung transplantation.[7-9]

Furthermore, conflicting evidence exists regarding the reference ranges of TAC C_0 in lung transplant recipients. Some lung transplantation centers recommend target ranges of C_0 to be 10–25 ng/mL within the first two weeks, 10–20 ng/mL for the subsequent 6–10 weeks, and 10–15 ng/mL thereafter,[10] or 12–15 ng/mL during the first year, and lowered to 9–12 ng/ml thereafter. [11, 12] However, recent evidence suggests that these recommendations may need to be revised downwards due to an increased risk of acute kidney injury (AKI) associated with elevated C_0 levels following lung transplantation, particularly when they exceeded 15 ng/mL.[13,14] On the other hand, emerging data indicates a potential correlation between lower C_0 and inferior outcomes. For instance, Ryu reported an increased risk of rejection when C_0 was below 9 ng/mL at one month after lung transplantation, [15] while Gallagher found that lower C_0 at 6–12 months post-transplantation was a significant risk factor for chronic lung allograft dysfunction (CLAD).[7]

Previous studies have also indicated that the combined effect of dose corrected concentration (C/D) and high IPV may exert a more pronounced influence on adverse allograft outcomes. However, this combined effect has only been observed in kidney transplant recipients thus far; therefore, it remains to be fully explored in the context of lung transplantation. [16, 17]

The cytochrome P450 (CYP) 3A5 isoenzyme plays a crucial role in the metabolism of TAC. The presence of *CYP3A5*3* (rs776746) mutation leads to reduced CYP3A5 activity, thereby influencing TAC concentration and dose requirement. However, at present, the association between *CYP3A5* genotype and TAC IPV has not been definitely recognized and further validation is required. [18]

Therefore, the primary aim of this study was to investigate and validate the impact of TAC C_0 and IPV on clinical outcomes, including the development of DSA, CLAD, and mortality, with an objective to establish optimal TAC exposure values. Furthermore, we conducted an assessment on the synergistic effect of TAC C_0 and IPV. Additionally, we examined how the *CYP3A5* genotype influenced TAC exposure during different time periods following lung transplantation.

2 Materials and Methods

2.1 Study design and population

This is a retrospective, single center study. Patients who received lung transplantation at China-Japan Friendship Hospital from August 2016 to August 2022 were selectively enrolled according to the criteria for the study. The inclusion criteria were: (1) lung transplantation for the first time; (2) received TAC-based immunosuppressive therapy. The exclusion criteria were: (1) unable to participate in *CYP3A5* genotyping; (2) switched to cyclosporine or sirolimus; (3) incomplete baseline data; (4) survived less than three months.

All procedures in this study were in accordance with the 1964 Helsinki declaration and its amendments, and was approved by the Ethics Committee of China-Japan Friendship Hospital in June, 2022 (No. 2022-KY-056-1).

2.2 Immunosuppressive regimens

For induction therapy, patients received methylprednisolone alone or in combination with basiliximab. Methylprednisolone was given intraoperatively at a dose of 500–1000 mg. Basiliximab were given both before transplantation and on post-operative day (POD) 4 at a dose of 20 mg.

The maintenance immunosuppressive regimen consists of immediate-release capsules of TAC, immediate-release tablets of mycophenolate mofetil, and prednisone. TAC administration was initiated on POD 1 at a standard dosage of 2 mg per day, divided into two doses, unless the patient had an exceptionally high or low body weight. Monitoring of C_0 commenced on POD 3 and continued thrice weekly during hospitalization, with additional blood draws conducted monthly or during follow-up visits. Per protocol in our center, the target ranges for TAC C_0 were set at 8–10 ng/mL within the first year (0–12 months) and 6–8 ng/mL within the second year (12–24 months) after lung transplantation, respectively. Initially, mycophenolate mofetil was

administered in a dose of 1000 mg/day divided into two doses. Prednisone was intravenously administered at a dosage of 1 mg/kg/day from POD 1 to POD 3, followed by oral administration at a dosage of 0.5 mg/kg/day which gradually tapered down to a long-term maintenance dose ranging between 5–10 mg/day.

