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Tacrolimus exposure after liver transplantation for alcohol-related liver disease: Impact on complications

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ABSTRACT

Background: Alcohol-related liver disease (ALD) is one of the main indications for liver transplantation (LT). For 20 years, tacrolimus (Tac) is the cornerstone immunosuppressive drug used after LT and is very efficient for the prevention of rejection. Nevertheless, the major drawback of long-term use of Tac is the risk for developing dose-dependent adverse effects.

Objective: The aim of the present study was to assess the impact of Tac exposure (trough concentrations and concentration/dose (C/D) ratio) during the first year after LT, on short- and long-term complications after LT for ALD.

Methods: All patients who underwent a LT for ALD at Lyon Edouard Herriot Hospital from October 1990 to September 2010, and who were treated with Tac for at least one year after LT, were analyzed.

Results: The study population consisted in 251 patients, mean age 53.4 ± 7.3 years, and followed during 11.6 ± 4.8 years. Post-LT complications included severe infectious events (44.6%), malignancies (41.4%), arterial hypertension (49.4%) dyslipidemia (44.2%), diabetes (18.7%) and cardiovascular events (15.5%). De novo hypertension, cardiovascular event, CMV infection, non-melanoma skin cancers and HCC recurrence after transplantation were significantly associated with higher Tac trough blood concentration. In addition, Tac fast-metabolizers (defined as C/D < 1.8) had significantly more impaired renal function at 1, 5, and 10 years and more cardiovascular events, PTLD, diabetes and hypertension than slow-metabolizers.

Conclusion: Our results strongly support that, in addition to blood trough concentrations, Tac metabolism, as estimated by the simple C/D ratio, could be an efficient parameter in daily practice to identify LT patients at risk to develop long term general complications of Tac.

List of abbreviations

ALD	Alcohol-related liver disease
LT	Liver transplantation

1. Introduction

Since the mid 80's, since cyclosporine became available, liver transplantation (LT) became the effective treatment of end-stage liver diseases, including alcohol-related liver disease (ALD) [1]. Over the years, the number of LT increased considerably and nowadays there are > 10,000 recipients of a liver graft in France. During the past 30 years, several improvements were made in surgical techniques and postoperative management, allowing a better survival of both grafts and patients. This includes the development of new immunosuppressive drugs and strategies. In particular, tacrolimus (Tac), a calcineurin inhibitor (CNI), replaced cyclosporine in the late 90's, as the cornerstone of immunosuppressive regimen for LT recipients, because of its higher ability to prevent acute cellular rejection (ACR) [2]. Nevertheless, the major drawback of long-term use of Tac is the risk for developing dose-dependent adverse effects, including malignancies, renal failure, hyperlipidemia, diabetes mellitus, arterial hypertension and cardiovascular diseases [3–7].

Tac pharmacokinetics is characterized by a high inter- and intra-patient variability; therefore, therapeutic drug monitoring is used to

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adjust the dosage in order to provide an effective immunosuppression and prevent toxicity [8]. New strategies have emerged to reduce doses of Tac ("minimizing tacrolimus strategy") [9–11]. The exposure to Tac can be evaluated from different ways in transplant recipients. In daily practice, trough blood concentration is used to adapt Tac doses. Therefore, overall mean blood concentration of Tac (for a defined period of time) can be used as an estimation of Tac exposure, and it has been suggested that this could have a significant impact on renal impairment and risk of de novo malignancies [12]. Recently, Tac metabolism rate has been defined as the Tac blood concentration (C) divided by the daily dose of Tac (D). This C/D ratio leads to separate Tac fast- and low-metabolizers, and it has been suggested that fast-metabolizers could develop more frequently renal impairment than slow-metabolizers [13,14]. The impact on other Tac-related adverse events has not been investigated yet.

In France, ALD (with or without hepatocellular carcinoma (HCC)) is the main indication for LT [7]. In this specific population, past or persistent tobacco/alcohol intake has been linked with a dramatic higher risk of developing several malignancies [7,15], especially cancers of the upper aero-digestive tract. Moreover chronic alcohol consumption is associated in higher risk of developing cardiovascular [16,17] and infectious diseases [18]. The aim of the present retrospective study was to assess the impact of Tac exposure during the first year after LT, on short- and long-term complications after LT for ALD.

