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ARTICLE

High intrapatient variability of tacrolimus exposure associated with poorer outcomes in liver transplantation

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Abstract

Tacrolimus (TAC) is a dose-dependent immunosuppressor with considerable intrapatient variability (IPV) in its pharmacokinetics. The aim of this work is to ascertain the association between TAC IPV at 6 months after liver transplantation (LT) and patient outcome. This single-center cohort study retrospectively analyzed adult patients who underwent transplantation from 2015 to 2019 who survived the first 6 months with a functioning graft. The primary end point was the patient's probability of death and the secondary outcome was the loss of renal function between month 6 and the last follow-up. TAC IPV was estimated by calculating the coefficient of variation (CV) of the dose-corrected concentration (C_0/D) between the third and sixth months post-LT. Of the 140 patients who underwent LT included in the study, the low-variability group (C_0/D CV < 27%) comprised 105 patients and the high-variability group (C_0/D CV ≥ 27%) 35 patients. One-, 3-, and 5-year patient survival rates were 100%, 82%, and 72% in the high-variability group versus 100%, 97%, and 93% in the low-variability group, respectively ($p = 0.005$). Moreover, significant impaired renal function was observed in the high-variability group at 1 year (69 ± 16 ml/min/1.73 m^2 vs. 78 ± 16 ml/min/1.73 m^2, $p = 0.004$) and at 2 years post-LT (69 ± 17 ml/min/1.73 m^2 vs. 77 ± 15 ml/min/1.73 m^2, $p = 0.03$). High C_0/D CV 3–6 months remained independently associated with worse survival (hazard ratio = 3.57, 95% CI = 1.32–9.67, $p = 0.012$) and loss of renal function (odds ratio = 3.47, 95% CI = 1.30–9.20, $p = 0.01$). Therefore, high IPV between the third and sixth months appears to be an early and independent predictor of patients with poorer liver transplant outcomes.

Abbreviations: BPAR, Biopsy proven acute rejection; BMI, Body mass index; CKD-EPI, chronic kidney disease epidemiology collaboration; CV, coefficient of variation; C_0/D, dose-corrected concentration; CMV, cytomegalovirus; eGFR, estimated glomerular filtration rate; HR, hazard ratio; HCC, hepatocellular carcinoma; ICU, intensive care unit; IPV, intrapatient variability; i.v., intravenously; LC–MS/MS, liquid chromatography-tandem mass spectrometry; LT, liver transplantation; MELD, model for end-stage liver disease; MMF, mycophenolate mofetil; NASH, Non-Alcoholic Steatohepatitis; OR, odds ratio; PCR, polymerase chain reaction; SD, Standard Deviation; TAC, tacrolimus; 3–6 M, three–six months.

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Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
There is high intrapatient variability of tacrolimus and its correlation with liver transplantation (LT) outcomes.

WHAT QUESTION DID THIS STUDY ADDRESS?
Could the intrapatient variability of tacrolimus between months 3 and 6 post-LT be a potential prognostic tool for poor outcomes?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?
Those patients with dose-corrected concentration coefficient of variation greater than or equal to 27% between months 3 and 6 post-LT have worst overall survival and impaired renal function.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?
If we promptly identify those patients, a closer therapeutic drug monitoring program should be imperative with the possibility to make therapeutic interventions to improve outcomes.

INTRODUCTION

Liver transplantation (LT) has achieved a 5- and 10-year survival of ~70% and 50%, respectively,[1] with long-term complications of immunosuppression (IS) being the most common causes of death.[2,3] In addition, short-term survival after LT has successfully improved over time, but, regrettably, long-term survival remains unchanged.[4] The ideal scenario would be to establish an optimal balance between the prevention of graft rejection and IS-related side effects, particularly nephrotoxicity.

In fact, early high-dose tacrolimus (TAC) exposure[5,6] after LT and intrapatient variability (IPV) in TAC exposure[7–9] have been related to poor long-term outcomes. TAC C_0 levels between 6 and 10 ng/ml during the first month post-LT are currently recommended.[3,6,10] However, its pharmacokinetics is characterized by a high IPV leading to an unpredictable dose–response relationship. IPV increase in kidney transplantation has been related to a reduction in renal function, higher rejection rates, development of de novo donor-specific antibody, progression of histologic lesions in renal allografts, and graft loss.[11–14] Evidence is more limited in LT recipients. Supelana et al.[7] were the first to identify the clinical impact of IPV in TAC exposure observing a rejection rate almost twice as high as the rejection rate described in LT recipients. Rayar et al.[8] found a correlation between IPV in TAC exposure measured in the first month post-LT and graft loss. Recently, Van der Veer et al.[9] collected IPV data between 6 and 18 months post-LT as a more stable time frame but no association between high IPV and immune-mediated graft injury could be demonstrated; however, a greater loss of renal function per year was observed.

Ideally, IPV should be determined in a stable clinical period with a sufficient number of TAC C_0 determinations. On the other hand, this should be early enough after transplantation to allow for diagnosing and carrying out therapeutic interventions when high IPV is observed.[15] For this reason, we considered that IPV in TAC exposure between the third and sixth months post-LT may be a useful tool for the early prediction of poor outcomes.

Therefore, we aimed to ascertain the correlation between TAC IPV at the sixth month post-LT and patient outcome.

