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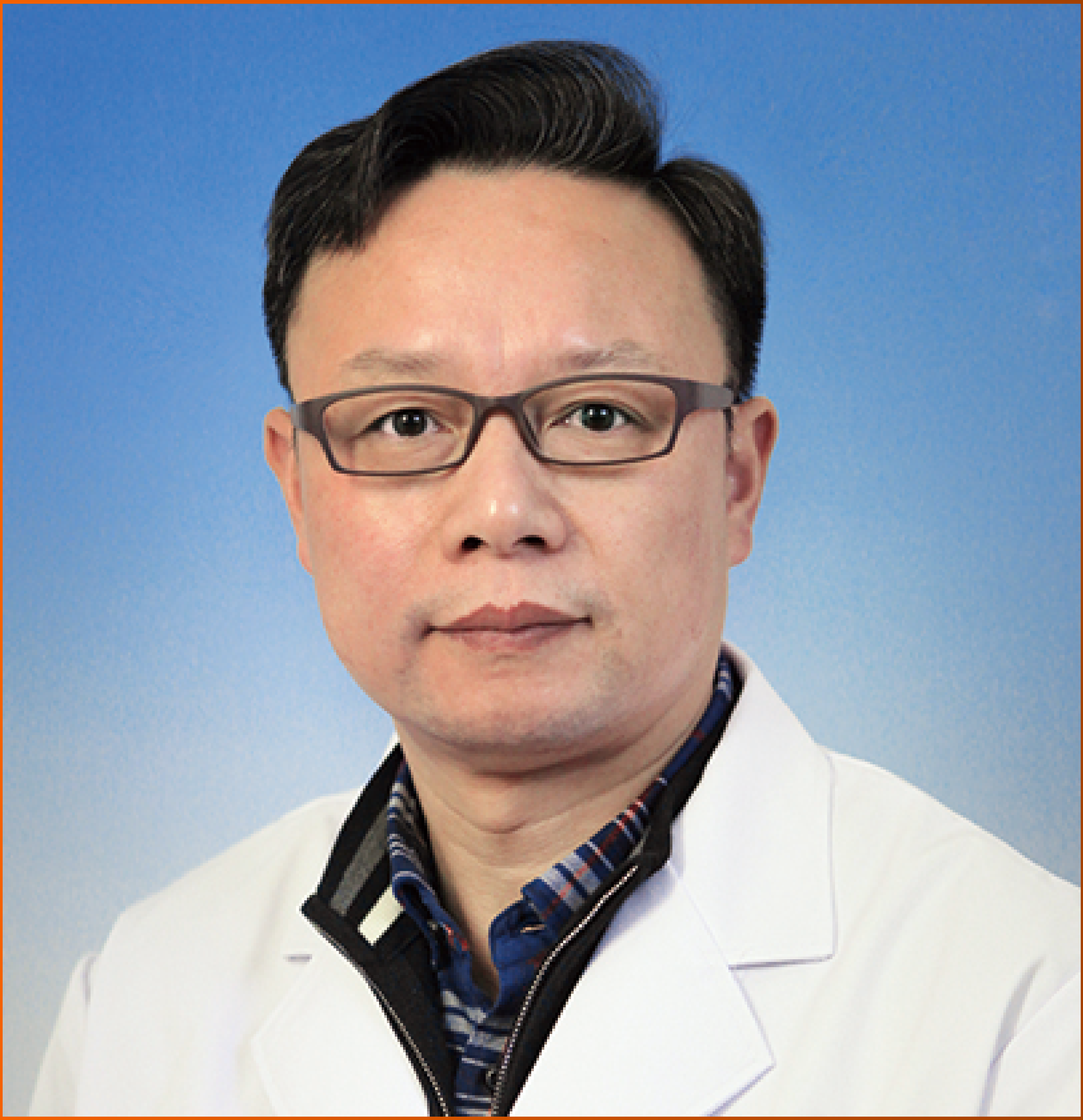

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Contents

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ABOUT COVER

Editorial Board Member of *World Journal of Hepatology*, Dr. Guang-Hua Luo is Director of the Clinical Medical Research Center in the Third Affiliated Hospital of Soochow University (China). He graduated from the Laboratory Medicine Department of Zhenjiang Medical College in 1996, obtained a PhD in 2013 at Soochow University, and was nominated to professorship in 2014. Currently, he is an editorial board member of Endocrine, Metabolic & Immune Disorders-Drug Targets. He has published more than 240 papers, 86 of which are included in SCI. Since 2009, four of his inventions have obtained patent authorization. His research activities mainly focus on molecular diagnostics in personalized medicine and on the regulatory role and mechanism of apolipoprotein M in liver diseases and chronic inflammatory diseases. (L-Editor: Filipodia)

AIMS AND SCOPE

The primary aim of *World Journal of Hepatology* (*WJH*, *World J Hepatol*) is to provide scholars and readers from various fields of hepatology with a platform to publish high-quality basic and clinical research articles and communicate their research findings online.

WJH mainly publishes articles reporting research results and findings obtained in the field of hepatology and covering a wide range of topics including chronic cholestatic liver diseases, cirrhosis and its complications, clinical alcoholic liver disease, drug induced liver disease autoimmune, fatty liver disease, genetic and pediatric liver diseases, hepatocellular carcinoma, hepatic stellate cells and fibrosis, liver immunology, liver regeneration, hepatic surgery, liver transplantation, biliary tract pathophysiology, non-invasive markers of liver fibrosis, viral hepatitis.