2. Methods

2.1. Study population

Since October 1990, we set up a prospective database in our medical center with the aim of generating detailed survival data. The present retrospective study included all adult patients who were transplanted for ALD (with or without HCC), from October 1990 to September 2010 at Edouard Herriot Hospital, Lyon, France, and who received Tac (twice-daily), beginning during the first 10 days following LT and maintained for more than one year. Steroids, azathioprine, mycophenolate mofetil (MMF), or everolimus (EVR) were either administrated as a part of initial triple immunosuppressive regimen or introduced during follow-up as maintenance immunosuppressive agents. Patients receiving combined kidney, heart or lung transplantation were not included.

Four hundred and eight patients were transplanted for ALD. We excluded 41 patients because they did not receive Tac as initial immunosuppressive therapy, 3 patients who received multi-organ transplant, 22 patients who died early (less than a year after LT), and 28 patients because alcohol was associated with another cause of liver disease (mainly HCV infection). On the 314 patients treated with Tac, 24 patients were exposed less than one year and 32 did not received Tac during the first month after LT. Finally, we excluded 7 patients who were lost to follow up and the study population consisted in 251 patients.

The aim of the study was to assess the impact of Tac exposure, during the first year after LT, on short- and long-term complications after LT for ALD.

2.2. Clinical and biological data collection

Data collection was based on written medical records and digital information, thereby providing a record of medical history of the patients. Recorded clinical data included: gender, age at LT, body mass index (BMI) at LT, Child-Pugh class at LT, Model for End Stage Liver Disease (MELD) score (retrospectively calculated from our data base for all the patients since 1997) at LT, presence of HCC at LT, initial immunosuppressive regimen after LT, smoking (defined as never, current, or past - in case of cessation of smoking before LT), alcohol relapse, occurrence of severe infections (requiring hospital stay and including cytomegalovirus (CMV) infections, and classified as early post-operative infection during the first month after LT, late post-operative infection between one and 6 months after LT or late infection after 6 months after LT), de novo post-LT malignancies (cases reported in the first 6 months following LT were excluded because it can be strongly assumed that the malignancies were probably present at the time of transplantation and undiagnosed), HCC recurrence, diabetes, dyslipidemia, hypertension, and cardiovascular events (CVE). Severe hypertension was defined as hypertension needing 3 or more antihypertensive drugs to be controlled.

Cardiovascular event included were stroke, peripheral arterial disease (usually obliterative arterial disease of the lower limb or carotid stenosis) and coronary heart disease.

We separated cancer in several categories: all de novo malignancies, upper aerodigestive tract cancers, HCC recurrence, non-melanoma skin cancers, and post-transplant lymphoproliferative disorders (PTLD).

Renal function was assessed at time of LT, and at 1 month, 6 months, 12 months, 5 years and 10 years after LT. It was estimated by calculating the glomerular functional rate (eGFR) using the MDRD (modification of diet in renal disease) formula [19].

$$[\mathbf{186 \times (creatinine\ (\mu mol/l) \times 0.0113)^{-1.154} \times age^{-0.203}}]\ x0.742\ if\ a\ woman.$$

Patients were categorized depending on the eGFR as follows:

- Mild renal impairment: eGFR = 60–90 mL/min
- Moderate renal impairment: eGFR = 30–60 mL/min
- Severe renal impairment: eGFR < 30 mL/min

2.3. Exposure to tacrolimus

After LT, Tac was routinely monitored using the Tac trough blood concentration (ng/mL). Tac concentration measurements were performed using liquid chromatography coupled with tandem mass spectrometry. Dose adaptations were made depending on several criteria including occurrence of rejection episodes, renal failure, infections or depending on the comorbidities of patients.

We collected Tac trough blood concentrations at different time points: day 3 (D3), D7, D14, D30, then at month 2 (M2), M3, M4, M5, M6, M7, M8, M9, M10, M11, M12, M15, M18, and then every year following LT.

We also estimated the mean exposition by calculating the overall mean concentration of Tac during the 1st, 3rd, 6th and 12th months after LT for each patient, using the trapezoidal method as described by Vivarelli [20].

