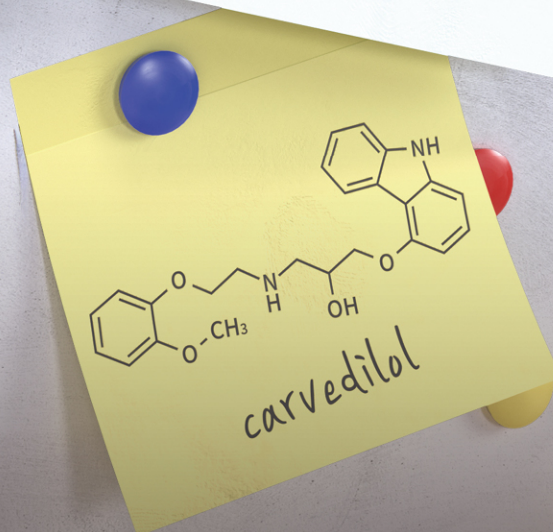
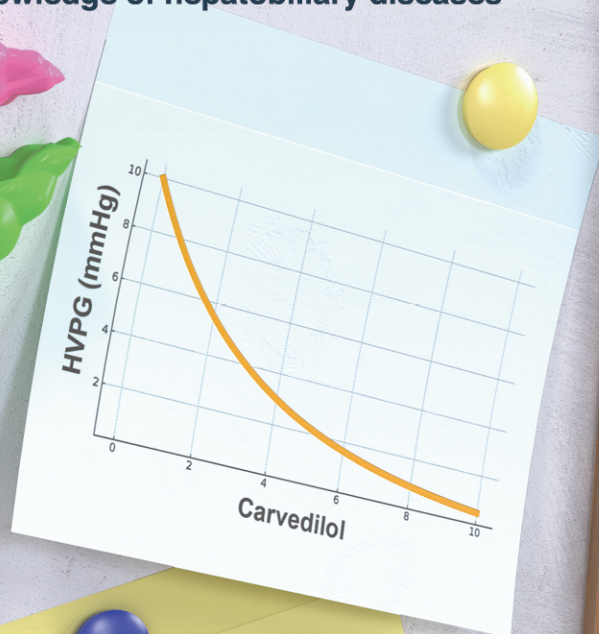


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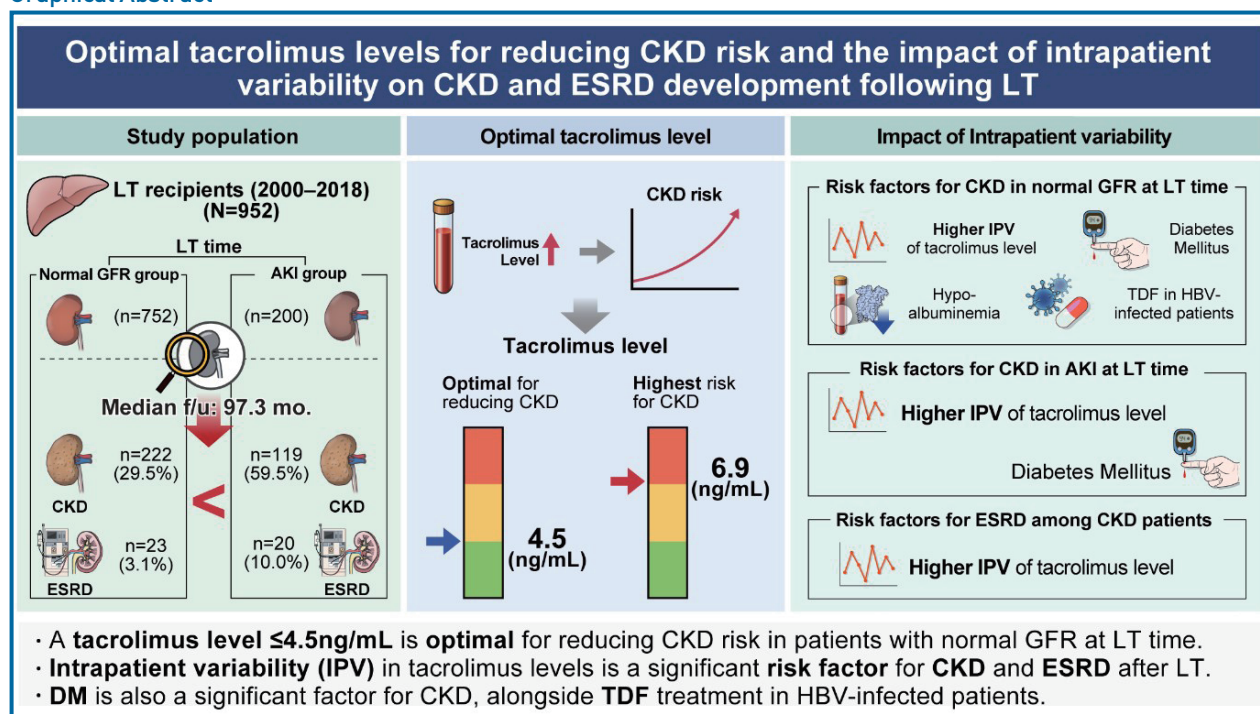
Original Article

Optimal tacrolimus levels for reducing CKD risk and the impact of inpatient variability on CKD and ESRD development following liver transplantation

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Graphical Abstract



Study Highlights

- We determined that a tacrolimus level ≤ 4.5 ng/mL is optimal for reducing CKD risk, with the highest risk observed at ≥ 6.9 ng/mL, in patients with normal GFR at LT time.
- Detailed analysis underscored the significant role of IPV in tacrolimus levels in CKD development, across both normal GFR and AKI groups.
- IPV in tacrolimus levels was also identified as a significant factor for end-stage renal disease development among CKD patients post-LT.
- Diabetes mellitus also emerged as a significant factor for CKD, alongside tenofovir disoproxil fumarate treatment in HBV-infected patients.

Background/Aims: This study aimed to identify the risk factors for chronic kidney disease (CKD) and end-stage renal disease (ESRD) following liver transplantation (LT), with a specific focus on tacrolimus levels and inpatient variability (IPV).

Methods: Among the 1,076 patients who underwent LT between 2000 and 2018, 952 were included in the analysis. The tacrolimus doses and levels were recorded every 3 months, and the IPV was calculated using the coefficient of variability. The cumulative incidence rates of CKD and ESRD were calculated based on baseline kidney function at the time of LT. The impact of tacrolimus levels and their IPV on the development of CKD and ESRD was evaluated, and the significant risk factors were identified.

Results: Within a median follow-up of 97.3 months, the 5-year cumulative incidence rates of CKD (0.58 vs. 0.24) and ESRD (0.07 vs. 0.01) were significantly higher in the acute kidney injury group than in the normal glomerular filtration rate (GFR) group. In the normal GFR group, the tacrolimus levels were identified as a risk factor for CKD, with a level of ≤ 4.5 ng/mL suggested as optimal for minimizing the risk of CKD. Furthermore, the IPV of tacrolimus levels and doses emerged as a significant risk factor for CKD development in both groups ($P < 0.05$), with tenofovir disoproxil fumarate also being a risk factor in HBV-infected patients. The IPV of tacrolimus levels was also a significant factor in ESRD development ($P < 0.05$).