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The *WJH* is now abstracted and indexed in PubMed, PubMed Central, Emerging Sources Citation Index (Web of Science), Scopus, China National Knowledge Infrastructure (CNKI), China Science and Technology Journal Database (CSTJ), and Superstar Journals Database. The *WJH*'s CiteScore for 2019 is 5.8 and Scopus CiteScore rank 2019: Hepatology is 22/61.

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ORIGINAL ARTICLE

Retrospective Study

Early tacrolimus exposure does not impact long-term outcomes after liver transplantation

Mikel Gastaca, Patricia Ruiz, Javier Bustamante, Lorea Martinez-Indart, Alberto Ventoso, José Ramón Fernandez, Ibone Palomares, Mikel Prieto, Milagros Testillano, Patricia Salvador, Maria Senosiain, Maria Jesus Suárez, Andres Valdivieso

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Abstract

BACKGROUND

Tacrolimus trough levels (TTL) during the first weeks after liver transplantation (LT) have been related with long-term renal function and hepatocellular carcinoma recurrence. Nevertheless, the significance of trough levels of tacrolimus during the early post-transplant period for the long-term outcome is under debate

AIM

To evaluate the effect of TTL during the first month on the long-term outcomes after LT.

METHODS

One hundred fifty-five LT recipients treated *de novo* with once-daily tacrolimus were retrospectively studied. Patients with repeated LT or combined transplantation were excluded as well as those who presented renal dysfunction prior to transplantation and/or those who needed induction therapy. Patients were classified into 2 groups according to their mean TTL within the first month after transplantation: ≤ 10 (n = 98) and > 10 ng/mL (n = 57). Multivariate analyses were performed to assess risk factors for patient mortality.

RESULTS

CEIC E13/08.

Informed consent statement: Patients gave written consent to be included in the liver transplantation prospective data base. The requirement for specific informed consent for this study was waived because of the retrospective nature of the study.

Conflict-of-interest statement: MG is a member of advisory boards and has received honoraria from Astellas, Novartis and Chiesi. JB has received honoraria from Astellas and Novartis. AV has received honoraria from Astellas and Novartis. All other authors have no conflicts to declare.

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Mean levels within the first month post-transplant were 7.4 ± 1.7 and 12.6 ± 2.2 ng/mL in the ≤ 10 and > 10 groups, respectively. Donor age was higher in the high TTL group 62.9 ± 16.8 years *vs* 45.7 ± 17.5 years (P = 0.002) whilst mycophenolate-mofetil was more frequently used in the low TTL group 32.7% *vs* 15.8% (P = 0.02). Recipient features were generally similar across groups. After a median follow-up of 52.8 mo (range 2.8-81.1), no significant differences were observed in: Mean estimated glomerular filtration rate (P = 0.69), hepatocellular carcinoma recurrence (P = 0.44), *de novo* tumors (P = 0.77), new-onset diabetes (P = 0.13), or biopsy-proven acute rejection rate (12.2% and 8.8%, respectively; P = 0.50). Eighteen patients died during the follow-up and were evenly distributed across groups (P = 0.83). Five-year patient survival was 90.5% and 84.9%, respectively (P = 0.44), while 5-year graft survival was 88.2% and 80.8%, respectively (P = 0.42). Early TTL was not an independent factor for patient mortality in multivariate analyses.

CONCLUSION

Differences in tacrolimus levels restricted to the first month after transplant did not result in significant differences in long-term outcomes of LT recipients.

Key Words: Liver transplantation; Tacrolimus levels; Prolonged released tacrolimus; Once-daily tacrolimus; Renal function; Survival; Outcomes

Core Tip: This is a retrospective study to evaluate the effect of early tacrolimus trough levels (TTL) on the long-term outcomes after liver transplantation. Patients were classified into 2 groups according to mean TTL within the first month: ≤ 10 (n = 98) and > 10 ng/mL (n = 57). After a median follow-up of 52.8 mo (range 2.8-81.1), no significant differences were observed in: Mean estimated glomerular filtration rate, hepatocellular carcinoma recurrence, *de novo* tumors, biopsy-proven acute rejection rate and five-year patient and graft survival. Differences in tacrolimus levels within the first month after liver transplant did not result in significant differences in long-term outcomes.

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INTRODUCTION

Tacrolimus represents the keystone of current immunosuppressive regimens after liver transplantation (LT)[1]. Monitoring of trough drug levels is required to maintain them within the therapeutic range[2]. In the case of LT, there is some debate regarding the significance of trough levels of tacrolimus in the early post-transplant period for the long-term outcome. Initial recommendations were extrapolated from kidney transplantation, but LT does not require the high doses needed to prevent acute cellular rejection (ACR) in other allografts[3]. In this regard, various studies have explored the idea of minimizing initial tacrolimus trough levels (TTL)[4-6].

Mean TTL < 10 ng/mL within the first month after LT was associated with less renal impairment within 1 year in a recent meta-analysis[7]. In this study, tacrolimus concentration between 6 and 10 ng/mL were recommended as more appropriate after LT. Mean TTL > 10 ng/mL within the first month after LT but not thereafter has been also associated with increased risk of hepatocellular carcinoma (HCC) recurrence[8]. High exposure to calcineurin inhibitors was an independent predictor of HCC recurrence by multivariate analysis in this study (RR: 2.82; P = 0.005). Moreover, Rodríguez-Perálvarez *et al*[3] reported that mean TTL of 7-10 ng/mL during the first

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two weeks after LT was effective in preventing ACR, and was related with significantly superior results in graft survival than TTL above or below this range. More recently, the survival time of patients with mean TTL < 5 ng/mL during the first four weeks after LT was observed to be significantly shorter than that of patients with higher mean TTL[9]. Despite these studies, the actual role of initial TTL on long-term outcomes after LT is difficult to assess. Retrospective studies did not report TTL during the follow-up period[3,9], and therefore the influence of potential differences among groups in tacrolimus exposure throughout the follow-up cannot be ruled out. In addition, in some reports TTL were maintained different in the study groups not only during the first month but throughout the whole follow-up, though not significantly, with the consequent difference of long-term tacrolimus exposure and the potential influence on the outcomes[4,6,8].