Daily doses of Tac were recorded at 1, 3 and 6 months, and at the end of the first year after LT. Tac metabolism rate was determined by dividing the Tac blood trough concentration (C) by the corresponding daily Tac dose (D), at M1, M3, M6 and M12 [13].

$$C/D\ ratio\ (ng/mL \times 1/mg) = \frac{\text{blood Tac trough concentration (ng/mL)}}{\text{daily Tac dose (mg)}}$$

2.4. Statistical analysis

The main clinical parameters of the patient were determined using mean and median for quantitative variable (for age, time from LT to diagnosis cancer or cardiovascular event or severe infectious episodes, eGFR, Tac blood concentration, weight) and frequencies and percentages for qualitative variable (for the incidence of diabetes, hypertension, dyslipidemia, alcohol consumption, tobacco consumption, severe infectious events and such).

Shapiro-Wilk test was used to assess whether the distribution of each data was normal. If so, Student's *t*-test was used to compare the data and if not, we used the Mann-Whitney *U* test. To evaluate the

Table 1
Characteristics of the study population (n = 251).

Characteristics		n (%) or median
Sex (male/female, n)	193/58	76.9%/23.1%
Age at the time of LT (year, mean ± SD)	53.4 ± 7.3	53.5
HCC (n)	75	29.9%
eGFR before LT (mL/min/1,73m^2, mean ± SD)	91.6 ± 28.1	89.7
Follow-up time (year, mean ± SD)	11.6 ± 4.8	12.0
Alive 5 years after LT (n)	233	92.8%
Alive 10 years after LT (n)	170	67.7%
Initial immunosuppressive regimen (1st month after LT)		
Tac only (n)	83	33.1%
Tac + MMF (n)	161	64.1%
Tac + azathioprine (n)	5	2.0%
Tac + EVR + MMF (n)	2	0.8%
Steroids (n and median dose, mg/d)		
1st month	163	64.9% (15.1)
3rd month	150	59.8% (8.1)
6th month	55	21.9% (5.6)
12th month	26	10.4% (5.2)
CMV prophylactic treatment (n)	247	98.4%
Tacrolimus dose after LT (mg/d, mean ± SD)		
1st month	7.0 ± 3.8	6.0
3rd month	6.6 ± 4.1	6.0
6th month	5.5 ± 3.6	5.0
12th month	4.3 ± 2.4	4.0
Maintenance immunosuppressive regimen (last follow-up)		
Tac (n)	144	57.3%
MMF (n)	133	53.0%
Azathioprine (n)	0	0.0%
Cyclosporine (n)	11	4.4%
EVR (n)	75	29.9%
Co-morbidities		
Before LT		
Tobacco smoking (n)	179	71.3%
Weight (kg, mean ± SD)	75.0 ± 15.2	75.0
BMI (kg/m^2, mean ± SD)	26.0 ± 4.4	25.3
Obesity (n)	41	16.3%
Dyslipidemia (n)	9	3.6%
Diabetes (n)	51	20.3%
Diabetes treated with insulin	25	10.0%
Hypertension (n)	54	21.5%
Severe hypertension (n)	1	0.4%
After LT		
Severe alcohol relapse after LT (n)	60	23.9%
Last BMI (kg/m^2, mean ± SD)	26.0 ± 5.3	25.5
Obesity (n)	60	23.9%
Last eGFR (mL/min/1,73m^2, mean ± SD)	66.1 ± 31	64.4
Dialysis (n)	17	6.8%
Dyslipidemia (n)	118	47.0%
De novo dyslipidemia (n)	111	44.2%
Diabetes (n)	95	37.8%
De novo diabetes (n)	47	18.7%
Diabetes treated with insulin	53	21.1%
De novo diabetes treated with insulin (n)	33	13.1%
Hypertension (n)	174	69.3%
De novo Hypertension (n)	124	49.4%
Severe hypertension (n)	50	19.9%
Severe Infectious event (n)	112	44.6%
Time to first infectious event (months, mean ± SD /median)	35.8 ± 54.1 / 5.2	
< 1 month (n)	41	16.3%
between 1st and 6th month (n)	16	6.4%
after the 6th month (n)	55	21.9%
CMV Infection (n)	34	13.6%
Cardiovascular event (CVE) (n)	39	15.5%
Time to first CVE (months, mean ± SD /median)	94.6 ± 60.1 / 87.7	
Cancer		
Malignancies (all) after LT (n)	104	41.4%
Time to first malignancy (months, mean ± SD /median)	91.7 ± 47.3 / 86.1	
HCC recurrence (n)	7	9.3%
PTLD (n)	8	3.2%
De novo malignancies (excl. PTLD) (n)	90	35.9%
De novo upper aerodigestive tract malignancies (n)	55	21.9%
Non-melanoma skin cancers (n)	22	8.8%
Multiple cancers (n)	19	7.6%

Table 2
Immunosuppressive drugs used after LT in the study population (n = 251).