2.3 Data collection

The pertinent parameters were collected through a retrospective chart review of all patients, including demographic data (age, sex, height, weight, comorbidities), laboratory data (alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, serum creatinine, urea, fasting blood glucose, hemoglobin, hematocrit), transplant-related data (indication for transplant, transplant type, use of basiliximab and use of extracorporeal membrane oxygenation (ECMO)), TAC C_0, Forced expiratory volume in 1 second (FEV1), presence of donor-specific antibodies (DSA) and mortality were obtained.

The TAC C_0 values were determined using micro-particle enzyme immunoassay (ARCHITECT i1000SR immunoassay analyzer, Abbott U.S.). All TAC C_0 data within the 0–3 months, 3–12 months, and 12–24 months were included. The average TAC C_0 were calculated for each patient within each respective epoch. TAC IPV was calculated as the coefficient of variation (CV) using the formula TAC IPV = (standard deviation/mean) $\times$ 100%, as previously reported.[19] A minimum of three measurements per each post-transplant epoch were required to calculate IPV. Respiratory function test was performed at four weeks post-transplantation and then every 3 months thereafter, or based on physicians' discretion.

2.4 Genotyping

The *CYP3A5*3* (rs776746) genotype was determined using the Sanger dideoxy DNA sequencing method. Genomic DNA was extracted from EDTA-anticoagulated peripheral blood samples using a commercially available DNA purification kit (EasyPure Blood Genomic DNA Kit, Transgene Biotech, Beijing, China). Subsequently, genomic DNA samples were amplified via polymerase chain reaction (PCR) and sequenced utilizing the ABI 3730XL DNA Sequencer (ABI Co; Majorbio Biotechnology Co.,Ltd., Beijing, China). The forward primer was 5'-CAGCATTTAGTCCTTGTGAG-3', and the reverse primer was 5'-ACGACACACAGCAACCTTAG-3'. Data analysis was performed using Chromas software (Technelysium, South Brisbane, Australia). Patients carrying the **1* allele (*CYP3A5*1/*1* or **1/*3*) were categorized as *CYP3A5* expressers, while those with the*CYP3A5 *3/*3* genotype were classified as *CYP3A5*Non-expressers.

2.5 Outcome definitions

Our composite end-point outcomes consisted of the incidence of DSA, chronic lung allograft dysfunction (CLAD), and mortality. Serum samples were routinely collected post-transplantation to screen for the presence of anti-human leukocyte antigen (HLA) antibodies. The presence of DSA was determined by comparing the measured HLA types with donor HLA typing. CLAD was defined as a 20% decline from the best post-transplant FEV1 according to the International Society of Heart and Lung Transplant Guidelines.[20] Additionally, we examined the distribution characteristics of TAC exposure, represented by average C_0 and IPV, across 3 distinct time periods (0–3 months, 3–12 months and 12–24 months).

2.7 Statistical analysis

Data was processed using Statistical Package for Social Science (SPSS) 19.0. Continuous variables were expressed as mean $\pm$ standard deviation (SD) or median (interquartile range, IQR). Categorical variables were presented as count (percentage). The differences between groups were assessed using Student's t-test for normally distributed continuous variables, and Mann-Whitney U tests for non-normally distributed continuous variables, as appropriate. Predefined outcomes stratified by average TAC C_0, IPV or *CYP3A5* genotype were analyzed by Kaplan-Meier analysis and compared using the Log-rank test. Time from transplant to DSA, CLAD or death was modeled using Cox proportional hazards regression. Potential risk factors included age at transplant, sex, body mass index (BMI), comorbidities (hypertension, hyperlipidemia and diabetes), transplant-related data and TAC exposure. The variables with a P value < 0.2 in the univariate analysis were included in the multivariate analysis, which employed a forward likelihood ratio-test approach for

constructing final Cox survival models. $P < 0.05$ was considered of statistical significance.