Our experience with the use of once-daily tacrolimus (Tac-QD) *de novo* after LT has been published[10]. Outstanding long-term patient and graft survival was achieved with the use of *de novo* Tac-QD in a minimizing immunosuppression protocol in LT recipients. With the aim of assessing the significance of the early post-transplant period in the outcomes of LT, we conducted this study to determine the real role of early TTL within the first month on long-term outcomes after LT.

MATERIALS AND METHODS

Design and patients

We conducted a retrospective analysis of a prospectively collected database of patients transplanted between April 2008 and May 2013. A total of 237 consecutive LTs were performed during the study period. Patients in the database with repeated LT (n = 13) or combined transplantation (n = 8) were excluded from this analysis, as were those who died within the first week after LT (n = 5) and those who did not receive Tac-QD for various reasons (n = 11). Patients who presented renal dysfunction prior to transplantation, defined as estimated glomerular filtration rate (eGFR) < 60 mL/min/1.73 m^2, and/or those who needed induction therapy (n = 45 overall) were also excluded to avoid bias in the early TTL measurements due to their particular immunosuppressive protocol with induction therapy and delayed initiation of tacrolimus. Finally, 155 adult LT recipients, whose immunosuppressive therapy was based on Tac-QD *de novo*, were eligible for this study and were followed up until December 31, 2015. Patients with HCC met the preoperative Milan criteria. To determine the effect of early exposure to tacrolimus on long-term outcomes and renal function, patients were classified into two groups according to their mean TTL during the first month after LT: ≤ 10 ng/mL or > 10 ng/mL. All TTL obtained during the first month were used to define the mean values.

The study was performed in accordance with relevant guidelines and regulation. No organs were procured from prisoners. The prospective database received the approval of the Research Ethics Committee of the Hospital Universitario Cruces, No. CEIC E13/08. All patients gave informed consent to be included in the prospective database; the requirement for specific informed consent was waived because of the retrospective nature of the study.

Early post-transplantation immunosuppressive therapy

Initial immunosuppression included Tac-QD and steroids 20 mg/day, except in those patients with diabetes mellitus who were treated with Tac-QD and mycophenolate-mofetil (MMF), avoiding the use of steroids. Tac-QD was administered within the first 24 h after LT, either orally or *via* a nasogastric tube. Patients considered at risk of renal dysfunction received MMF at a daily dose of 1000-2000 mg. Initial Tac-QD dose was 0.15 mg/kg *per* day (or 0.1 mg/kg *per* day if combined with MMF). Subsequent doses were adjusted according to trough levels. Serum tacrolimus levels were monitored regularly every 48 h until discharge. Target TTL were 5-10 ng/mL during the first 3 mo; however, if trough levels were lower but liver function tests were normal, the TacQD dose was not preventively increased. Azathioprine was not used in our patients.

Clinical follow-up and long-term immunosuppressive therapy

Biliary reconstruction in our patients is performed with end-to-end choledocho-choledochostomy with T-tube. When the patient progresses well, T-tube is closed on postoperative day 3 to avoid the potential effect that biliary diversion might have on TTL. Cholangiography is performed on day 7 and in the third postoperative month before T-tube removal. During these three months, patients are monitored weekly at home after hospital discharge, and also seen every two weeks at the outpatient clinic. Patients are monitored with liver function tests and TTL monthly afterwards until completion of the first year, and every 2-3 mo for a further two years. Stable patients with no relevant comorbidities are seen every 4 to 6 mo from the third year on.

The treating physicians adjusted immunosuppressive treatment according to their clinical judgment. Target TTL were progressively reduced: 4-9 ng/mL from month 3 to 6, 3-8 ng/mL from month 6 to 12, < 7 ng/mL after the first year and < 5 ng/mL after the second year onwards. Immunosuppressive protocol included steroids withdrawal 3-4 mo after transplantation, except in case of autoimmune disease (in which low-dose prednisone 5 mg/day was maintained), and in patients with hepatitis C virus (HCV), in whom withdrawal was delayed until months 12-18. Duration of treatment with MMF depended on side effects and/or clinical requirements. Adherence to treatment was assessed at each visit by asking the patients regarding any deviations from the prescribed regimen.

Endpoints and definitions

Outcome variables were: (1) Long-term renal function; (2) Immunosuppression-related morbidity; (3) Patient survival; and (4) Graft survival.

Long-term renal function was assessed by eGFR based on the modification of diet in renal disease formula. K/DIGO guidelines were used to define and classify chronic kidney disease[11]. Metabolic syndrome was defined according to already stablished definitions[12]. Fasting plasma glucose repeatedly > 126 mg/dL was used to define *de novo* diabetes whilst dyslipidemia was considered when treatment was prescribed for elevated blood cholesterol or triglycerides, and arterial hypertension when antihypertensive treatment was initiated. Patients with HCC met the Milan criteria. ACR was biopsy-proven acute rejection (BPAR) in all cases. BPAR were graded according to the Banff International Consensus Document[13]. Liver biopsy was not performed *per* protocol but indicated according to clinical evolution. In case of BPAR, tacrolimus exposure was further increased as the initial step. In case of severe rejection or if the graft dysfunction persisted after Tac-QD adjustments, three consecutive daily 500 mg corticosteroid boluses were used. Early graft dysfunction was defined according to previous specifications[14].