Conclusions: This study elucidated the optimal tacrolimus trough level and highlighted the impact of IPV on the CKD and ESRD development post-LT. (*Clin Mol Hepatol* 2024;30:131-146)

Keywords: Liver transplantation; Chronic kidney disease; Tacrolimus; Tenofovir; Diabetes mellitus

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Abbreviations:

AKI, acute kidney injury; CKD, chronic kidney disease; CNI, calcineurin inhibitor; CTP, Child-Turcotte-Pugh; CV, coefficient of variability; DM, diabetes mellitus; ESRD, end-stage renal disease; GFR, glomerular filtration rate; HD, hemodialysis; IPV, inpatient variability; IQR, interquartile range; ISs, immunosuppressants; KDIGO, Kidney Disease Improving Global Outcomes; LT, liver transplantation; MELD, Model for End-Stage Liver Disease; NA, neucleos(t)ide analogue; sHR, sub-distribution hazard ratio; TDF, tenofovir disoproxil fumarate

INTRODUCTION

Liver transplantation (LT) is the definitive treatment for early-stage hepatocellular carcinoma or end-stage liver disease. It is widely performed worldwide, with an incidence rate of approximately 4.5 LTs per million people.¹ The introduction of potent immunosuppressants (ISs), including tacrolimus—a calcineurin inhibitor (CNI)—has significantly improved the long-term management of LT patients by reducing the risk of rejection and mortality.² However, the prolonged use of ISs post-LT can lead to various complications, such as metabolic syndrome, malignancy, and chronic kidney disease (CKD).^{3,4}

Approximately 10–45% of patients undergoing LT develop CKD with a subset progressing to end-stage renal disease (ESRD), necessitating hemodialysis (HD).^{3,5} Consequently, the development of CKD amplifies mortality risk, especially when the glomerular filtration rate (GFR) falls below 30 mL/min/1.73 m², correlating with declining renal function.⁶ Given the high prevalence of CKD and its profound impact on mortality, identifying the risk factors for CKD is crucial for enhancing the clinical outcomes of LT patients.

In addition to the use of ISs, such as CNI, several other potential risk factors for CKD have been identified. These include age, hepatitis C, acute kidney injury (AKI), and the presence of diabetes mellitus (DM).^{3,7,8} In HBV-infected patients, treatment with tenofovir disoproxil fumarate (TDF)—a potent nucleos(t)ide analogue (NA)—has been identified as a risk factor for renal dysfunction, although data in LT patients are limited.⁹ Considering that HBV infection is a leading cause of LT in Asia, the effect of CNIs, particularly tacrolimus, on CKD development must be assessed, in conjunction with other potential CKD risk factors including the use of TDF.

Some studies have suggested the optimization of tacrolimus levels during the early post-LT period to achieve a balance between the efficacy of treatment and the reduction of renal dysfunction. During the first month following LT, maintaining the tacrolimus levels at 6–8 ng/mL, or below 10 ng/mL, may reduce the incidence of renal dysfunction 1 year after LT.^{3,10} However, the long-term effects of these levels on CKD development and the specific cut-off levels for minimizing CKD risk remain unclear. In addition to tacrolimus levels, the inpatient variability (IPV) in tacrolim-

us levels has been linked to poorer allograft outcomes following kidney transplantation.^{11,12} However, a notable discrepancy exists regarding the impact of tacrolimus IPV on clinical outcomes post-LT.^{13,14} Additionally, long-term studies evaluating the effect of tacrolimus IPV on the development of CKD and ESRD following LT, especially considering baseline kidney function at the time of LT, are lacking. This underscores the necessity for research in this area to enhance patient outcomes.

To address these critical gaps, we conducted a comprehensive analysis focusing on the long-term risks of CKD and ESRD following LT, particularly examining the effects of tacrolimus levels and IPV. Firstly, our longitudinal, large cohort study assessed changes in renal function and the development of CKD and ESRD according to the baseline kidney function at the time of LT. Subsequently, we meticulously examined the influence of tacrolimus levels and their IPV on the development of CKD and ESRD, considering baseline kidney function at LT. Furthermore, our study aimed to identify the concomitant risk factors, including DM and TDF, for CKD and ESRD post-LT by incorporating tacrolimus IPV to mitigate the risk of CKD and ESRD development in LT patients.

MATERIALS AND METHODS

Patients

A total of 1,076 patients who underwent LT from January 2000 to December 2018 at a tertiary university hospital were considered eligible for the study. Of them, 124 patients who either died or were lost to follow-up within 6 months (n=58), aged ≤18 years (n=5), underwent KT or HD before LT (n=8), and had pre-existing CKD before LT (n=53) were excluded. Finally, 952 patients were included in the final analysis (Supplementary Fig. 1). The study was approved by the Institutional Review Board of Seoul St. Mary's Hospital (KC24RISI0104) and conducted in accordance with the ethical standards of the Declaration of Helsinki.

Laboratory and demographic parameters

Demographic data collected at the time of LT included

age, sex, LT type, presence of DM, hypertension, and the cause of LT. Additionally, various laboratory parameters were recorded, including total bilirubin, albumin, aspartate transaminase, alanine transaminase, creatinine, international normalized ratio, Child-Turcotte-Pugh (CTP) score, and Model for End-Stage Liver Disease (MELD) score. AKI at the time of LT was diagnosed according to the Kidney Disease Improving Global Outcomes (KDIGO) guidelines and the International Liver Transplantation Society Consensus Statement, which define it as an increase in serum creatinine of more than 1.5 times the baseline, an increase of ≥ 0.3 mg/dL, or a urine output of < 0.5 mL/kg/h for more than 6 hours.^{3,15-17} The estimated GFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration formula.^{3,18} Patients with AKI at the time of LT were assigned to the AKI group, while patients with normal GFR at LT were classified as the normal GFR group.

Immunosuppressants and follow-up

Post-LT, immunosuppression involved the use of induction agents (where applicable), such as interleukin-2 receptor antibody (basiliximab), accompanied by triple-drug immunosuppressants consisting of CNIs, steroid, and mycophenolate mofetil (MMF). Steroid therapies were tapered and typically withdrawn by 1-month post-LT, as appropriate. MMF was planned to be withdrawn 6–12 months post-LT, though a small number of patients continued MMF along with CNIs, as appropriately determined by the clinician. Maintenance ISs, primarily CNIs with tacrolimus or cyclosporine monotherapy, were administered in accordance with treatment guidelines.³ Subsequently, patient follow-up was initially conducted every 1–3 months. After the early post-LT period, follow-up visits were scheduled every 3 months to conduct routine hepatobiliary function tests and monitor the IS trough levels.

Diagnosis of CKD and ESRD

Based on the KDIGO guidelines, CKD was defined as the presence of kidney structural abnormalities or an eGFR of < 60 mL/min per 1.73 m^2 ($\geq$ stage 3) for more than 3 months.¹⁹ Furthermore, ESRD development was characterized as having an eGFR of < 15 mL/min per 1.73 m^2 (stage 5) or the requirement for HD.¹⁹

Assessment of tacrolimus IPV

The mean tacrolimus dose and level were calculated every 3 months. The IPV for the tacrolimus levels was estimated by calculating the coefficient of variability (CV) using the following formula: $\text{CV} (\%) = (\text{standard deviation} / \text{mean tacrolimus level}) \times 100$. Similarly, the IPV for tacrolimus dose and level/dose ratio were assessed by determining the CV for each variable.