MATERIALS AND METHODS

Study design

This retrospective single-center cohort study (Hospital Vall d'Hebron, Barcelona, Spain) of adult LT recipients from a prospectively obtained database was designed to evaluate the impact of IPV in prolonged-release TAC on patient outcome using the coefficient of variation (CV) of dose-corrected concentration (C_0/D) between the third and sixth months post-LT.

Inclusion criteria

Adult patients who undergo LT receiving prolonged-release TAC-based IS from the first week post-LT and surviving the first 6 months with a functioning graft were included in this study.

Exclusion criteria

Patients with early retransplant (first 6 months post-LT), or receiving MeltDose-TAC, or with <5 TAC C_0 values between 3 and 6 months post-LT or with incomplete patient records were excluded. Hospitalized patients between the third to sixth months post-LT were also excluded from the study because they were considered to be nonclinically stable with the need for temporarily lowering immunosuppressive drugs or use of other interacting drugs.

All liver transplant recipients are followed up in our outpatient clinics and concentrations from whole-blood samples taken between 7 and 10 a.m. before receiving the TAC oral doses were considered for analysis. Clinical and laboratory information (liver and renal function, tacrolimus concentration, drug dosing, and mild and long-term outcomes) are incorporated into our electronic medical files through a centralized computer database.

The study was approved by our institutional review board and conducted in compliance with the provisions of the Declaration of Helsinki and Good Clinical Practice guidelines.

Intrapatient tacrolimus variability

Tacrolimus variability was estimated by the CV calculated as follows: CV (%) = SD of TAC trough concentrations/mean TAC trough concentrations ×100. Because dose corrections were made during follow-up to maintain TAC levels within range, C_0/D (C_0/D CV) variability was calculated using the total of TAC doses ingested on the day prior to each trough level measurement: C_0/D CV (%) = SD/mean C_0/D ×100.[14]

Three post-LT periods were identified: the first month, between the second and the third months and between the third and sixth months. Because the last period was considered the most stable, with minimal interference of postoperative complications and concomitant medications, it was chosen as the optimal period for C_0/D CV analysis. At least four TAC_0 trough sample concentrations were required to calculate the C_0/D CV for each patient.

This study population was divided into two groups according to the C_0/D CV during the third period (low C_0/D CV 3–6 months group and high C_0/D CV 3–6 months group). The cutoff was 27% corresponding to the third quartile of the C_0/D CV between the third and sixth months post-LT.

Surgical procedure

All patients underwent orthotopic LT from brain-death donors or donations after cardiac death using normothermic regional perfusion. The standardized technique with inferior vena cava preservation was used.[16]

Immunosuppression and monitoring

Standard immunosuppression included the use of TAC, mycophenolate mofetil (MMF), and methylprednisolone.

Tacrolimus was introduced at 0.05 mg/kg twice daily (Prograf; Astellas Pharma, Chertsey, UK) and dosage adjustments were made to achieve a trough level of 5 to 8 ng/dl for the first 6 months and <5 ng/dl thereafter if no rejection occurred. Methylprednisolone was started intra-operatively at doses of 500 mg i.v., followed by 200 mg i.v. per day, tapered to 20 mg orally per day over 6 days until 2017. From 2018, the intra-operative dose of 500 mg i.v. was followed by 20 mg i.v. per day. During follow-up, methylprednisolone was reduced to 16 mg orally per day at the fourth week, tapered to minimum doses for the following 3 months, and discontinued in all patients with normal liver function, except those with autoimmune disease. MMF was introduced at a dose of 500 g twice a day for 6 months post-transplant and discontinued promptly if hematologic events occurred.

Induction therapy with Basiliximab (Simulect; Novartis, Basel, Switzerland) 20 mg on days 0 and 4 was used in patients with pre-LT renal dysfunction defined as an estimated glomerular filtration rate (eGFR) < 60 ml/min/1.73 m^2 following the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula.

Patients were switched from twice-daily Prograf to prolonged-release TAC (Advagraf; Astellas Pharma, Meppel, The Netherlands) on a 1:1.1 mg basis prior to hospital discharge. A small group of patients received a MeltDose TAC formulation (Envarsus; Chiesi Pharmaceuticals, Parma, Italy), as they were included in a clinical study.

Everolimus (Certican, Novartis Pharma Setin AG) was introduced between 4- and 6-weeks post-LT for the following indications: prevention of hepatocellular carcinoma (HCC) recurrence in patients with microvascular invasion or beyond Milan criteria confirmed in the explanted liver, severe acute rejection, or neurotoxicity. The everolimus target trough level was targeted at 2–3 ng/dl and TAC at 4–6 ng/dl.

TAC whole-blood trough concentration was measured daily during the hospital admission. After discharge, TAC levels were monitored weekly for the first month post-LT and every 2 weeks or monthly thereafter until the sixth month post-LT. Most TAC concentrations (88 patients, 70 in the low C_0/D CV 3–6 M group and 18 in the high C_0/D CV 3–6 month group, $p = 0.9$) were analyzed in whole-blood samples using a validated liquid chromatography-tandem mass spectrometry (LC–MS/MS) method, with a lower limit of quantification of 0.5 ng/ml. The remaining TAC concentrations of the model building dataset (9%) were measured before the introduction of the LC–MS/MS method (April 2018) using the immunoassay with a lower limit of quantification of 1.0 ng/ml. The accuracy of the quality-control samples was between 85% and 115% and the intra- and interassay

imprecision was <15% during the study period. As it is known that there is a difference between TAC concentrations measured using an LC–MS/MS and an immunoassay, this was built into the residual error model.[10]

Concomitant drugs to immunosuppression

Other concomitant drugs were omeprazole and trimethoprim-sulfamethoxazole for pneumocystis prophylaxis. Cytomegalovirus (CMV) prophylaxis with ganciclovir was indicated when the donor was positive and the recipient negative. CMV viral load was monitored weekly by polymerase chain reaction (PCR) for the first month post-LT and monthly thereafter.