3 Results

3.1 Clinical characteristics of patients

A total of 241 patients underwent lung transplantation and participated in *CYP3A5* genotyping between August 2016 and August 2022. From this study population, 5 patients were excluded for using cyclosporine, 1 patient was excluded due to missing data and 1 patient did not survive the first month post-transplantation, resulting in a final analysis cohort of 234 patients. The baseline characteristics are presented in Table 1. The median (IQR) age was 60 (51–65) years old, with males accounting for 81.2% of the participants. The most prevalent pulmonary diagnosis was interstitial lung disease (ILD; 66.2%), followed by chronic obstructive pulmonary disease (COPD; 12.0%), pulmonary hypertension (PH; 9.8%), bronchiectasis (3.8%) and other indications including bronchiolitis obliterans, pneumoconiosis, hypersensitivity pneumonitis and cystic fibrosis (8.1%). The prevalence rates of diabetes mellitus, hypertension and hyperlipidemia before surgery were 27.8%, 19.7% and 18.8%, respectively. Fifty percent of the patients ($n = 117$) were *CYP3A5* expressers, while the remaining 50% ($n = 117$) were *CYP3A5* non-expressers.

3.2 TAC exposure after lung transplantation

The average TAC C_0 was 8.8 (IQR, 8.1–9.9) ng/mL, 8.5 (IQR, 7.7–9.8) ng/mL, and 7.8 ± 1.5 ng/mL for the epochs 0–3 months, 3–12 months and 12–24 months, respectively; whereas the corresponding TAC IPV were 36.7% (IQR, 30.2%–44.3%), 30.6% (IQR, 23.5%–39.0%), and 28.2% (IQR, 21.8%–35.0%) over the same corresponding period (Figure 1). Both parameters exhibited a descending trend over time.

The number of TAC C_0 measurements for the periods 0–3 months, 3–12 months and 12–24 months were 18 (IQR, 14–21), 30 (IQR,12–29), and 13 (IQR,7–21), respectively (Figure 1). Patients received more extensive monitoring in the early post-transplantation periods, especially in the first year.

Given that CYP3A5 is the primary enzyme responsible for TAC metabolism, we sought to investigate its potential impact on TAC exposure; the results are summarized in Table S1. Notably, significant difference was observed only during the 0–3 months period between *CYP3A5* expressers and non-expressers ($P = 0.011$ for TAC C_0, $P = 0.003$ for TAC IPV).

3.3 Correlation of TAC exposure across different time periods

The trend-lines for TAC C_0 and IPV are depicted in Figure 2, illustrating significant fluctuations in TAC concentration and consistently high IPV values across all time periods. Furthermore, the change trends of these parameters at different time points exhibit similarity. TAC C_0 and IPV showed significant correlation between 3–12 months and 12–24 months (TAC C_0: $r = 0.541$, $P < 0.001$; TAC IPV: $r = 0.231$, $P = 0.002$), so we used the data during 3–12 months for all further analyses ($n = 224$). The distribution of the TAC C_0 and IPV during this time period are shown in Figure S1. In the outcome analysis, TAC C_0 were categorized into two groups: High C_0 (TAC C_0 >= 8 ng/mL) and Low C_0 (TAC C_0 < 8 ng/mL), while TAC IPV values were classified as High IPV (TAC IPV >= 30%) and Low IPV (TAC IPV < 30%) (Supplementary Figure 1).

3.4 Occurrence and risk factors for clinical outcomes

3.4.1 DSA

Of the 224 patients in this study, 18 (8%) experienced DSA and the median time for DSA incidence was 1088 days (IQR, 592–1677 days). Kaplan-Meier analysis revealed that DSA incidence was 12.5% and 5.6% in the Low and High C_0 group, respectively ($P = 0.025$) (Figure 4A). Additionally, the High IPV group also demonstrated a higher frequency of DSA compared to the Low IPV group, although this difference did not reach statistical significance (11.1% versus 4.7%, $P = 0.056$) (Figure 4D). From the Cox proportional hazard model, Low C_0 was independently associated with increased risk for DSA (HR 2.820, 95% CI 1.093–7.276, $P = 0.032$) (Table 2).