	Cyclosporine	Azathioprine	MMF	EVR	Tac
Patients (n)	27	8	225	111	251
Mean time before introduction (months)	50.4	12.1	10.6	81.3	0.0
Median time before introduction (months)	50.2	0.0	0.0	78.0	0.0
Mean exposition time (months)	75.1	13.0	102.4	43.2	105.1
Median exposition time (months)	72.3	7.2	103.3	28.1	98.6

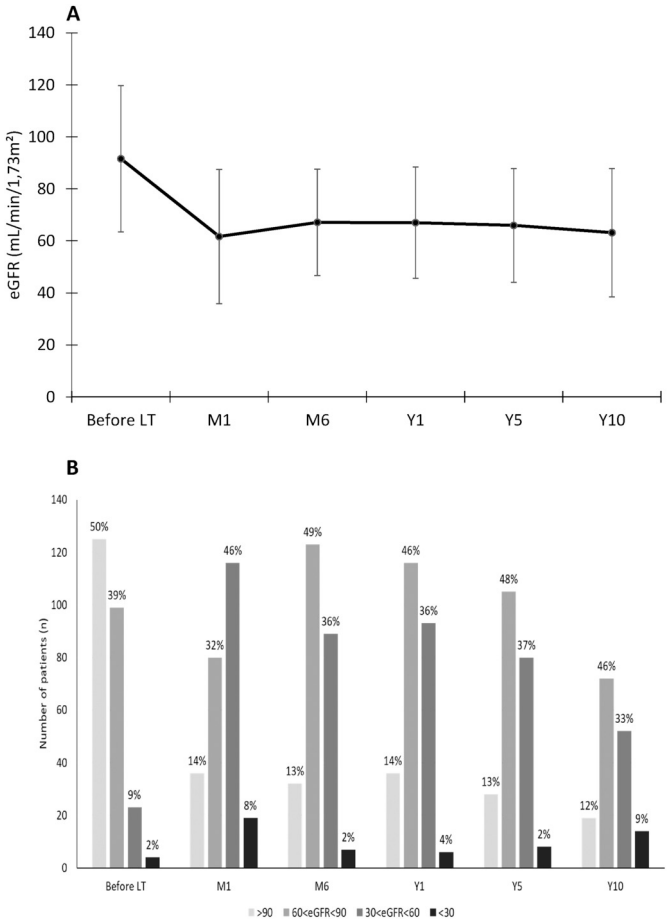


Fig. 1. Renal function after LT.
A: Evolution of eGFR after LT.
Before LT mean eGFR was 91.2 ± 28.1 mL/min/1.73m^2 and then decrease to 61.6 ± 25.9 the first month after LT. After there was a small improvement of eGFR: At 6 months, mean eGFR was 67.1 ± 20.5, then 67.0 ± 21.4 at 1 year, 65.9 ± 21.9 at 5 years and 63.2 ± 24.7 at 10 years after LT.
B: Evolution of stages of chronic kidney failure after LT.