Outcomes measurements

The primary outcome was the development of CKD after LT, with a median follow-up period of 97.3 months. The follow-up duration was calculated from the date of LT to either the date of CKD development or the last follow-up. Our analysis aimed at evaluating the effects of tacrolimus dose, level, and their IPV on CKD development in patients with and without AKI at the time of LT, specifically focusing on those whose primary IS was tacrolimus.

The secondary outcomes included ESRD development during follow-up and the influence of TDF on CKD development. Furthermore, this study aimed to identify the risk factors for CKD and ESRD development, particularly examining the effect of tacrolimus IPV and TDF in patients with and without AKI at LT, specifically focusing on those whose primary IS was tacrolimus.

Statistics

Categorical data were described using counts and proportions, while continuous data were summarized using medians with interquartile ranges (IQR) or means with standard deviations, as appropriate, based on the normality of data distribution. Descriptive analyses were used to compare groups using the chi-square, Fisher's exact, and Wilcoxon rank-sum tests. The cumulative incidence curves for CKD and ESRD were generated using the cumulative incidence function and Kaplan–Meier method, respectively. In the time-dependent competing risk analysis, tacrolimus levels and doses were included as time-varying covariates obtained at any point during follow-up. Smoothing splines were used to assess the non-linear relationships and to identify threshold among tacrolimus levels, doses, and CKD development. In a repeated measures approach, lin-

ear mixed-effects regression models with random intercepts and an unstructured covariance matrix were used to evaluate the changes in tacrolimus levels and doses over time. The analysis aimed to identify the differences between patients with and without CKD and the results were presented using least squares means and error bar plots.

The differences in the CV of tacrolimus levels and doses between patients with and without CKD were assessed using Wilcoxon's rank sum test, and the results were visually represented using beeswarm plots. The sub-distribution hazard ratio (sHR) and 95% confidence interval (CI) for tacrolimus level and dose were estimated using crude and adjusted Fine-Gray's competing hazards models, with death considered a competing event. Significant risk factors for ESRD development were identified using Cox regression analyses. Landmark analyses at 3- and 5-year post-LT were conducted for CKD development, with sub-analyses of HBV-infected patients. Variables with a *P*-value less than 0.1 in the univariable analysis were selected for multivariable analysis. The optimal cutoff tacrolimus values, including the CV of tacrolimus level, dose, and level/dose ratio, based on the log-rank statistics, were determined using the Contal and O'Quigley's method.²⁰ Higher IPV of tacrolimus values are determined based on these calculated optimal cutoff values. A *P*-value of <0.05 was considered significant. All analyses were performed using SAS software (version 9.4; SAS Institute, Inc., Cary, NC, USA) and R version 4.3.1 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Baseline characteristics

Of the 952 patients, 752 exhibited a normal GFR at LT time (normal GFR group), while 200 experienced AKI at the time of LT (AKI group) (Supplementary Fig. 1). The mean age was 51.0 years, and patients who developed CKD in both the normal GFR and AKI groups tended to be older. The majority of patients were men (69.5%), and HBV infection was the predominant cause of LT (64.6%). Following LT, tacrolimus was the primary IS in approximately 86% of patients during the follow-up period (Table 1). When the baseline characteristics were compared based on the kidney function status at the time of LT, the CTP and MELD

scores were significantly higher in the AKI group than in the normal GFR group (Supplementary Table 1).

CKD development and changes in kidney function based on baseline function

During a median follow-up of 97.3 months (IQR, 47.7–154.8 months; range 6.6–326.5 months), 341 patients (35.8%) developed CKD, comprising 222 patients (29.5%) in the normal GFR group and 119 patients (59.5%) in the AKI group (Supplementary Fig. 1). The incidence of CKD development did not differ between the living donor LT (LDLT) and deceased donor LT (DDLT) groups (Supplementary Fig. 2).

When the incidence of CKD development was examined according to the baseline kidney function status, the cumulative incidence rates of CKD in the normal GFR group at 1, 3, 5, and 10 years were 0.14, 0.20, 0.24, and 0.30, respectively (Fig. 1A). These rates were significantly lower than those in the AKI groups, where the incidence rates were 0.53, 0.57, 0.58, and 0.60 at the corresponding intervals (*P*<0.05; Fig. 1B). Additionally, the median times for CKD development were 15 months in the normal GFR group (Fig. 1C) and 9 months in the AKI group (*P*<0.05). Kidney function gradually declined in both groups, with the decrease being more rapid and pronounced in the AKI group (Fig. 1D and E).

Development of ESRD according to baseline kidney function

As kidney function gradually deteriorated post-LT, 43 patients developed ESRD: 23 (3.1%) in the normal GFR group and 20 (10.0%) in the AKI group. Among those with ESRD, baseline characteristics were similar between the two groups, except for higher baseline creatinine and MELD scores in the AKI group (Supplementary Table 2).

The median time to ESRD development was notably longer in the normal GFR group than in the AKI group (97.4 vs. 47.4 months, respectively; *P*<0.001; Fig. 2A and B). Furthermore, the cumulative incidence rates of ESRD in the normal GFR group were 0.003, 0.01, and 0.03 at 3, 5, and 10 years, respectively. By contrast, the incidence rates in the AKI group were significantly higher, with rates of 0.05, 0.07, and 0.14 in the corresponding years (*P*<0.001; Fig. 2C).

Table 1. Baseline characteristics of the study population

Variables	Total (n=952)	Normal GFR group at LT time (n=752)		AKI group at LT time (n=200)		P-value
		No CKD (n=530)	CKD (n=222)	No CKD (n=81)	CKD (n=119)	
Age, years	52.0 (46.0–57.0)	51.0 (44.0–56.0)	54.0 (47.0–58.0)	50.0 (42.0–56.0)	54.0 (48.0–60.0)	0.003
Female sex	290 (30.5)	144 (27.2)	71 (32.0)	38 (46.9)	37 (31.1)	0.023
LT type						0.333
LDLT	792 (83.2)	467 (88.1)	187 (84.2)	59 (72.8)	79 (66.4)	
DDDT	160 (16.8)	63 (11.9)	35 (15.8)	22 (27.2)	40 (33.6)	
DM	167 (17.5)	65 (12.3)	60 (27.0)	8 (9.9)	34 (28.6)	0.001
HTN	65 (6.8)	32 (6.0)	16 (7.2)	6 (7.4)	11 (9.2)	0.648
LT cause						0.023
HBV	615 (64.6)	371 (70.0)	136 (61.3)	39 (48.1)	69 (58.0)	
HCV	57 (6.0)	25 (4.7)	20 (9.0)	5 (6.2)	7 (5.9)	
Alcohol	143 (15.0)	68 (12.8)	36 (16.2)	12 (14.8)	27 (22.7)	
Others	137 (14.4)	66 (12.5)	30 (13.5)	25 (30.9)	16 (13.4)	
Presence of HCC	404 (42.4)	256 (48.3)	92 (41.4)	20 (24.7)	36 (30.3)	0.390
CTP score	9.0 (7.0–11.0)	8.0 (6.0–11.0)	9.0 (8.0–11.0)	11.0 (10.0–12.0)	11.0 (9.0–12.0)	0.144
MELD score	14.7 (9.0–23.0)	12.0 (7.0–18.0)	14.0 (10.0–20.0)	28.0 (18.0–36.0)	25.0 (16.0–33.3)	0.230
WBC, / μ L	3,810.0 (2,570.0–6,040.0)	3,540.0 (2,480.0–5,380.0)	3,405.0 (2,200.0–5,120.0)	7,090.0 (4,020.0–12,333.0)	4,770.0 (3,000.0–9,510.0)	0.010
Platelet, $\times 10^3/\mu$ L	59.0 (40.0–94.0)	63.5 (42.0–105.0)	52.0 (37.0–83.0)	67.0 (49.0–92.0)	53.0 (37.0–73.0)	<0.001
Cr, mg/dL	0.8 (0.7–1.0)	0.8 (0.6–0.9)	0.8 (0.7–1.0)	1.4 (0.9–2.3)	1.6 (1.2–2.2)	0.095
Albumin, g/dL	3.0 (2.7–3.4)	3.1 (2.8–3.6)	2.9 (2.7–3.3)	3.0 (2.7–3.3)	2.8 (2.6–3.2)	0.053
AST, U/L	51.0 (35.0–86.0)	48.0 (33.0–75.0)	52.0 (35.0–83.0)	94.0 (46.0–399.0)	58.0 (38.0–112.0)	0.003
ALT, U/L	34.0 (23.0–59.0)	33.0 (23.0–54.0)	33.0 (22.0–58.0)	44.0 (27.0–613.0)	35.0 (24.0–61.0)	0.001
Total bilirubin, mg/dL	2.9 (1.2–11.3)	2.1 (0.9–6.9)	3.0 (1.3–7.7)	9.8 (4.9–25.5)	9.0 (2.1–25.6)	0.346
Immunosuppressant						0.140
Cyclosporine	97 (10.2)	49 (9.2)	30 (13.5)	5 (6.2)	13 (10.9)	0.206
Tacrolimus	823 (86.4)	458 (86.4)	186 (83.8)	76 (93.8)	103 (86.6)	
Others	32 (3.4)	23 (4.3)	6 (2.7)	0 (0.0)	3 (2.5)	
INR	3.2 $\pm$ 34.5	2.1 $\pm$ 9.4	1.9 $\pm$ 2.6	2.3 $\pm$ 1.0	11.4 $\pm$ 95.5	0.299