Graft rejection and treatment

All biopsy-proven acute rejection (BPAR) episodes were collected and stratified according to BANFF criteria.[17] Treatment included increasing doses of TAC if rejection was mild and three boluses of methylprednisolone (500 mg i.v.) if episodes were moderate or severe.

Infectious complications

Cytomegalovirus infection was considered if the viral load by PCR was positive. Other infections requiring treatment with antiviral, oral, or i.v. antibiotics, percutaneous drainage, or surgical intervention were also collected until the last follow-up.

Other complications

The more frequent complications during follow-up were postoperative vascular and biliary complications, neurologic (confusion, tremor, seizures, and hepatic encephalopathy) and cardiovascular (acute coronary disease, acute pulmonary edema, and arrhythmia) complications.

End points

The primary end point was the patient's death after the sixth month post-LT until the last follow-up. Patient survival was defined from the date of transplantation to death or last follow-up (September 2021). Causes of death and graft loss were also collected.

The secondary end point was the loss of renal function defined as decrease of eGFR > 10 ml/min/1.73 m^2 at 1 year post-LT from the baseline (month 3 post-LT) and computed by the eGFR CKD-EPI formula. The incidence of acute rejection, infections, and other complications until the last follow-up were also analyzed.

Statistical analysis

Demographic and baseline data of recipients, donors, surgical procedure, and outcome were collected.

Categorical variables were summarized as counts and percentages and continuous variables as medians with range. Comparisons between low C_0/D CV 3–6 month and high C_0/D CV 3–6 month groups were made by Student's *t*-test or Wilcoxon test as appropriate for continuous data and chi-square test with Fisher's correction for categorical data. The Friedman test was used to detect differences among different values of one variable. Survival analysis was performed with the Kaplan–Meier curve and compared with the log rank test.

A multivariable Cox proportional hazard models was used to ascertain whether C_0/D CV 3–6 months was an independent predictor for impaired patient survival after LT. Patients with missing covariates were excluded from the final analysis. Seven covariates were selected after univariate analysis and according to the literature[18–20]: recipient age at transplantation, HCC as a main indication for LT, Model for End-Stage Liver Disease (MELD) score pre-LT, cardiovascular disease, diabetes mellitus pre-LT, and use of steroids between 3 and 6 months post-transplantation.

Regarding the study of the secondary end point, a multivariable logistic regression model with a stepwise selection method was used to test the association between C_0/D CV 3–6 months and loss of renal function. We excluded from the analyses those patients with eGFR < 60 ml/min/1.73 m^2 at month 3 post-LT for being considered a relevant confounder.

Differences were considered statistically significant when $p < 0.05$. Statistical analysis was performed using IBM SPSS Statistics 24.0 software.

RESULTS

Study population

One hundred seventy-nine liver transplantations were performed between January 2015 and September 2019. In the final analyses, 140 liver transplant recipients were included. Of the 39 excluded patients, 18 were on MeltDose TAC, 11 were hospitalized between the third and sixth months post-LT, four had insufficient samples to calculate the C_0/D CV between the third and sixth months, four died before the sixth month post-LT, and two were early retransplants (Figure 1).

Median follow-up was 46 months (range: 13–80 months).

Tacrolimus variability

The distribution of TAC trough level, CV, and C_0/D CV for the first 6 months post-transplant is shown in Table 1.

The low-variability group comprised 105 patients with a mean C_0/D CV 3–6 months of 14 ± 7% and median 14% (range: 0–27) whereas the high-variability group included 35 patients with a mean C_0/D CV 3–6 months of 35 ± 7% and median 32% (range: 27–55). The mean TAC C_0 and C_0/D at the third to sixth months was 7.4 ± 1.2 ng/ml and 1.9 ± 1.6 ng/ml/mg in the low-variability group compared to 6.7 ± 1.9 ng/ml ($p = 0.09$) and 2.0 ± 1.9 ng/ml/mg ($p = 0.85$) in the high-variability group, respectively. The correlation coefficient between CV and C_0/D CV 3–6 months was 0.562 ($p < 0.001$).