3.4.2 CLAD

The overall incidence of CLAD in the study population following lung transplantation was 19.6%, with a median time to CLAD onset of 952 days (IQR, 533–1480 days). TAC exposure was not associated with CLAD incidence both in Kaplan-Meier analysis and Cox proportional hazard model (Figure 4B and 4E, Table 2).

3.4.3 Survival

The survival rate of the study population was 79.5% and the median survival time was 1107 days (IQR, 622–1681 days). Kaplan-Meier analysis showed a statistically significant difference in survival rates between the High C_0 and Low C_0 group ($P = 0.025$), as well as between the High IPV and Low IPV group ($P = 0.007$) (Figure 4C and 4F). According to the Cox proportional hazard model, Low C_0 (HR 2.220, 95% CI 1.162–4.243, $P = 0.016$) and High IPV (HR 2.100, 95% CI 1.120–3.937, $P = 0.021$) was associated with increased risk of mortality (Table 2).

3.5 Impact of combined TAC exposure on clinical outcomes

Since both TAC C_0 and IPV showed significance in outcome analysis, we combined these 2 parameters together and further stratified the study population into 3 groups: High C_0/Low IPV group, High C_0/High IPV & Low C_0/Low IPV group and Low C_0/High IPV group (Figure 4). Notably, patients from High C_0/Low IPV group exhibited statistically significant lower incidence of DSA (Figure 5A, Log rank$P = 0.01$) and superior survival rate compared with Low C_0/High IPV group (Figure 5C, Log rank $P = 0.001$).

Table 3 shows the results of the Cox regression analysis of DSA and survival according to TAC C_0/IPV status. After adjusting for age, sex, BMI, and factors with a P value < 0.2 in univariate analysis, the adjusted HR of Low C_0/High IPV for DSA was 4.534 (1.326–15.507) ($P = 0.016$), while for survival it was 4.205 (1.739–10.168) ($P = 0.001$), surpassing the predictive power of TAC C_0 or IPV alone.

4 Discussion

Our findings have revealed that a combination of Low C_0 and High IPV of TAC, assessed between 3 and 12 months after transplantation, was associated with an increased risk of donor-specific antibodies (DSA), as well as mortality among lung transplant recipients. Furthermore, we observed that the *CYP3A5* genotype influenced TAC C_0 and IPV during the early post-transplantation period; however, no correlation was found with clinical outcomes (Supplementary Figure 2).

We concluded that patients with an average C_0 below 8 ng/mL during 3–12 months are at higher risk for DSA and mortality. Previous studies investigating the correlation between C_0 and graft outcomes in lung transplantation have yielded inconsistent findings. Ryu et al. reported an increased risk of mortality with a C_0 below 10 ng/ mL within one month post-transplantation, [15] while Gallagher found a negative association between mean TAC C_0 (median (IQR): 9.8 (8.56–10.75) ng/mL) between 6–12 months and CLAD.[7] Darley et al. reported a significantly higher proportion of rejection biopsies (85.7% versus 31.9%) with C_0 fell below their target range of 12–15 ng/mL within the first three months after transplantation.[21] The discrepancies in lower limits of TAC C_0 may be attributed to variations in target ranges employed across different studies. In our center, where a substantial number of elderly patients undergo transplantation and face high risk of infection resulting in mortality, we have observed a significant association between $C_0 < 8$ ng/mL and inferior outcomes, thereby suggesting this lower limit remains acceptable. Meanwhile, higher TAC concentration has been linked to increased drug toxicity such as acute kidney injury, chronic kidney disease and post-transplant diabetes mellitus; [22, 23] however, limited studies exist to assess the upper limit of TAC C_0 in lung transplant recipients, thus further investigations are warranted.