Data are presented as number (%) for categorical variables and median (interquartile ranges) for continuous variables.

CKD, chronic kidney disease; LT, liver transplantation; BMI, body mass index; DM, diabetes mellitus; HTN, hypertension; HCC, hepatocellular carcinoma; CTP, Child-Turcotte-Pugh; MELD, model for end-stage liver disease; INR, international normalized ratio.

Impact of tacrolimus level on CKD development

Considering the critical role of AKI as a baseline risk fac-

tor for both CKD and ESRD, we evaluated the effect of tacrolimus on CKD development, specifically in patients with tacrolimus as their primary IS, while accounting for base-

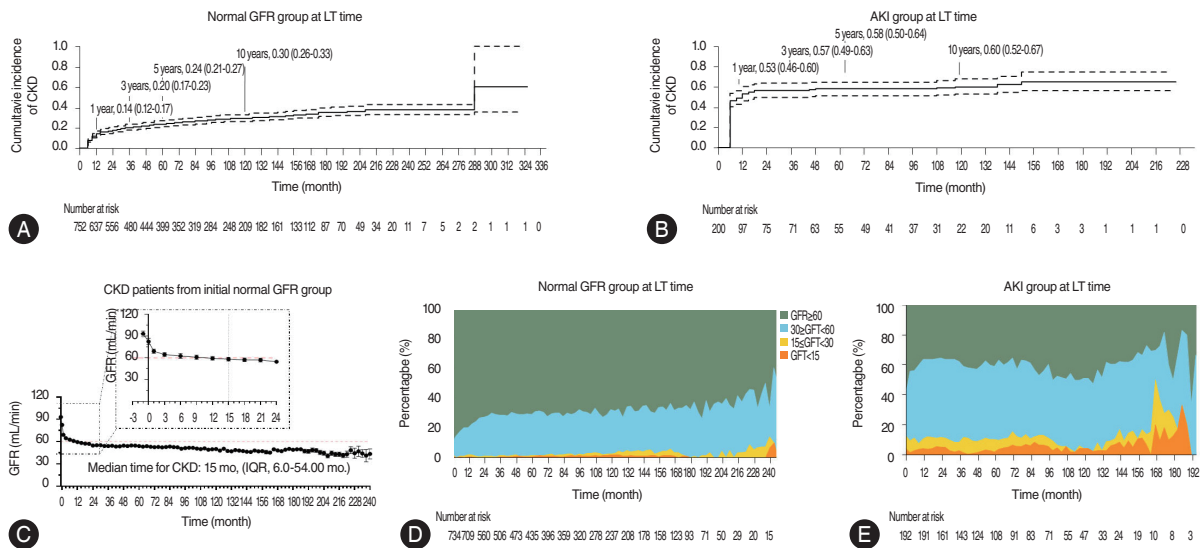


Figure 1. (A, B) Cumulative incidence of CKD based on the baseline GFR at the time of LT. (C) Changes in the kidney function in CKD patients from the normal GFR group. (D, E) Changes in the distribution of kidney function according to the baseline GFR at LT time. CKD, chronic kidney disease; GFR, glomerular filtration rate; LT, liver transplantation.

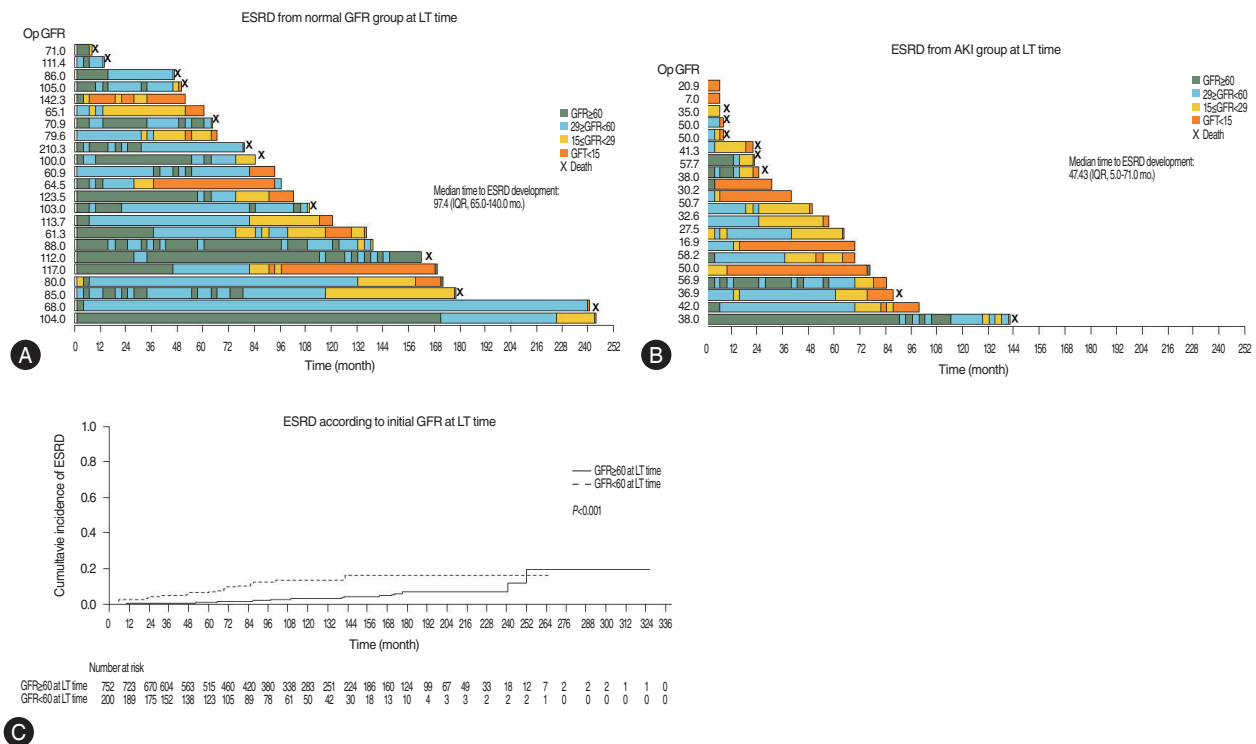


Figure 2. (A, B) Changes in the eGFR in ESRD patients and (C) cumulative incidence of ESRD according to the baseline GFR at the time of LT. eGFR, estimated glomerular filtration rate; CKD, chronic kidney disease; LT, liver transplantation; ESRD, end-stage renal disease.