Main characteristics of the study population

The main characteristics of the study population are summarized in Table 2. There were no differences in recipient, donor, and transplant characteristics between the groups. The main factors associated with TAC variability in the literature[10] were also analyzed (body mass index [BMI], albumin, and hemoglobin) between the third and sixth months post-transplant and no significant differences

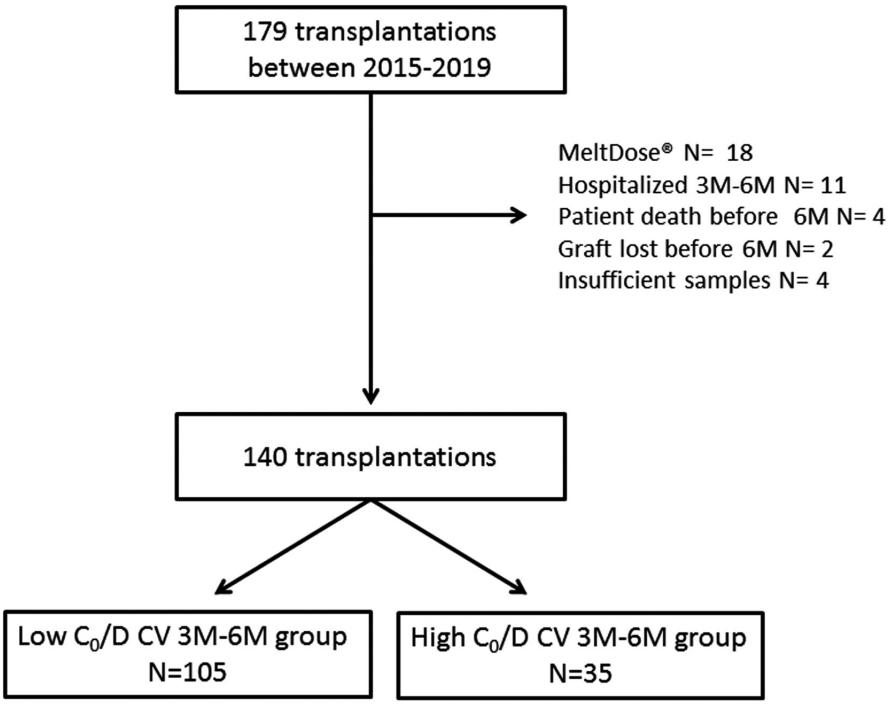

FIGURE 1 Flowchart of the study population. C_0/D, dose-corrected concentration; CV, coefficient of variation; M, month

TABLE 1 Tacrolimus concentration variability during the first 6 months post-transplant

N = 140	TAC C_0 ≤1 M (ng/ml)	TAC C_0 1–3 M (ng/ml)	TAC C_0 3–6 M (ng/ml)	TAC CV ≤1 M (%)	TAC CV 1–3 M (%)	TAC CV 3–6 M (%)	TAC C_0/D CV 3–6 M (%)
Mean	7.77	8.24	7.29	31.19	29.50	25.26	19.54
Median	7.68	8.57	7.15	25.29	25.16	23.00	17.82
Standard deviation	2.24	2.30	1.98	19.30	16.91	17.00	11.18
Minimum	2.26	2.32	1.25	4.26	2.25	0.00	0.00
Maximum	13.32	16.62	16.81	98.84	95.45	88.24	55.49
Quartile							
1st	6.21	6.63	6.30	17.22	17.37	11.89	10.70
2nd	7.68	8.57	7.15	25.29	25.16	23.00	17.82
3rd	9.43	9.69	8.45	40.19	38.61	35.50	27.00

Abbreviations: C_0/D, concentration/dose; CV, coefficient of variation; 1 M, 1 month; 1–3 M, 1–3 months; 3–6 M, 3–6 months; TAC, tacrolimus.

were found when the low and high TAC C_0/D CV 3–6 months groups were compared (Table S1). Moreover, both groups were similar regarding TAC maintenance therapy received during this period (Table S1).

Post-operative outcomes during the early period

No differences were observed in the incidence of arterial or biliary complications between the low and high C_0/D CV 3–6 months group at the end of follow-up.

A higher frequency of overall infections during the first 3 months post-LT was observed in the low TAC C_0/D CV 3–6 month group (25% vs. 9%, $p = 0.04$). No other significant differences were reported between groups regarding the frequency of acute rejection and CMV infection, or the presence of neurologic and cardiovascular disorders (Table 3).

Primary end point

The probability of death is shown in Figure 2. Eight patients died in the low C_0/D CV 3–6 month group (8/105, 8%) owing to de novo solid tumor ($n = 3$), HCC recurrence ($n = 2$), lympho-proliferative disease ($n = 1$), and cardiovascular disease ($n = 2$). Causes of death in the high C_0/D CV 3–6 month group (8/35, 23%) were: HCC recurrence in four patients, de novo solid tumor in three patients, and infection in one patient.