In addition to routine C_0 monitoring, TAC IPV has emerged as a novel marker for identifying transplant recipients at risk for suboptimal clinical outcomes.[24] Various methodologies have been proposed to assess IPV, including coefficient of variation (CV), standard deviation (SD) and time in therapeutic range (TTR).[25] In our study, we employed the most prevalent parameter in solid organ transplantation, CV, and observed that

patients with CV >= 30% during 3–12 months was associated with increased risk of mortality. Meanwhile, current studies on evaluating TAC IPV in lung transplantation used different methodologies, and conclusions remains controversial.

Gallagher conducted a retrospective analysis of 110 lung transplant recipients, using SD to characterize IPV. Their findings suggest that TAC SD calculated during 6–12 months independently predicted the time to development of CLAD and mortality. With each unit increase in SD, there was a corresponding 46% and 27% increase in the risk of CLAD and death, respectively. [7] Ensor et al. conducted a retrospective study involving 292 patients and reported that a 10% increase in TTR was significantly associated with reduced risk of acute cellular rejection (ACR), CLAD and mortality at 1 year.[9] In contrast, Kao et al analyzing TTR in 157 lung transplant patients during the first 6 months, did not find any correlation between TTR and ACR. [8]These studies differed in their target ranges; Ensor and his colleagues employed a narrower range of 12–15 ng/mL for 0–6 months followed by 10–12 ng/mL for 6–12 months, while Kao used a range of 10–15 ng/mL throughout the first year. Due to TAC's high variability, maintaining a strict therapeutic range can be challenging; therefore, adopting a more liberal range may allow more time within the therapeutic range without adverse effect on ACR, leading to negative outcomes.

The majority of studies evaluating the impact of TAC exposure on clinical outcomes have primary focused on the stable period, typically defined as at least 3 months post-transplantation. During the early post-transplant phase, patients experience clinical instability and necessitate frequent dose adjustments of TAC. This results in highly variable concentrations, which can introduce biases in statistical analysis and complicate result interpretation. Nonetheless, efforts have been made to investigate this phenomenon. Gallagher et al. found no association between TAC SD within 0–6 months and CLAD or mortality. Similarly, Evens et al. reported that high TAC variability within 0-3 months did not correlate with an increased acute rejection score at 12 months. In our study, we also observed no correlation between TAC IPV during 0–3 months and DSA, CLAD, or mortality (data not shown). Furthermore, there is limited literature exploring long-term TAC exposure beyond 12 months after lung transplantation. Our data suggest TAC exposure during 3–12 months predicted the developing trend of exposure in the second year, which aligns with previous study.[7]

The average C_0 effectively reflects the levels over specific time periods, while IPV captures fluctuations between high and low levels, similar to accuracy and precision in analytical science. Hence, a combination of C_0 and IPV may provide a more comprehensive characterization of TAC exposure. Stefanović et al enrolled 104 Caucasian kidney transplant patients, and found patients with high IPV/low C_0/D during 6–12 months had significantly reduced graft survival compared to the other combinations.[16] Park et al further validated this conclusion in a larger cohort with 1080 kidney transplant recipients, by reporting higher incidences of death censored graft loss (DCGL), biopsy-proven allograft rejection (BPAR) and overall graft loss in the high IPV/low C/D group. [17] Our study was the first study in lung transplantation to investigate the combinational effect of TAC C_0 and IPV, and we also observed a stronger predictive power of Low C_0/High IPV combination than TAC C_0 or IPV alone.

The impact of *CYP3A5* polymorphism on TAC metabolism has been extensively studied; however, the definitive association between *CYP3A5* genotype and TAC IPV remains to be established.[26] Seiber et al reported each additional loss-of-function allele (*CYP3A5*3* , **6* and *7) reduced TAC CV by 1.82% in the first six months following kidney transplantation in European Americans. [18] On the other hand, studies conducted between 6 and 12 months suggested no significant influence of *CYP3A5* genotype on TAC IPV. [27, 28] Similarly, we only observed a higher IPV in *CYP3A5* expressers within the first three months. Other factors such as noncompliance, drug-drug or drug-food interactions may play a more prominent role in determining IPV during the stable phase after transplantation.