line kidney function status. Initially, our analysis focused on the impact of tacrolimus levels in the normal GFR group,

revealing tacrolimus levels as a significant factor for CKD development ($P=0.01$). To determine the optimal tacrolimus

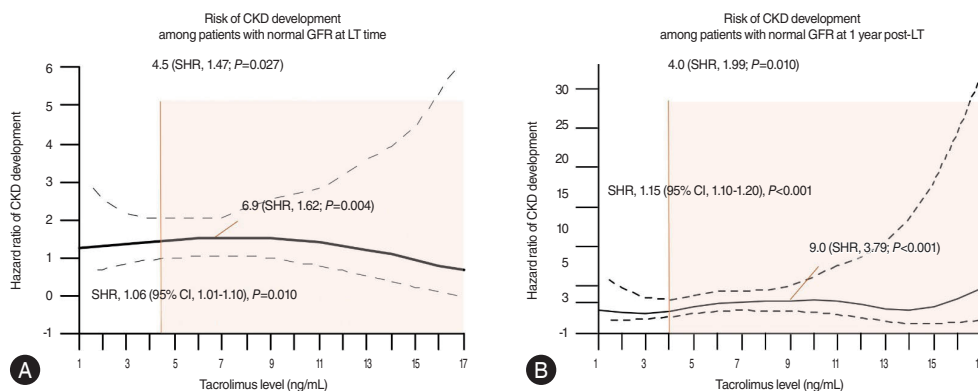


Figure 3. Changes in the risk of CKD development according to the tacrolimus level among patients with normal GFR at (A) the time of LT and (B) 1-year post-LT time. GFR, glomerular filtration rate; LT, liver transplantation; CKD, chronic kidney disease.

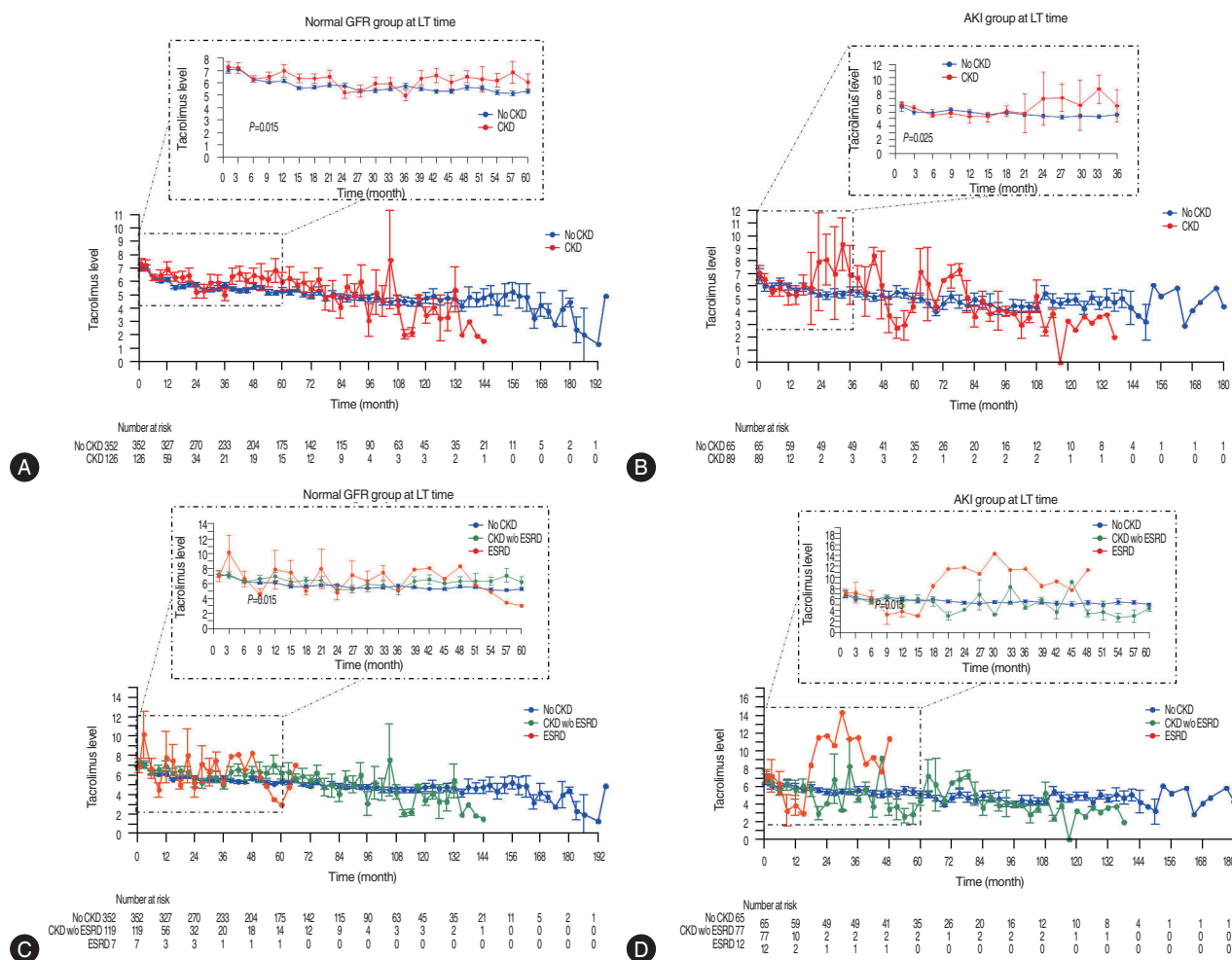


Figure 4. Comparison in the serial changes of the tacrolimus level according to the development of (A, B) CKD and (C, D) ESRD. CKD, chronic kidney disease; ESRD, end-stage renal disease.

level that minimizes CKD risk, we explored the relationship between tacrolimus levels and CKD development in a time-dependent manner (Fig. 3). The risk of CKD development began to increase significantly when the tacrolimus level was >4.5 ng/mL (HR 1.47; $P=0.027$), with the highest risk observed at a level of ≥ 6.9 ng/mL (HR 1.62; $P=0.004$) (Fig. 3A).

Furthermore, we further evaluated the optimal tacrolimus level to reduce CKD risk, only including patients with normal GFR at 1-year post-LT, a time point when levels are expected to be more stable and less affected by perioperative complications. We found that a level ≤ 4.0 ng/mL (sHR 1.99; $P=0.01$) is optimal, with the highest risk observed at tacrolimus levels ≥ 9.0 ng/mL (sHR 3.79; $P<0.001$) (Fig. 3B).