Statistical analysis

Qualitative variables are summarized as percentages and quantitative variables using means and standard deviations or median and interquartile range. Comparisons between frequencies of characteristics among trough-level groups were performed using the Chi-squared test or Fisher test, and continuous variables were compared using the Kruskall-Wallis test. Patient and graft survival were analyzed using the Kaplan-Meier method, in which patients lost to follow-up were censored at their last recorded visit. Graft loss was defined as retransplantation or death with non-functioning graft. Death with functioning graft was censored for the analysis of graft survival. The log-rank test was used to compare survival among the three groups. A univariate Cox regression analysis was performed to identify clinical and treatment factors related with patient survival including all patients in the cohort. Those variables with a P < 0.200 were included in a multivariate Cox regression model. Variables with the higher P value were excluded one by one until all variables had a P < 0.05. The proportional hazard assumption was tested. The statistical methods of this study were reviewed by Lorea Martinez-Indart from Bioinformatics and Statistics Platform, Biocruces Bizkaia Health Research Institute. Statistical analysis was performed using SPSS version 23.0.

RESULTS

All patients were Caucasian and received whole grafts from donation after brain-death. Ninety-eight were included in the ≤ 10 ng/mL group and 57 in the > 10 ng/mL group. A median of 7 samples of TTL (range 5-12) were used to obtain the mean TTL during the first month after transplant. Donor and recipient characteristics of the two groups are summarized in Table 1. Recipient features were generally similar across groups, including age, cause of liver disease, model for end-stage liver disease (MELD) score, baseline kidney function and pre-transplant comorbidities. The only significant difference between groups was the age of the graft donor (older for recipients whose early TTL were > 10 ng/mL); consequently, stroke as the cause of death was more

Table 1 Donors and recipients characteristics

	≤ 10 ng/mL, *n* = 98	> 10 ng/mL, *n* = 57	*P* value
Donors			
Age, year (mean ± SD)	54.7 ± 17.5	54.7 ± 17.5	0.002
Male	58 (59.2%)	35 (61.4%)	0.786
Cause of death			0.004
Stroke	57 (58.2%)	48 (84.2%)	
Trauma	27 (27.6%)	6 (10.5%)	
Other	14 (14.3%)	3 (5.3%)	
Graft steatosis	19 (19.6%)	12 (21.1%)	0.827
Recipients			
Age, years (mean ± SD)	55.3 ± 8.4	53.2 ± 9.8	0.227
Male	81 (82.7%)	48 (84.2%)	0.802
MELD (mean ± SD)	13.1 ± 5.6	12.7 ± 5.3	0.618
Hepatocellular carcinoma	45 (45.9%)	29 (50.9%)	0.551
Cause of liver disease			0.283
Alcohol abuse	45 (45.9%)	24 (42.1%)	
HCV	40 (40.8%)	18 (31.6%)	
HBV	3 (3.1%)	5 (8.8%)	
Cho/estatic liver disease	3 (3.1%)	4 (7%)	
Other	7 (7.1%)	6 (10.5%)	
Medical history (*pre* LT)			
MDRD-4 (mean ± SD)	107.8 ± 35.7	16.7 ± 33.7	0.223
Diabetes mellitus	18 (18.4%)	9 (15.8%)	0.683
Arterial hypertension	12 (12.2%)	10 (17.5%)	0.362
Mean tacrolimus trough levels 1 mo (ng/mL)	7.38 ± 1.68	12.62 ± 2.25	NA
Corticosteroids	80 (82.5%)	49 (86.0%)	0.571
Mycophenolate mofetil	32 (32.7%)	9 (15.8%)	0.024

MELD: Model for end-stage liver disease; MDRD-4: Modification of diet in renal disease; HCV: Hepatitis C virus; HCC: Hepatocellular carcinoma; LT: Liver transplantation.

frequent among those donors. Corticosteroids were similarly used in all groups; however, MMF use was significantly more common in the group with TTL ≤ 10 ng/mL.

Evolution of mean TTL during the follow-up of the two groups is shown in Figure 1. Mean levels within the first month post-transplant were 7.4 ± 1.7 and 12.6 ± 2.2 ng/mL in the ≤ 10 and > 10 groups, respectively (Table 1). Levels decreased in the > 10 mg/mL group within the first three months and were similar in both groups by the third month. From the third month on, a steady decrease in TTL was observed in both groups. Of note, for the purpose of this study, TTL were significantly different among groups only during the first month after LT, but not during the rest of the follow-up ($P = 0.65$).