TABLE 2 Donor, recipient, and peri-transplant characteristics

	Entire population ($n = 140$)	Low C_0/D CV 3–6 M ($n = 105$)	High C_0/D CV 3–6 M ($n = 35$)	*p* Value
Age (years)	56 (18–71)	56 (18–71)	58 (34–68)	0.29
Male, *n* (%)	106 (76%)	77 (73%)	29 (83%)	0.18
Primary liver disease, *n* (%)				
HCC	66 (47%)	50 (48%)	16 (46%)	0.84
Alcoholic	22 (16%)	16 (15%)	6 (17%)	0.78
Hepatitis C	12 (9%)	8 (8%)	4 (11%)	0.48
Hepatitis B	9 (7%)	9 (9%)	–	0.11
Cholestatic	9 (7%)	7 (7%)	2 (6%)	0.84
NASH	4 (3%)	2 (2%)	2 (6%)	0.24
Pretransplant arterial hypertension, *n* (%)	38 (27%)	31 (30%)	7 (20%)	0.27
Pretransplant diabetes mellitus, *n* (%)	46 (33%)	34 (32%)	12 (34%)	0.83
Pretransplant heart disease, *n* (%)	11 (8%)	8 (8%)	3 (9%)	0.85
Pretransplant eGFR (ml/min/1.73 m^2)	79 ± 21	79 ± 23	77 ± 18	0.61
Pretransplant eGFR < 60 ml/min/1.73 m^2	24 (17%)	18 (17%)	6 (17%)	0.98
MELD score	16 (6–42)	16 (6–42)	15 (6–26)	0.30
Donor age	53 (16–87)	53 (16–83)	56 (16–87)	0.28
Cold ischemia time (min)	347 (155–660)	342 (155–660)	361 (234–547)	0.24
Warm ischemia time (min)	40 (20–113)	39 (20–65)	43 (20–113)	0.08
Intra-operative transfusion				
Red blood cells (unit)	4 (0–16)	4 (0–16)	4 (0–14)	0.84
Fresh frozen plasma (unit)	3 (0–22)	4 (0–18)	3 (0–22)	0.21
Platelets (unit)	1 (0–7)	1 (0–6)	1 (0–7)	0.71
Moderate–severe histologic reperfusion injury	42/123 (34%)	30/93 (32%)	12/30 (40%)	0.43
ICU stay	5 (1–45)	5 (1–45)	4 (1–8)	0.14
Total hospitalization stay	17 (5–160)	16 (5–63)	18 (6–160)	0.54

Abbreviations: C_0/D, concentration/dose; CV, coefficient of variation; 3–6 M, 3–6 months; eGFR, estimated glomerular filtration rate; HCC, hepatocellular carcinoma; ICU, intensive care unit; MELD, Model for End-Stage Liver Disease; NASH, non-alcoholic steatohepatitis.

TABLE 3 Post-transplant patient outcomes

	Entire population ($n = 140$)	Low C_0/D CV 3–6 M ($n = 105$)	High C_0/D CV 3–6 M ($n = 35$)	p Value
Arterial complications	1 (0.7%)	1 (1%)	–	0.56
Biliary complications	10 (7%)	8 (8%)	2 (6%)	0.70
Acute rejection				
<3 months	31 (22%)	22 (21%)	9 (26%)	0.55
3–6 months	2 (1.4%)	1 (1%)	1 (3%)	0.41
≥6 months	11 (8%)	7 (7%)	4 (11%)	0.36
Infection rate				
<3 months	29 (21%)	26 (25%)	3 (9%)	0.04
3–6 months	4 (3%)	3 (3%)	1 (3%)	0.99
≥6 months	14 (10%)	11 (11%)	3 (9%)	0.74
Cytomegalovirus infection				
<3 months	50 (36%)	39 (37%)	11 (31%)	0.54
3–6 months	7 (5%)	5 (5%)	2 (6%)	0.82
≥6 months	4 (3%)	3 (3%)	1 (3%)	0.99
Neurologic complications				
<3 months	10 (7%)	7 (7%)	3 (9%)	0.70
3–6 months	–	–	–	–
≥6 months	15 (11%)	11 (11%)	4 (11%)	0.87
Cardiovascular complications				
<3 months	2 (1.4%)	1 (1%)	1 (3%)	0.41
3–6 months	–	–	–	–
≥6 months	9 (6.4%)	7 (7%)	2 (6%)	0.84

Abbreviations: C_0/D, concentration/dose; CV, coefficient of variation.

One-, 3-, and 5-year unadjusted cumulative patient survival rates were 100%, 97%, and 93% in the low C_0/D CV 3–6 month group versus 100%, 82%, and 72% in the high C_0/D CV 3–6 month group, respectively (log rank $p = 0.005$).

The rate of re-transplantation was 4% ($n = 4$) in the low C_0/D CV 3–6 month group owing to chronic acute rejection. Similar results were observed in high C_0/D CV 3–6 month group ($n = 1$, 3%) with no significant differences ($p = 0.79$).

Seven potential variables were candidates for inclusion in the multivariable Cox regression analysis and, after backward stepwise elimination, C_0/D CV 3–6 months, recipient age, and pretransplant cardiovascular disease remained in the final model (Table 4). The multivariate Cox regression model showed high C_0/D CV 3–6 months (hazard ratio [HR] = 3.57, 95% confidence interval [CI] = 1.32–9.67, $p = 0.012$), recipient age >60 years (HR = 3.39, 95% CI = 1.05–10.99, $p = 0.04$) and pretransplant cardiovascular disease (HR = 6.13, 95% CI = 1.58–23.75, $p = 0.04$) as the main risk factors for patient death (Table 4).

Secondary end point

Regarding renal function, the mean of eGFR at month 3 post-LT was similar in both groups ($p = 0.24$), as shown in Figure 3. However, significant impaired renal function was demonstrated in the high C_0/D CV 3–6 month group at 1 year (69 ± 16 ml/min/1.73 m^2 vs. 78 ± 16 ml/min/1.73 m^2, $p = 0.004$) and at 2 years post-LT (69 ± 17 ml/min/1.73 m^2 vs. 77 ± 15 ml/min/1.73 m^2, $p = 0.03$).

Loss of renal function at 1 year post-LT from the baseline (month 3 post-LT) was observed in 19 of 105 patients (18%) in the low C_0/D CV 3–6 month group (−16 ± 6 ml/min/1.73 m^2) compared with 12 of 35 patients (34%) in the high C_0/D CV 3–6 month group (−20 ± 9 ml/min/1.73 m^2; $p = 0.04$).