In the multivariable Fine-Gray's competing risk model analysis, tacrolimus level remained a significant factor in CKD development ($P=0.018$), along with the presence of DM, albumin levels, and LT cause (Supplementary Table 3). Moreover, among HBV-infected patients, TDF treatment emerged as a significant factor in CKD development. However, in the AKI group, tacrolimus level did not emerge as a significant factor for CKD development.

Serial changes in tacrolimus level and dose and their inpatient variability

Next, we evaluated and compared the serial changes in tacrolimus levels and level/dose ratios between patients with and without CKD and ESRD, specifically focusing on those whose primary IS was tacrolimus. Patients with CKD exhibited more pronounced variability in tacrolimus levels than those without CKD in the normal GFR and AKI groups (Fig. 4A and B). When further classifying CKD patients based on ESRD development, those with ESRD exhibited the highest variability in tacrolimus levels, followed by CKD patients without ESRD, and then patients without CKD, in both groups (Fig. 4C and D).

Based on these observed differences in tacrolimus levels and dose patterns, we also assessed and compared the IPV of tacrolimus levels and doses using the CV among patients with and without CKD and ESRD. Patients with CKD showed significantly higher CV values for tacrolimus levels and doses in the normal GFR and AKI groups (Fig. 5A and B). These trends persisted in the first 3-year CV values (Fig. 5C and D). Additionally, ESRD patients exhibited the highest CV values, followed by CKD patients without ESRD and patients without CKD in both groups (Fig. 5E and F).

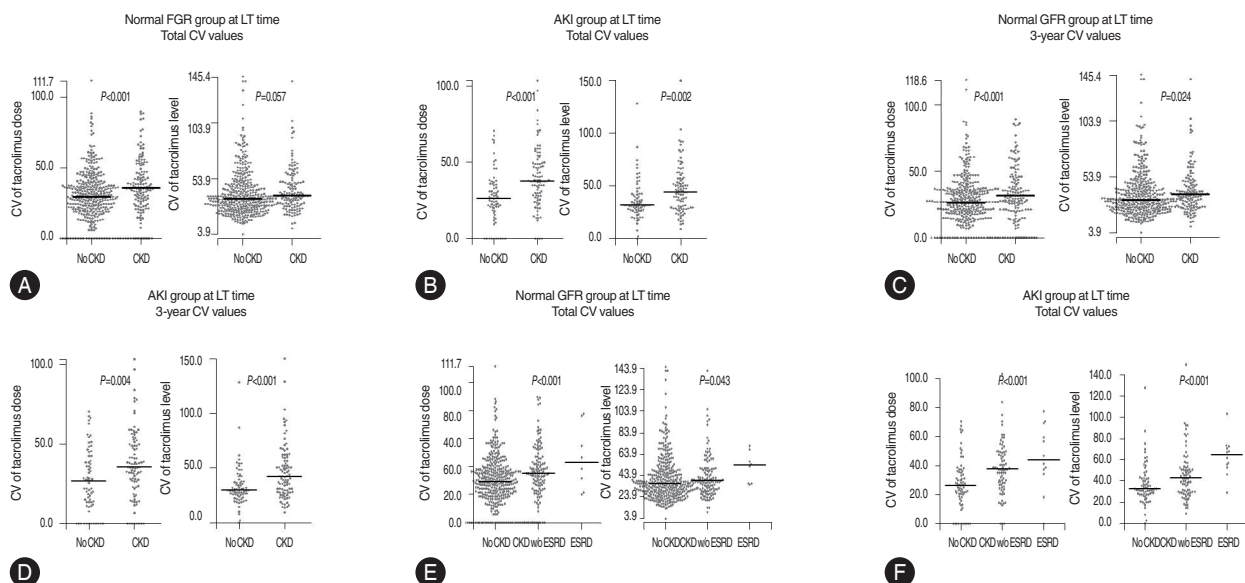


Figure 5. Comparison of (A, B) total and (C, D) 3-year inpatient variability between patients with and without CKD development during follow-up. (E, F) Comparison of total inpatient variability between patients with ESRD, CKD without ESRD, and without CKD development during follow-up. CKD, chronic kidney disease; ESRD, end-stage renal disease; CV, coefficient of variants.

Table 2. Effect of the inpatient variability of tacrolimus level on the development of CKD based on their cut-off level

Variables	Normal GFR group at LT time				AKI group at LT time			
	Total follow-up		Variables		Total follow-up		Variables	
	No CKD (n=359)	CKD (n=130)	sHR (95% CI)	P-value	No CKD (n=69)	CKD (n=91)	sHR (95% CI)	P-value
Total CV of tacrolimus level				0.054				<0.001
<37.7	196 (54.6%)	56 (43.1%)	Reference		51 (73.9%)	40 (44.0%)	Reference	
≥37.7	163 (45.4%)	74 (56.9%)	1.39 (0.99–1.96)		18 (26.1%)	51 (56.0%)	1.89 (1.37–2.61)	
First 1-year CV of tacrolimus level				0.486				0.005
<31.1	188 (53.4%)	64 (49.6%)	Reference		48 (71.6%)	46 (50.5%)	Reference	
≥31.1	164 (46.6%)	65 (50.4%)	1.13 (0.81–1.58)		19 (28.4%)	45 (49.5%)	1.57 (1.14–2.15)	
First 3-year CV of tacrolimus level				0.002				<0.001
<35.1	201 (56.3%)	50 (38.5%)	Reference		52 (76.5%)	40 (44.0%)	Reference	
≥35.1	156 (43.7%)	80 (61.5%)	1.73 (1.22–2.44)		16 (23.5%)	51 (56.0%)	1.96 (1.41–2.71)	
First 5-year CV of tacrolimus level				0.005				0.001
<36.4	207 (57.8%)	55 (42.3%)	reference		46 (66.7%)	34 (37.4%)	Reference	
≥36.4	151 (42.2%)	75 (57.7%)	1.64 (1.16–2.30)		23 (33.3%)	57 (62.6%)	1.79 (1.27–2.52)	
1-year landmark analysis								
No CKD (n=401)	CKD (n=80)	sHR (95% CI)	P-value	No CKD (n=77)	CKD (n=81)	sHR (95% CI)	P-value	
First 1-year CV of tacrolimus level			0.056				0.007	
<18.4	65 (16.2%)	20 (25.0%)	Reference		62 (80.5%)	51 (63.0%)	Reference	
≥18.4	336 (83.8%)	60 (75.0%)	0.62 (0.39–1.01)		15 (19.5%)	30 (37.0%)	1.54 (1.12–2.10)	
3-year landmark analysis								
No CKD (n=384)	CKD (n=103)	sHR (95% CI)	P-value	No CKD (n=72)	CKD (n=87)	sHR (95% CI)	P-value	
First 3-year CV of tacrolimus level			0.031				<0.001	
<35.3	209 (54.4%)	44 (42.7%)	reference		52 (72.2%)	35 (40.2%)	Reference	
≥35.3	175 (45.6%)	59 (57.3%)	1.52 (1.04–2.21)		20 (27.8%)	52 (59.8%)	1.96 (1.40–2.75)	
5-year landmark analysis								
No CKD (n=375)	CKD (n=113)	sHR (95% CI)	P-value	No CKD (n=72)	CKD (n=88)	sHR (95% CI)	P-value	
First 5-year CV of tacrolimus level			0.012				0.001	
<36.4	213 (56.8%)	49 (43.4%)	reference		47 (65.3%)	33 (37.5%)	Reference	
≥36.4	162 (43.2%)	64 (56.6%)	1.59 (1.10–2.28)		25 (34.7%)	55 (62.5%)	1.81 (1.28–2.56)	

GFR, glomerular filtration rate; LT, liver transplantation; CKD, chronic kidney disease; sHR, sub-distribution hazard ratio; CV, coefficient of variant; CI, confidence interval.