Median follow-up was 52.8 mo (range 2.8-81.1) for those patients with early levels ≤ 10 ng/mL and 52.6 mo (10.8-79.1) for patients with tacrolimus mean levels > 10 ng/mL. Patient outcomes after transplantation are summarized in Table 2. There were no statistically or clinically relevant differences among groups. Mean TTL during the early post-transplant period did not affect renal function. Creatinine clearance fell in parallel in both groups ($P = 0.67$), decreasing similarly during the first 6 mo to remain steady thereafter until the end of follow-up, at mean levels of approximately 80

Table 2 Recipients outcomes after liver transplantation

	≤ 10 ng/mL, *n* = 98	> 10 ng/mL, *n* = 57	*P* value
Biopsy-proven acute rejection	12 (12.2%)	5(8.8%)	0.505
Arterial complications	12 (12.2%)	7(12.3%)	0.995
Biliary complications	13 (13.3%)	8 (14%)	0.893
Infection (any)	49(50.0%)	26 (45.6%)	0.598
Cytomegalovirus infection	26 (26.5%)	12 (21.1%)	0.445
Retransplantation	5 (5.1%)	5 (8.8%)	0.500
HCC recurrence[1]	1 (2.3%)	0	0.999
HCV recurrence[2]	35 (87.5%)	14 (77.8%)	0.438
De novo tumor	10 (10.2%)	5 (8.8%)	0.771
New-onset arterial hypertension	35(36.1%)	19 (36.5%)	0.827
New-onset diabetes	21 (21.6%)	6 (12.7%)	0.127
Tacrolimus withdrawal. Causes:	18 (18.4%)	8 (14.0%)	0.486
Kidney failure	7	1	
Neurotoxicity	1	2	
Metabolic syndrome	6	4	
Metabolic synd + kidney failure	1	-	
Other	3	1	
MDRD-4 at 5 yr (mean ± SD)	82.5 ± 19.4	80.32 ± 14.7	0.686
Deaths. Causes:	10 (10.2%)	8 (14.0%)	0.827
HCV recurrence	5	3	
De novo tumor	1	2	
Sepsis	2	1	
Stroke	0	1	
Other	2	1	

[1]Including variables with $P < 0.2$ in univariate analysis, highlighted in bold.

[2]Renal dysfunction during hospitalization. HCC: Hepatocellular carcinoma; HCV: Hepatitis C virus; MDRD-4 stands for: Modification of Diet in Renal Disease.

mL/min/1.73 m^2 in all groups (Figure 2). Patients with higher levels within the first month after LT did not present more immunosuppression-related toxicity including new-onset diabetes, hypertension, HCC recurrence or *de novo* tumors. BPAR occurred with low and similar frequency in all groups (12.2%, and 8.8% in ≤ 10 and > 10 mg/mL, respectively; P = 0.50). Only 10 patients were treated with corticosteroid boluses (8 (66.7%) and 2 (40.0%), respectively; P = 0.99), and the rest responded to tacrolimus dose escalation. There was no relationship between the decision to withdraw tacrolimus during follow-up and the initial trough level.

Eighteen patients died during the follow-up and were evenly distributed across groups (P = 0.83) (Table 2). The most common cause of death was HCV recurrence. Five-year patient survival in the study groups was 90.5% and 84.9%, respectively (P = 0.44) (Figure 3A), while 5-year graft survival was 88.2% and 85.8%, respectively (P = 0.42) (Figure 3B).

Univariate and multivariate analysis

All patients were included in a univariate and multivariate Cox regression analysis to study factors associated with patient mortality. Multiple variables from donor and recipients were considered in the univariate analysis, as well as various outcomes and adverse events. This analysis was performed considering the two mean TTL groups described in methods, and also dividing the sample into two groups using the cut-off

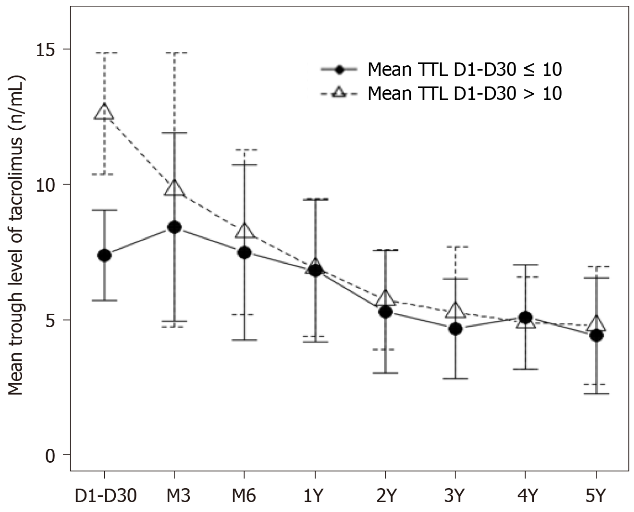

Figure 1 Mean serum tacrolimus levels according to the mean tacrolimus trough levels for each group within 1 mo after transplantation (mean ± SD). P = 0.65 comparing means from month 3 (M3) to year 5 (5Y). TTL: Tacrolimus trough levels.

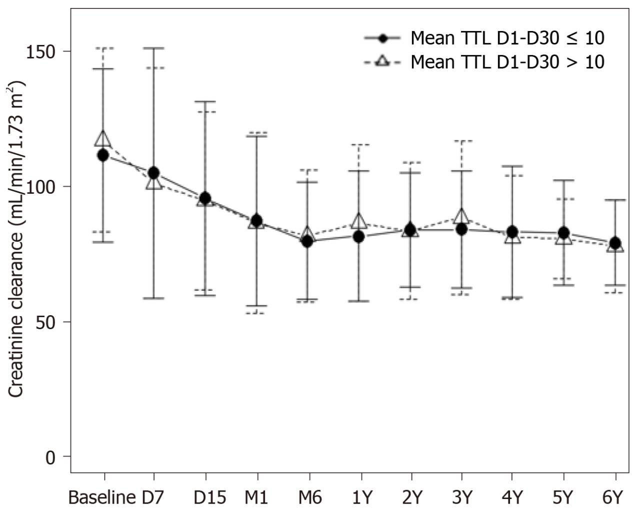

Figure 2 Mean creatinine clearance according to the mean tacrolimus trough levels for each group within 1 mo after transplantation (mean ± SD) (P = 0.67). TTL: Tacrolimus trough levels.