There are several limitations in our study. Firstly, the study design was single-center, retrospective and observational. Secondly, the sample size is relatively small compared to previous research in kidney transplantation. Thirdly, we did not exclude inpatient data in our study. Lung transplant recipients usually face higher risk of infection compared to other solid organ transplantation and have a high likelihood of hospital readmission, particularly within the first year. To maintain an adequate sample size, we included

all measurements in our study.

In conclusion, the present study suggested using a combination of Low C_0 (< 8 ng/mL) and High IPV (>=30%) of TAC calculated during 3–12 months after lung transplantation may help predict adverse clinical outcomes. Monitoring TAC C_0 and IPV in routine clinical practice is a convenient tool that may assist in identifying patients at high risk for inferior long-term outcomes.

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Conflicts of interests

There are no competing interests to declare.

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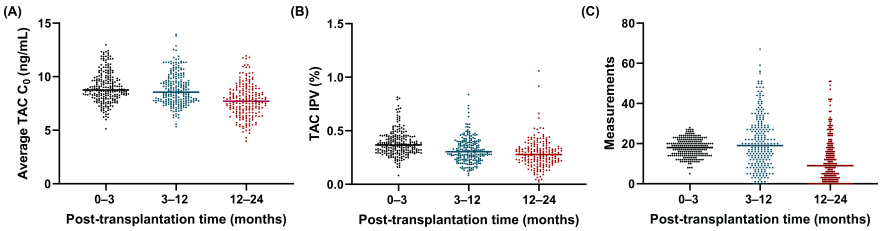