Table 3. Univariable and multivariable Fine-Gray's competing risk model analyses of CKD development in the normal GFR and AKI groups at LT time including the inpatient variability of tacrolimus levels

Variables	Normal GFR group at LT time				AKI group at LT time			
	Univariate		Multivariable 1		Univariate		Multivariable 1	
	sHR	P-value	Adjusted HR (95% CI)	P-value	sHR	P-value	Adjusted HR (95% CI)	P-value
Age (≥ 50)	1.11	0.557			1.43	0.048	1.14 (0.81–1.60)	0.462
Sex (female)	1.24	0.218			0.73	0.074	0.76 (0.55–1.05)	0.094
DDLT (vs. LDLT)	1.69	0.021	1.51 (0.96–2.37)	0.073	1.09	0.596		
DM	2.08	<0.001	2.11 (1.44–3.11)	<0.001	1.69	0.001	1.43 (1.02–2.00)	0.039
HTN	1.48	0.183			1.13	0.638		
LT cause (Others vs. HBV)	1.47	0.026	1.27 (0.90–1.79)	0.179	0.84	0.280		
HCC before LT	0.89	0.497			1.36	0.072	0.94 (0.66–1.34)	0.742
CTP score (≥ 10)	1.08	0.684			0.69	0.030	0.93 (0.66–1.34)	0.681
MELD (> 20)	0.89	0.605			0.81	0.199		
WBC ($\geq 4,000/\mu\text{L}$)	0.84	0.321			0.71	0.040	0.90 (0.63–1.27)	0.537
Platelet ($\geq 75 \times 10^3/\mu\text{L}$)	0.69	0.042	0.86 (0.60–1.24)	0.426	0.65	0.033	0.78 (0.54–1.14)	0.194
Albumin (≥ 3.0 g/dL)	0.50	<0.001	0.56 (0.39–0.81)	0.002	0.83	0.261		
AST (≥ 160 U/L)	1.02	0.949			0.45	0.002	0.94 (0.50–1.77)	0.841
ALT (≥ 160 U/L)	0.75	0.440			0.34	0.001	0.47 (0.21–1.07)	0.071
Total bilirubin (≥ 8 mg/dL)	0.93	0.716			0.84	0.267		
INR (> 2.3)	0.91	0.708			0.66	0.039	0.85 (0.56–1.28)	0.337
TDF (vs. no or other NAs)	1.16	0.487			0.76	0.277		
Total CV of TAC level (≥ 37.7 , $\geq 42.2^*$)	1.39	0.054	1.46 (1.04–2.05)	0.027	Not included		1.62 (1.17–2.24)	0.004
First 3-year CV of TAC level (≥ 35.1 , $\geq 39.3^*$)	1.73	0.002	Not included		1.73 (1.23–2.44)	0.002	Not included	
					1.89	<0.001	1.62 (1.17–2.24)	0.004
					1.96	<0.001	Not included	
							1.75 (1.28–2.39)	<0.001

AKI, acute kidney injury; GFR, glomerular filtration rate; LT, liver transplantation; sHR, sub-distribution hazard ratio; HR, hazard ratio; CI, confidence interval; DDLT, deceased donor liver transplantation; LDLT, living donor liver transplantation; DM, diabetes mellitus; HTN, hypertension; HCC, hepatocellular carcinoma; CTP, Child-Turcotte-Pugh; MELD, model for end-stage disease; WBC, white blood cells; AST, aspartate transaminase; ALT, alanine transaminase; INR, international normalized ratio; TDF, tenofovir disoproxil fumarate; NAs, nucleos(t)ide analogues; CV, coefficient of variant; TAC, tacrolimus.

*CV cut-off value for AKI.

We also compared complications, including rejection, graft survival, and critical infection, between patients with higher and lower CV values (Supplementary Table 4). No significant differences were observed in rejection or critical infection, but graft survival was lower in patients with higher CV values.

Effect of inpatient variability of tacrolimus level and dose on CKD development

Considering the observed differences in the IPV of tacrolimus levels and doses between patients with and without CKD, we further investigated their impact on CKD after establishing the optimal cut-off levels. In the normal GFR and AKI groups, the CV of the IS levels was a significant risk factor for CKD development (Table 2). Notably, in the AKI group, the CV of tacrolimus levels was significant in the first 1-year of CV and 1-year landmark analysis, while this significance was marginal in the normal GFR group. These results suggest that the CV of tacrolimus level is a critical factor in CKD development in both groups, exhibiting an earlier effect in the AKI group.

Considering the importance of the CV of tacrolimus level, we conducted a multivariable Fine-Gray' competing risk model analysis (Table 3). In the normal GFR group, the CV ($P<0.05$) of tacrolimus levels was a significant risk factor for CKD, along with the presence of DM and low albumin levels. Similarly, in the AKI group, the significance of the CV of the tacrolimus level persisted ($P<0.01$).

Moreover, we assessed the impact of the CV of tacrolimus dose on CKD development. In line with the findings for tacrolimus levels, the CV of tacrolimus doses was a significant factor for CKD development in the normal and AKI groups (Supplementary Table 5), underscoring the critical role of the CV of tacrolimus levels and doses in CKD development.

Further Analysis of the impact of inpatient variability in tacrolimus levels on CKD development

Considering the predominance of HBV infection as the primary cause of LT, we specifically analyzed the effect of IPV of tacrolimus levels on the incidence of CKD in HBV-infected patients in the normal GFR group (Supplementary

Table 6). The CV values of the tacrolimus levels consistently emerged as significant factors for CKD development. Additionally, TDF treatment emerged as a significant factor ($P=0.05$) underscoring the associated risk of CKD development.

Additionally, we also assessed the impact of the CV of tacrolimus levels on CKD among patients with normal GFR at 1-year post-LT, a time point when levels are expected to be less affected by perioperative complications. Consistent with our previous findings, the CV value of tacrolimus remained a significant predictor of CKD development (Supplementary Table 7).

The influence of inpatient variability in tacrolimus levels on ESRD development among CKD patients

Finally, further analyses were conducted to assess the effect of IPV of tacrolimus levels on the development of ESRD in patients with CKD. The CV ($P<0.05$) of tacrolimus levels was a significant factor in the normal GFR group (Supplementary Table 8). Moreover, the significance persisted in the AKI group, highlighting the influence of the IPV of tacrolimus levels on ESRD development in patients with CKD in both groups.

DISCUSSION

Our comprehensive long-term cohort study demonstrated the substantial burden of CKD and ESRD following LT, with a notably high incidence observed in the AKI group. Specifically, in patients whose primary IS was tacrolimus, our study not only revealed the impact of tacrolimus levels on CKD development but also identified an optimal level to minimize the risk of CKD. In HBV-infected patients, TDF treatment is also associated with an increased risk of CKD alongside the tacrolimus. Furthermore, our detailed analysis consistently revealed the pivotal role of the IPV of tacrolimus levels and doses in CKD and ESRD development in both the normal GFR and AKI groups. To the best of our knowledge, this study is the first to thoroughly elucidate the significance of the IPV of tacrolimus levels in CKD and ESRD development following LT.