After adjusting for potential confounders, high C_0/D CV 3–6 month (odds ratio [OR] = 3.47, 95% CI = 1.30–9.20, $p = 0.01$) and pretransplant diabetes mellitus (OR = 2.55, 95% CI = 1.03–6.32, $p = 0.04$) were the main predictors for loss of renal function in the multivariable logistic regression model (Table 4).

High C_0/D 3–6 months CV was not statistically significant as a predictor for acute cellular rejection (OR = 1.78,

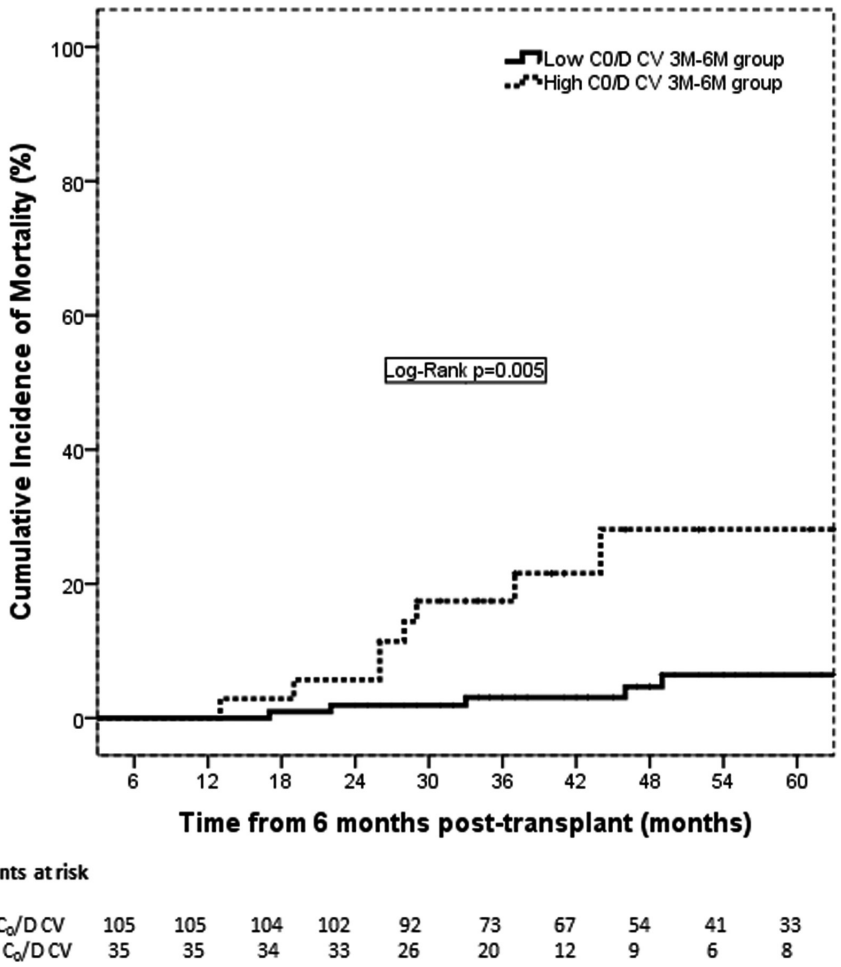

FIGURE 2 Probability of death. C_0/D, dose-corrected concentration; CV, coefficient of variation

95% CI = 0.49–6.51, $p = 0.37$), CMV viremia (OR = 0.99, 95% CI = 0.10–9.84, $p = 0.99$), or overall infections (OR = 0.79, 95% CI = 0.20–3.02, $p = 0.73$) after the sixth month post-LT. No other relationships regarding cardiovascular or neurologic disorders were found.

DISCUSSION

High TAC IPV has emerged as a major prognostic risk factor after solid organ transplantation; however, data in LT are scant and on occasions with contradictory results when kidney and liver transplantations are compared.[8,9,11–14] In the present study, higher mortality and worse renal function post-LT were found in patients with high TAC IPV compared with those with low TAC IPV. However, we could not prove its impact on acute cellular rejection or other complications associated with calcineurin inhibitor-related adverse events (neurologic and cardiovascular complications) as published by other authors.[7,8,21]

One of the main reasons for this disparity in results could be the heterogeneity of the TAC IPV studies in LT, including how IPV was calculated, the period chosen for IPV analyses, the IPV target, and even the type of primary end point evaluated.[15]

In an attempt to determine the association between TAC metabolism and clinical outcomes, different pharmacokinetic and pharmacodynamic models taking into consideration clinical variables, such as hemoglobin, albumin, BMI, and polymorphisms of the most relevant genes encoding cytochrome-P450 enzymes have been developed.[10] However, it has been shown that a simple measurement of IPV using CV can predict the risk of developing TAC side effects.[22] Most studies use CV because it normalizes SD to the mean; however, it does not consider dose correction during follow-up.[8,9] Only Van der Veer et al.[9] stated that every obtained sample was normalized for the dose; however, they did not explain how. We opted to use TAC C_0/D CV which could more accurately reflect TAC variability.[14]

Regarding the period chosen for IPV analyses, the C_0/D CV between the third and sixth months was preferred because the patients are in a more stable situation considering that the hospitalization period is characterized by greater fluctuation in TAC C_0 due to concurrent disease and concomitant medications, among others, as was already reported.[15,23] Moreover, it is early enough to make therapeutic interventions to optimize outcomes.