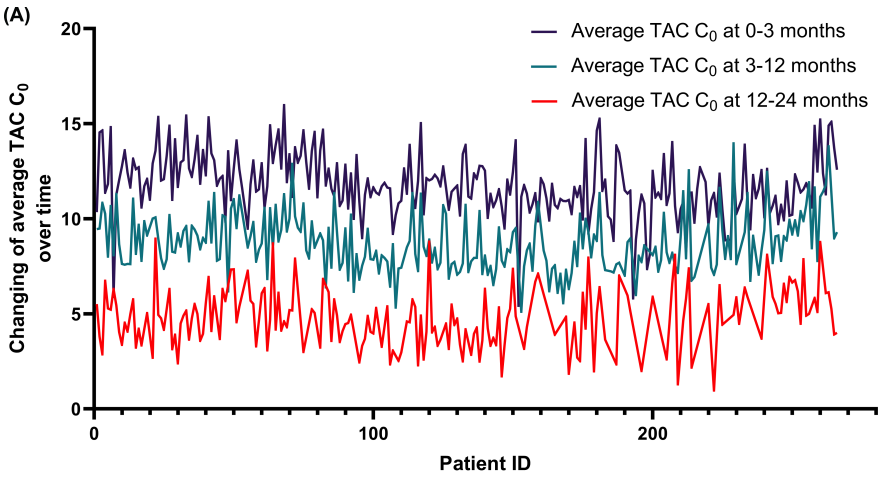

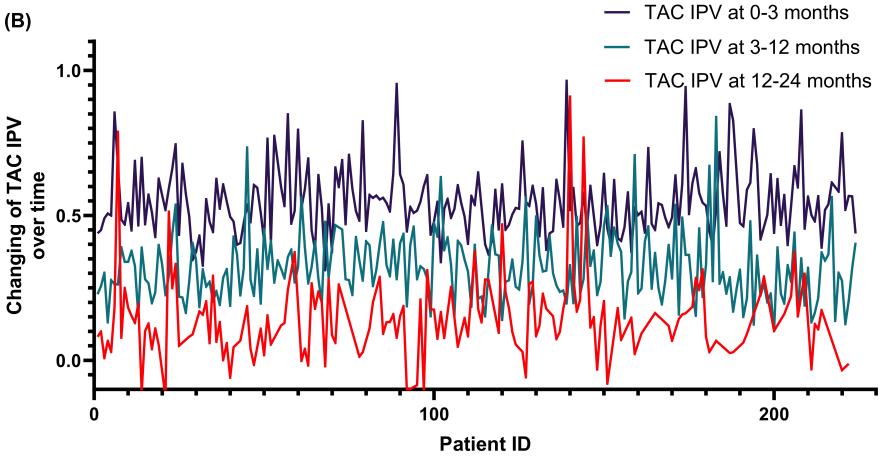

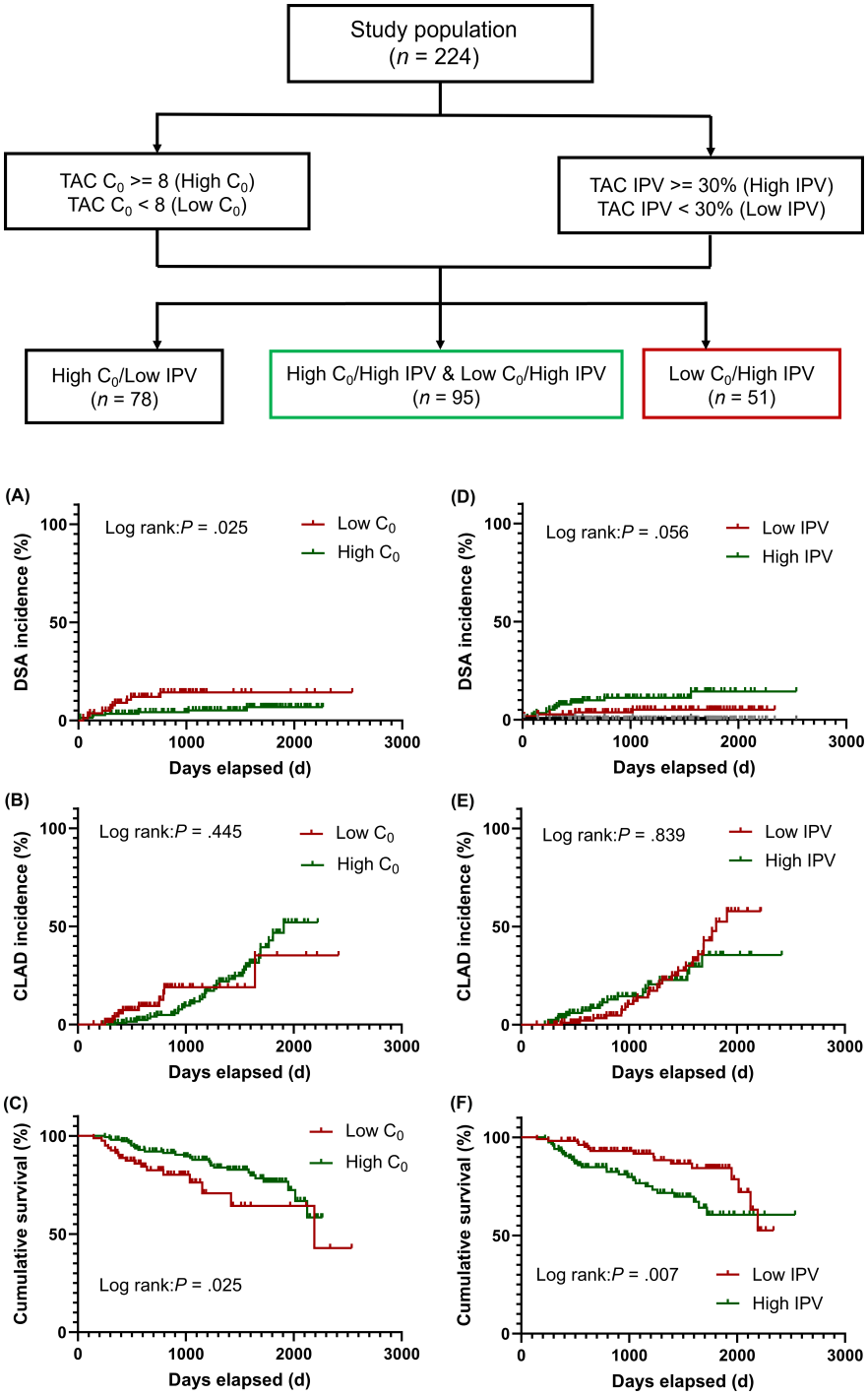