level 8 ng/mL or three groups using cut-off levels of < 7 ng/mL, 7-10 ng/mL and > 10 ng/mL. Multivariate analysis revealed that factors independently related with patient mortality were *de novo* tumor (HR = 13.8; 95%CI: 4.1-46.9; P < 0.001), MELD score ≥ 20 (HR = 6.1; 95%CI: 1.9-19.6; P = 0.003), HCV infection as the cause of liver disease (HR = 4.9; 95%CI: 1.7-14.1; P = 0.003) and arterial complications (HR = 3.7; 95%CI: 1.1-12.6; P = 0.03) (Table 3). Early TTL was not an independent factor for patient mortality.

DISCUSSION

This analysis aimed to further explore factors related to long-term clinical outcomes in our LT patients treated *de novo* with Tac-QD, with particular interest in the effect of mean TTL during the early post-transplant period. In order to have an adequate follow-up time to study long-term outcomes, patients transplanted between 2008 and 2013 were included in the study. Considering the time when LTs were performed, we followed a policy of immunosuppression minimization with target TTL of 5-10 ng/mL during the first 3 mo; however, a significant number of patients in this cohort were outside our target levels during the first month after LT, although this was corrected afterwards, as shown in Figure 1. We divided our cohort into two groups of early TTL (within 1 mo) as previously done by Rodríguez-Perálvarez *et al*[7,8] who found a

Table 3 Univariate and multivariate analysis of factors associated with patient mortality

	Univariate analysis		Multivariate analysis[1]	
	P value	HR (95%CI)	*P* value	HR (95%CI)
Age of donor ≥ 70 years	0.55	0.73 (0.26-2.05)		
Recipient				
Liver steatosis	0.1	0.408 (0.09-1.79)		
Age ≥ 60 years	0.94	1.04 (0.39-2.78)		
HCV infection as cause of liver disease	0.02	3.02 (1.17-7.81)	0.003	4.94 (1.72-14.17)
Presence of hepatocellular carcinoma	0.57	1.31 (0.52-3.34)		
MELD score ≥ 20	0.02	3.16 (1.12-8.91)	0.003	6.06 (1.88-19.56)
Diabetes before transplantation	0.63	1.32(0.43-4.01)		
Hypertension before transplantation	0.05	2.78 (0.98-7.90)	-	-
MDRD-4 at baseline	0.35	0.99 (0.97-1.01)		
Mycophenolate mofetil at initial therapy	0.89	1.07 (0.38-3.02)		
Outcomes and complications				
BPAR	0.20	2.08 (0.67-6.43)		
Arterial complications	0.06	2.91 (0.94-9.06)	0.03	3.76 (1.12-12.62)
Biliary complications	0.59	1.41 (0.40-4.92)		
Renal dysfunction early after transplant[2]	0.08	2.40 (0.90-6.38)	-	-
Renal hypertension	0.82	1.12 (0.42-3.02)		
De novo diabetes	0.02	3.25 (1.24-8.55)	-	-
Cardiovascular	0.14			
Arterial hypertension	0.08	0.32 (0.09-1.15)	-	-
Heart failure	0.26	0.31 (0.04-2.37)		
De novo tumor	0.005	4.20 (1.56-11.32)	< 0.001	13.82 (4.06-46.98)
HCV recurrence	0.22	1.79 (0.70-4.53)		
HCC recurrence	0.008	16.61 (2.10-131.07)	-	-
Any infection	0.71	1.12 (0.47-3.03)		
Bacterial infection	0.04	2.71 (1.04-7.07)	-	-
Viral infection	0.39	0.61 (0.20-1.87)		
Fungal infection	0.87	1.19 (0.16-9.03)		
Cytomegalovirus infection	0.79	0.86 (0.28-2.62)		
Normal renal function at last visit (MDRD-4 ≥ 60 mL/min/1.73 m^2)	0.92	1.08 (0.23-5.08)		
Mean tacrolimus levels at days 1-30 after LT				
> 10 ng/mL *vs* ≤ 10 ng/mL	0.44	1.44 (0.57-3.65)		
< 7 ng/mL (reference)[3]	0.32			
7-10 ng/mL	0.31	0.49 (0.12-1.96)		
> 10 ng/mL	0.59	1.33 (0.47-3.73)		
> 8 ng/mL *vs* < 8 ng/mL[3]	0.78	1.14 (0.44-2.95)		
Early graft dysfunction	0.08	2.44 (0.890-6.63)	< 0.001	6.02 (2.34-15.49)

[1]Including variables with $P < 0.2$ in univariate analysis, highlighted in bold.
[2]Renal dysfunction during hospitalization.

[3]Additional analysis modifying cut-off values. MELD: Model for end-stage liver disease; HCV: Hepatitis C virus; HCC: Hepatocellular carcinoma; BPAR: Biopsy-proven acute rejection; MDRD-4 stands for: Modification of Diet in Renal Disease; LT: Liver transplantation.