TABLE 4 Multivariable Cox proportional hazard model for the impact of tacrolimus variability on the probability of death and multivariable logistic regression model for the impact of tacrolimus variability on the loss of renal function

Multivariable Cox proportional hazard model				
	Univariate		**Multivariate**	
	Hazard ratio (95% CI)	***p* Value**	**Hazard ratio (95% CI)**	***p* Value**
Recipient age ≥ 60 years	*3.34 (1.07–10.41)*	*0.03*	*3.39 (1.05–10.99)*	*0.041*
HCC	*2.66 (0.91–7.69)*	*0.07*		
MELD score	0.24 (0.05–1.09)	0.06		
Pretransplant cardiovascular disease	4.72 (1.29–17.30)	0.01	6.13 (1.58–23.75)	0.009
Pretransplant diabetes mellitus	0.65 (0.21–2.03)	0.65		
Use of steroids 3–6 M post-transplant	*1.99 (0.62–6.304)*	*0.23*		
High C_0/D CV 3–6 M post-transplant	*3.80 (1.41–10.23)*	*0.008*	*3.57 (1.32–9.67)*	*0.012*
Multivariable logistic regression model				
	Univariate		**Multivariate**	
	Odds ratio (95% CI)	***p* Value**	**Odds ratio (95% CI)**	***p* Value**
Recipient age ≥ 60 years	*1.71 (0.72–4.01)*	*0.21*		
Recipient male	0.47 (0.18–1.19)	0.11		
MELD score	2.03 (0.84–4.86)	0.11		
Pretransplant cardiovascular disease	1.62 (0.37–6.95)	0.51		
Pretransplant diabetes mellitus	*2.48 (1.03–5.94)*	*0.04*	*2.55 (1.03–6.32)*	*0.04*
Renal function at 3 M post-transplant	1.01 (0.98–1.04)	0.35		
Acute rejection ≤ 3 M post-transplant	0.91 (0.32–2.56)	0.86		
Overall infections ≤ 3 M post-transplant	0.83 (0.31–2.26)	0.72		
CMV infection ≤ 3 M post-transplant	1.50 (0.62–3.58)	0.36		
High C_0/D CV 3–6 M post-transplant	*3.37 (1.305–8.72)*	*0.01*	*3.47 (1.30–9.20)*	*0.01*

Abbreviations: C_0/D, concentration/dose; CI, confidence interval; CMV, cytomegalovirus; CV, coefficient of variation; 3–6 M, 3–6 months; HCC, hepatocellular carcinoma; MELD, Model for End-Stage Liver Disease.

p Value in italics are statistically significant.

The few studies published in this regard have shown different approaches. Rayar et al.[8] calculated the CV between postoperative days 8 and 30, Van der Veer et al.[9] collected at least five TAC C_0 between 6 and 18 months post-transplant and, in another two studies, a low number of TAC C_0 samplings over a time period of 2 or 3 years was used.[7,21] Despite the different outcomes, it is clear that performing CV calculation during the early postoperative phase is not ideal, but not too late if CV as a prognostic factor is to be considered.

The third interesting point for discussion is the target of CV TAC. The recommended CV TAC target is ≤20%, attempting to avoid values over 30%.[15,24] In the literature, the cutoff varies from 40% in the first month post-LT to 28% after the sixth month post-LT.[7,8] During the early period in our series, mean CV was over 30% owing to the different interactions, as explained previously. However, this value dropped below 30% after the third month post-LT. Between the third and sixth months post-LT, mean CV was 25 ± 15% and mean C_0/D CV 19 ± 11%. The cutoff chosen in the present study was 27% corresponding to the third quartile of the C_0/D CV.

Therefore, it is important to achieve a consensus on the best IPV TAC calculation, the post-transplant time frame and the best target for consistent results regarding the real impact of TAC variability in post-transplant outcomes.

The strength of our study lay in its homogeneous population; all patients were on prolonged-release TAC formulation (Advagraf) from hospital discharge to minimize the risk of nonadherence, one of the main causes