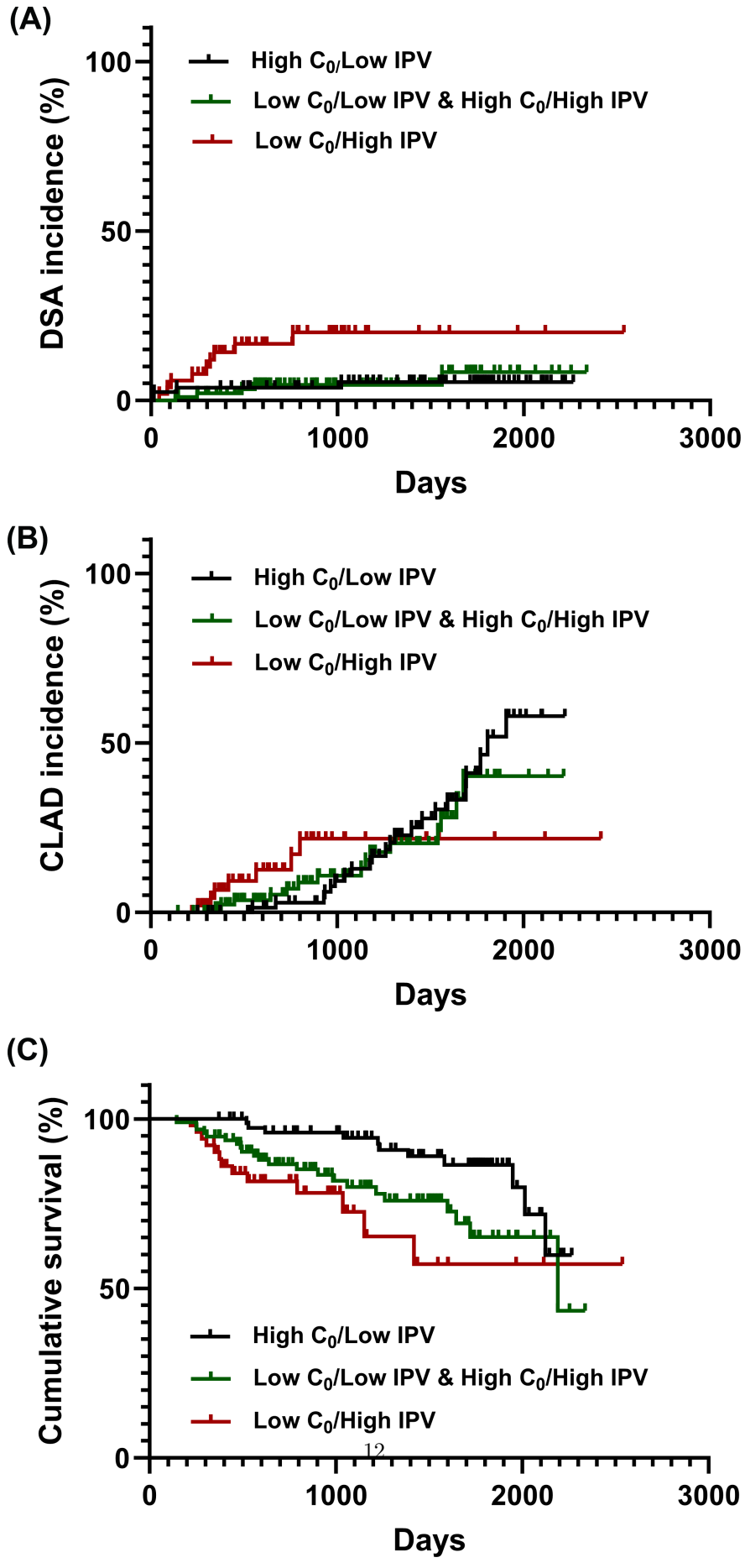

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