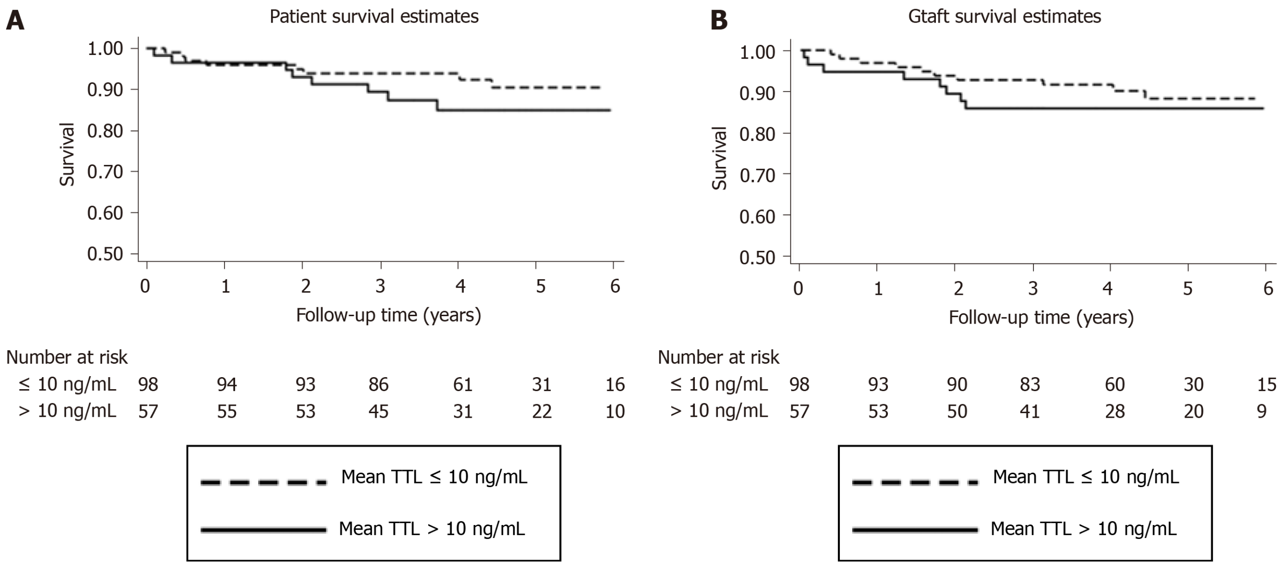

Figure 3 Kaplan-Meier survival curves after liver transplantation according to the mean tacrolimus trough levels for each group within 1 mo after transplantation. A: Patient survival (*P* = 0.44); B: Graft survival (*P* = 0.42). TTL: Tacrolimus trough levels.

significant improvement of outcomes when mean TTL within the first month post-LT were ≤ 10 ng /mL, compared with patients with > 10 ng/mL[7,8]. Of note, patients treated with induction therapy and delayed introduction of low-dose tacrolimus, namely those with pretransplant renal dysfunction, were excluded in our study to avoid bias as most of these patients would have probably ended in the low mean TTL group. In contrast to the published studies, we did not find significant differences in long-term renal function, HCC recurrence, immunosuppression-related toxicity or patient and graft survival in both groups of early TTL. In addition, multivariate analysis in our study, performed three times with different cut-off values for early TTL, demonstrated the lack of influence of early TTL on long-term patient survival.

In our study, donor age was significantly higher in the group with high TTL. Aging is characterized by a decline of liver cellular function that could determine alterations in immunosuppressants liver metabolism and pharmacokinetic. In this sense, it has been suggested that aged donor livers might exhibit lower drug clearance with consequently higher TTL[15]. Nevertheless, this circumstance was not detrimental in our experience as both TTL groups achieved comparable long-term outcomes.

According to the literature, the relative risk of death more than 1 year after LT suffers a 4-fold to 5-fold increase when renal dysfunction is present[16,17]. In our study, renal function evolved similarly in the two groups, with an expected 20% decrease in eGFR during the initial period after LT-as already described by other authors[18]-and maintenance of renal function from month 6 onwards. This contrasts with the progressive decline in renal function in the Mid/long-term repeatedly reported in literature[19-21]. Although, some authors have found no relationship between TTL within 15 d after LT and chronic renal impairment[3,9], high TTL within the first month after LT has been associated with worse renal function in different studies[7,20]. Karie-Guigues *et al*[20] found that the introduction of MMF significantly reduced the TTL at the end of the first month after LT, and this was associated with a significantly less marked reduction of the eGFR at 12 and 60 mo. Rodríguez-Perálvarez *et al*[7] also observed in a meta-analysis that reduced TTL (< 10 ng/mL) within the first month after LT were associated with less renal impairment at 1 year[7]. Nevertheless, both studies can be discussed. In the former study, TTL were shown at months 1, 12 and 60 after LT; however, no data were shown on the evolution of TTL between those time points and so, results could be biased due to different exposition to tacrolimus in both groups[20]. In the latter study, only two clinical trials were used in the meta-analysis and TTL were maintained higher in both study groups along the whole follow-up although differences did not achieve significance[7].

We can hypothesize that TTL early after LT have little effect on the evolution of long-term renal function when a tacrolimus minimization policy is implemented during long-term follow-up, as in our case. A longer period of high TTL in the post-transplant period might be needed to negatively affect the mid/long-term renal function. In accordance with this idea, the role of cumulative exposure to tacrolimus in eGFR decline after LT has been recently addressed[22]. In this study, conventional/high exposure to tacrolimus within the first 3 mo resulted in a more pronounced eGRF decline as compared with minimization (23.3 mL/min *vs* 9.5 mL/min; $P \leq 0.001$).