In our study, the 5-year cumulative incidence rates of

CKD post-LT were 0.24 in the normal GFR group and 0.58 in the AKI group. These results align with those of previous studies, which reported the incidences rates of 17% and 37% in patients with MELD scores of <20 and ≥ 20 , respectively.²¹ Furthermore, our study observed a higher incidence and a shorter time to ESRD development in the AKI group, underscoring the impact of baseline AKI on CKD and ESRD development. Given the increased risk of mortality associated with CKD and ESRD,⁶ our findings underscore the critical importance of managing baseline kidney function to improve patient outcomes. Additionally, identifying the risk factors for CKD and ESRD is crucial for enhancing post-LT patient care.

Regarding risk factors for CKD, our study highlights the crucial role of tacrolimus levels in its development with detailed analyses that included setting tacrolimus levels as a time-dependent variable. We identified the optimal through tacrolimus levels, suggesting that a level of ≥ 4.5 ng/mL significantly increases the risk for CKD, with the highest risk observed at a level above 6.9 ng/mL in the normal GFR group. Furthermore, since tacrolimus levels are usually higher and more influenced by perioperative complications during 1-year post-LT, and tend to become lower and more stable after 1-year post-LT, we conducted further analyses that included only patients with normal GFR at 1-year post-LT. Finally, we also identified a tacrolimus level ≤ 4.0 ng/mL is optimal for reducing CKD risk after 1-year post-LT, which is slightly lower than 4.5 ng/mL identified earlier (Fig. 3). Although current guidelines recommend maintaining the tacrolimus level below 5 ng/mL after the first year post-LT,³ our findings suggest that maintaining the tacrolimus level below 4.0 ng/mL could further minimize the risk of CKD after the first year post-LT. Additionally, given the heightened CKD risk at a level exceeding 6.9 ng/mL, it is crucial to avoid surpassing this threshold, even during the early post-LT period. Consequently, in clinical practice, considering the potential risk of rejection due to low tacrolimus levels, especially in the first month post-LT, it's crucial to avoid both excessively low and high levels, adhering to the cut-off suggested in our study. In patients with high risk for CKD, combining other immunosuppressants, such as MMF or mammalian target of rapamycin inhibitors, with tacrolimus can be an option to lower tacrolimus levels, helping to achieve the optimal range for reducing CKD development without increasing the risk of rejection.^{3,22,23} Meanwhile, in

the AKI group, tacrolimus levels did not significantly influence CKD development, possibly linked to the higher IPV of tacrolimus level observed in this group. This suggests that controlling tacrolimus levels alone may not be sufficient to minimize the post-LT CKD risk, underscoring the influence of IPV of tacrolimus level in the CKD development.

Remarkably, our detailed analyses consistently demonstrated the significant effect of the IPV of tacrolimus levels on CKD in the normal GFR and AKI groups. Previous studies have shown that the IPV of tacrolimus levels is associated with poorer survival, particularly in kidney transplant patients.²⁴ Although the effects of the IPV of tacrolimus levels on patient outcomes post-LT remained controversial,^{13,14} a recent study suggested a potential association between the 1-year IPV of tacrolimus levels and renal dysfunction.¹³ Finally, our comprehensive analysis conclusively demonstrated that the IPV of tacrolimus levels is a risk factor for both CKD and ESRD. The consistent impact of IPV on CKD development across both the normal GFR and AKI groups underscores its role in CKD progression in LT patients, irrespective of their baseline kidney function at the time of LT. Furthermore, given the significant influence of IPV on ESRD development among CKD patients, attention to IPV in tacrolimus levels is crucial to prevent further renal function decline post-LT. In addition, the lower graft survival rates observed in patients with higher IPV may be partially associated with higher rates of CKD and ESRD development, which consequently increase mortality.⁶ While the exact mechanisms underlying the observed differences in IPV of tacrolimus levels were not fully elucidated, they might partially stem from the variations in patient's tacrolimus metabolism.²⁵ Additionally, in clinical practice, reducing the CKD risk by minimizing the IPV of tacrolimus levels requires meticulous care, especially in situations vulnerable to tacrolimus level fluctuations, such as suspected rejection or biliary strictures.³ Additionally, our findings indicate that the IPV of tacrolimus doses is also linked to CKD development. These insights suggest that avoiding hasty adjustments in tacrolimus dosage could reduce the subsequent IPV of both tacrolimus levels and doses, crucial for minimizing the risk of CKD and ESRD.

In addition to the IPV of tacrolimus levels, TDF treatment has emerged as a significant risk factor for CKD in patients with HBV infection. Although TDF is a well-known risk fac-

tor for decreasing renal function in non-LT settings, real-world evidence in LT patients has been limited. Our detailed analysis highlights the influence of TDF treatment alongside tacrolimus on CKD development in LT patients with HBV infection, aligning with recent studies that indicate a higher risk of CKD with TDF treatment compared to entecavir treatment.²⁶ Therefore, based on these results, in HBV-infected patients, opting for treatments with entecavir or tenofovir alafenamide treatment instead of TDF may reduce the CKD risk post-LT, similar to the non-LT setting.⁹ Furthermore, our study corroborates DM as a well-known risk factor for CKD.³ In LT patients with DM, a careful approach to tacrolimus management, including reducing the IPV of tacrolimus levels, is essential for minimizing the risk of CKD.

Our study has several limitations. First, this retrospective study may have introduced an inherent selection bias. Due to its retrospective design, there might be unmeasured variables, including potentially nephrotoxic drugs, which could affect the development of CKD. Additionally, we were unable to evaluate the impact of post-LT complications and newly onset diseases, which may influence kidney function. Second, this was a single-center cohort study, the generalizability of our findings may be limited. Particularly, our study was conducted in a setting where HBV infection was the predominant cause of LT and LDLT was the preferred method over DDLT. Consequently, our results should be validated in environments where different causes and types of LT are more prevalent. Despite these limitations, our detailed analysis, encompassing a large number of participants and a long-term follow-up, effectively demonstrated the risks of CKD and ESRD in LT patients. Furthermore, we investigated the effects of IPV of tacrolimus levels on the risk of CKD development. To further substantiate our findings, additional studies are necessary to validate the risks associated with the IPV of tacrolimus levels in CKD and ESRD development in post-LT patients.

In conclusion, our study highlights the significant burden of CKD and ESRD based on baseline kidney function. This study provides insights into the optimal trough level of tacrolimus and emphasizes the impact of the IPV of tacrolimus levels on CKD and ESRD development. Consequently, our findings underscore the importance of the meticulous management of tacrolimus, including avoiding high levels and steep fluctuations in tacrolimus doses and levels, as a

strategy to reduce the risk of CKD and ESRD.

Authors' contribution

S.K.L., and J.Y.C. designed the experiments. S.K.L., H.J.C., Y.K.Y., and P.S.S. collected the data. S.K.L., S.K.Y., J.W.J., and J.Y.C. analyzed and interpreted the data. S.K.L. wrote the manuscript. J.Y.C. edited the manuscript and supervised the study.

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Conflicts of Interest

The authors have no conflicts to disclose.

SUPPLEMENTARY MATERIAL

Supplementary material is available at Clinical and Molecular Hepatology website (<http://www.e-cmh.org>).

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