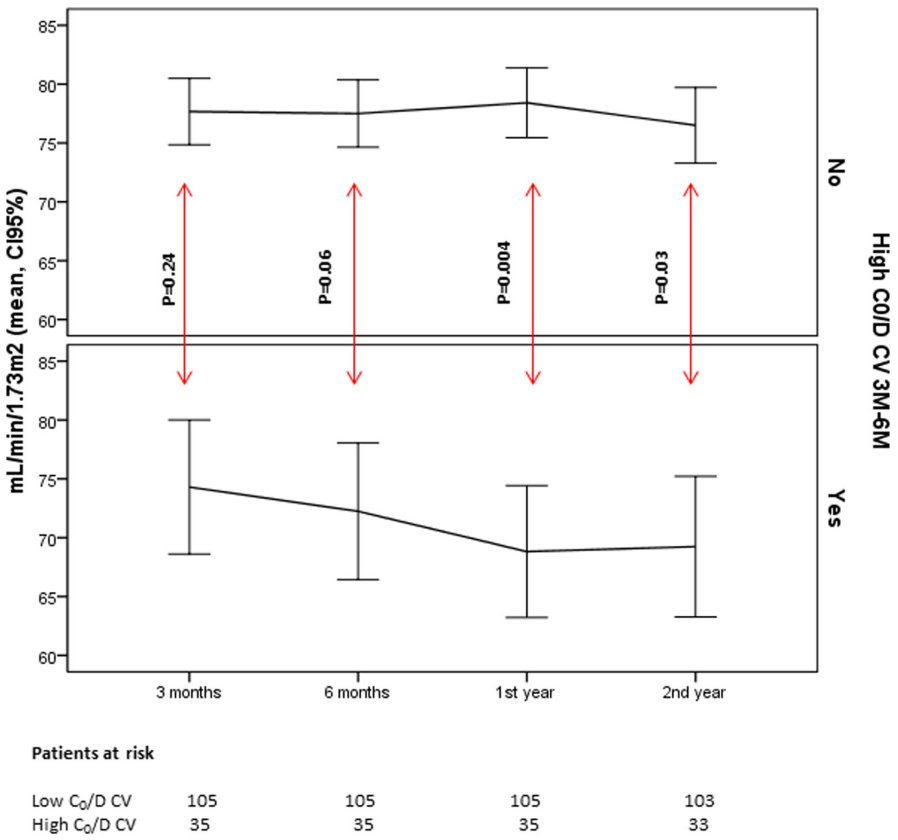

FIGURE 3 Renal function during post-transplant follow-up. C_0/D, dose-corrected concentration; CI, confidence interval; CV, coefficient of variation

of IPV.[15,24] TAC trough concentrations were similar in the low and high C_0/D CV groups for the first 6 months. Moreover, patients hospitalized between the third and sixth months post-LT were excluded to avoid clinical events that could interfere with TAC exposure. During this period, no differences were found between groups regarding BMI, albumin, and hemoglobin, which could also interfere with TAC levels.[10] Regarding early outcomes (episodes of rejection, infection, cardiovascular, or neurologic complications), only a slightly high rate of overall infections in the low C_0/D CV group during the first 3 months post-LT was observed, with no differences between groups thereafter.

Exactly how high C_0/D CV between the third and sixth months post-transplant is associated with poor long-term outcomes remains to be elucidated. Patients with fluctuating TAC concentrations likely undergo alternating episodes of under- and overexposure. These periods of overexposure could predispose to patient death owing to related causes (recurrence of original tumor, de novo tumor, cardiovascular disease, and infection) and TAC nephrotoxicity. On the other hand, we observed once again that liver grafts have lower allore-activity compared to kidney transplantation, requiring a lower target level of anticalcineurinic inhibitors and are less vulnerable to TAC underexposure, have been unable to support a relationship with acute rejection as the first studies published on IPV and LT.[7,21] In fact, the low rate of infections and rejections after the sixth month post-LT in our series could explain the absence of significant differences between patients with high and low CV. The same occurs with cardiovascular and neurologic complications. These results concur with those of recent published studies.[8,9]

Furthermore, patients at higher risk could be identified promptly and consequently and closer therapeutic drug monitoring should be undertaken to include measures to prevent the main causes associated with TAC IPV (i.e., the timing of TAC dosing in relation to food ingestion[25,26] or avoidance of certain types of food or herbal preparations[27,28] as a part of liver recipient education). The clinician should also be aware of drug–drug interactions[29,30] that can interfere with TAC metabolism and should promptly identify medication nonadherence for remedy of this clinical problem effectively from the beginning.[2] Less well-documented causes of IPV are the potential effects of different drug formulations and pharmacogenetics. In fact, some studies showed that the CYP3A5 genotype had no impact on the IPV of TAC clearance in kidney transplant recipients[31,32]; nevertheless, it may impact on kidney rejection.[33] We may hypothesize that having recipient CYP3A5 genotypes prior to LT could be useful to lower dose adjustments in the post-LT period and consequently minimize TAC IPV.

Our results must be interpreted with some caution as our study also had limitations. The sample size was

smaller than previous studies; however, it sufficed to detect significant differences in our primary composite end point. It may have had insufficient power to detect small significant differences owing to low event rates of acute rejection, infections, and neurologic or cardiovascular complications after the sixth month post-LT. Second, the retrospective nature of this study may have introduced bias and even complete certainty of medication adherence was lacking. Indeed, the immunosuppressive regimen was constant over the study period, and the close follow-up of these patients using a prolonged-release TAC formulation and the strict selection criteria should minimize bias due to the retrospective nature. Third, identifying a definitive CV cutoff is challenging due to center differences in the TAC therapeutic windows and it is possible that the extent of variability may be larger before becoming clinically significant among certain population (e.g., liver transplant recipients or pediatric recipients).[23] Current data are not yet strong enough to support a definitive CV cutoff and for this reason we chose the third quartile.

In conclusion, high IPV between the third and sixth months appears to be an early and independent predictor of poorer outcomes thus an intensified follow-up policy should be required. Further studies are needed to establish the clinical benefit of IPV-based strategies together with some pharmacogenetic clusters (CYP3A4 genotype) to improve long-term results.

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CONFLICT OF INTEREST

The authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS

C.D., I.B., S.G., I.C.V., and R.C wrote the manuscript. C.D., I.B., S.G., B.M., F.M., and R.C designed the research. C.D., S.G., B.M., C.G.G., M.C., L.C., and E.H. performed the research. C.D., S.G., and B.M. analyzed the data.

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SUPPORTING INFORMATION

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