The role of tacrolimus exposure in HCC recurrence has been also addressed in different studies. High TTL (> 10 ng/mL) within the first month after LT but not thereafter was associated with increased risk of HCC recurrence at 5 years by Rodríguez-Perálvarez *et al*[8] (RR = 2.8; $P = 0.005$). Of note, in this study, tacrolimus levels were consistently lower during the 3-year follow-up in the non-recurrence group, although differences did not achieve significance. In another study, high exposure to tacrolimus was followed by a 50% recurrence rate *vs* 9.1% in patients with low exposure ($P = 0.001$)[23]. In this study, high exposure was described as > 10 ng/mL during the first year and not only during the first month reflecting a significant higher exposure to tacrolimus along the follow-up. In our study, overall HCC recurrence rate was extremely low and no differences were found between groups. Low exposure to tacrolimus not only during the early post-transplant period but in the long term, and our strict selection policy, all patients fulfilled Milan criteria prior to transplantation, might have positively influenced these remarkable results in our study. Recently, other authors have also reported the lack of effect of the first fifteen days of calcineurin inhibitor exposure in the development of HCC recurrence or *de novo* tumors after LT[24]. Again, it seems that longer periods of high exposure to tacrolimus-and not only during the first month after transplant-are needed to influence the development of *de novo* tumors or HCC recurrence.

Early TTL were not related with an increase in BPAR rates in our study. Reduction in early TTL was associated with the use of MMF and this could explain why the BPAR rate was not higher in patients with lower early TTL. Immunosuppression therapy with tacrolimus, MMF and steroids is currently the most common combination following LT[1], and has been demonstrated to be effective in reducing TTL while maintaining or even reducing the acute rejection rate[4,6].

We observed a relatively low rate of immunosuppression-related toxicity in terms of *de novo* diabetes or arterial hypertension and no differences were seen according to early TTL. In addition, development of *de novo* tumors was not influenced by TTL during the first month in our study.

In our study, factors associated with patient survival in multivariate analysis were *de novo* tumor, higher severity of liver disease (MELD score > 20), baseline HCV infection and arterial complications after LT. These factors have been repeatedly reported to be related to patient and graft survival after LT in the pre-direct-acting antivirals era[16,25,26]. Of note, early TTL were not an independent risk factors for patient survival in our study.

We recognize some limitations in our study. It is retrospective, although the data were retrieved from a prospective database. Indeed, the number of patients included in the different groups are limited and hence the number of patients who experienced adverse events of interest such as impairment of renal function or HCC recurrence are also limited. In addition, MMF was more frequently used in the lower TTL group although immunosuppression-related morbidity is more likely related with tacrolimus exposure rather than to the use of MMF. Nevertheless, our study has several strengths: (1) Median follow-up was more than 4 years in both groups, which seems sufficient to assess the long-term outcomes and draw meaningful conclusions; and (2) Regarding TTL, our study groups were significantly different only within the first month after LT, which was the target period of time in the study, but not during the rest of the follow-up, what reinforces the adequacy of the study for our purpose and avoids the significant potential bias of having not only different early TTL but different TTL during the study period.

CONCLUSION

In summary, TTL within the first month after LT had no significant effect on long-term renal function, immunosuppression-related morbidity and 5-year patient or graft survival in our study. Early post-transplant tacrolimus level was not an independent factor for long-term patient in multivariate analysis. We conclude that relatively small differences in mean tacrolimus levels restricted to the first month after LT do not determine differences in long-term immunosuppression-related morbidity and patient survival and therefore, larger exposure to tacrolimus seems to be needed to influence long-term outcomes. Larger studies should be advisable to confirm our results; however, these studies should be done on the basis of different TTL only during the early post-transplant period and not along the follow-up to avoid potential biases.

ARTICLE HIGHLIGHTS

Research background

Immunosuppression is a cornerstone in liver transplantation (LT) and current immunosuppressive regimens are mostly based on tacrolimus. At present, side effects relating anticalcineurin inhibitors are one of the main concerns for long-term outcomes after LT. Side effects are commonly related with drug dose and trough levels.

Research motivation

Tacrolimus trough levels (TTL) above 10 ng/mL during the first weeks after liver transplant have been related with mid and long-term outcomes including impairment of renal function and an increased rate of hepatocellular recurrence, *de novo* tumors and new-onset diabetes.

Research objectives

The aim of this study was to assess the influence of the TTL during the early post-transplant period in the long-term outcomes of LT.

Research methods

This was a retrospective study of 155 consecutive liver transplants treated with an immunosuppressive regimen based on *de novo* once-daily tacrolimus. Patients were classified into 2 groups according to their mean TTL within the first month after transplantation: ≤ 10 ng/mL (n = 98) and > 10 ng/mL (n = 57). All TTL obtained during the first month were used to define the mean values. Multivariate analyses were performed to assess risk factors for patient mortality.

Research results

TTL were significantly different among groups only during the first month after transplantation, but not during the rest of the follow-up After a median follow-up of 52.8 mo (range 2.8-81.1), no significant differences were observed in the evolution of the mean estimated glomerular filtration rate, hepatocellular carcinoma recurrence, development of *de novo* tumors, new-onset diabetes, new-onset arterial hypertension or biopsy-proven acute rejection rate. Five-year patient and graft survival were comparable. Early tacrolimus trough level was not an independent factor for patient mortality in multivariate analyses.

Research conclusions

Differences in tacrolimus levels restricted to the first month after transplantation did not result in significant differences in long-term outcomes of liver transplant recipients.

Research perspectives

Mid and long-term calcineurin inhibitors-related side effects after LT should be studied considering the cumulative exposure to tacrolimus along the follow-up and not only the trough levels observed during the early post-transplant period.

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