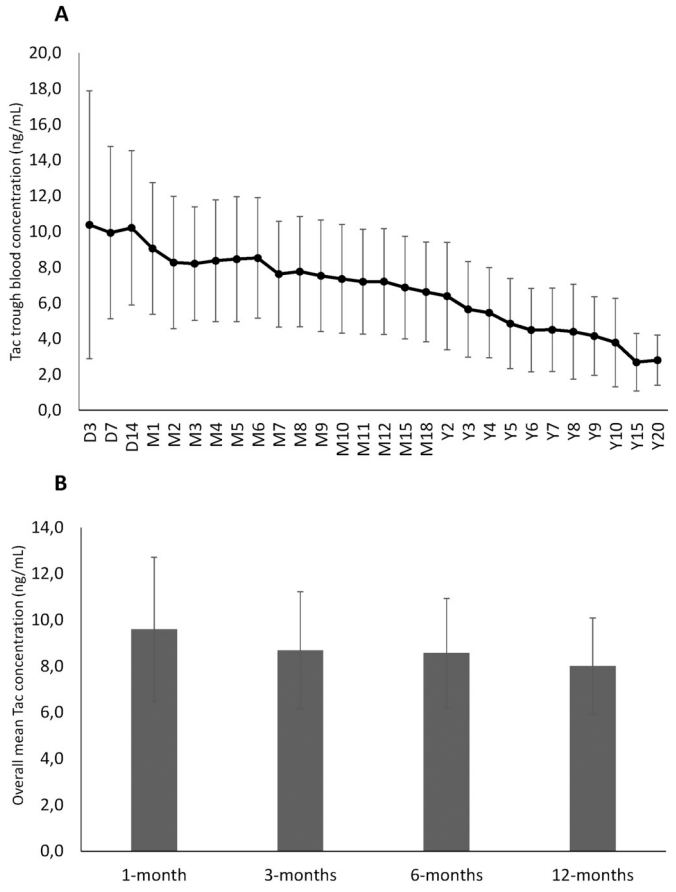


Fig. 2. Tac trough blood concentration and overall mean Tac concentration after LT.
A: Evolution of overall mean Tac concentration after LT.
At day 3, Tac trough blood concentration was 10.4 ± 7.5 ng/mL and then decrease to 9.1 ± 3.7 ng/mL at 1 month, then 8.2 ± 3.2 ng/mL at 3 month, 8.5 ± 3.4 ng/mL at 6 months, 7.2 ± 3.0 at 12 months, 6.4 ± 3.0 ng/mL at 2 years, 4.9 ± 2.5 ng/mL at 5 years, 3.8 ± 2.5 ng/mL at 10 years and finally 2.8 ± 1.4 ng/mL at 20 years.
B: Evolution of overall mean Tac concentration after LT.
During the 1st month, the overall mean Tac concentration was 9.6 ± 3.1 ng/mL, then 8.7 ± 2.5 ng/mL during the 3rd month, 8.6 ± 2.4 ng/mL during the 6th months, and 8.0 ± 2.1 ng/mL during the 12th month.

independence between 2 variables, chi-square was used. All the analyses were performed using the SPSS software, version 13.0 (IBM, Armonk, NY) and *p*-values under 0.05 were considered significant.

3. Results

3.1. Study population and post-LT complications

Characteristics of the 251 patients included in the study are summarized in Table 1. There was a majority of males (76.9%) and almost all patients were Caucasian (248/251, 98.8%). The median age at LT was 53.5 years and the median follow-up after LT was 12.0 years. Most of patients (71.3%) were smoker before LT and severe alcohol relapse occurred in 23.9% of the patients after LT. During follow up, 44.6% of patients experienced a severe infectious event, 41.4% a malignancy, and 15.5% a cardiovascular event. In addition, 47.0% of patient had dyslipidemia after LT, 37.8% had diabetes and 69.3% had arterial hypertension.

After LT, patients were usually treated with double immunosuppressive regimen using Tac with mycophenolate mofetil (MMF). During follow-up, immunosuppressive therapy was modified. From the initial cohort of 251 patients, Tac was stopped in 107 (42.6%); Table 2 summarizes immunosuppressive drugs used after LT.

Fig. 1 describes the evolution of eGFR at and after LT and the evolution of stages of chronic kidney disease before and after LT: the

Table 3
Tac trough blood concentration and complications after LT.

Tac trough blood concentration									
		M1	p	M3	p	M6	p	Y1	p
Cardiovascular event	Yes	9.2 ± 3.4	0.45	9.3 ± 3.7	0.02	8.7 ± 3.6	0.54	6.9 ± 2.4	0.67
	No	9.0 ± 3.8		8.0 ± 3.1		8.5 ± 3.3		7.3 ± 3.1	
Infections									
Severe infection	Yes	8.9 ± 3.7	0.78	8.1 ± 3.2	0.62	8.3 ± 3.4	0.18	7.3 ± 3.2	0.89
	No	9.2 ± 3.7		8.2 ± 3.1		8.7 ± 3.4		7.1 ± 2.8	
CMV infection	Yes	8.4 ± 3.5	0.29	7.7 ± 3.3	0.32	9.6 ± 4.1	0.04	7.7 ± 3.2	0.31
	No	9.2 ± 3.7		8.2 ± 3.1		8.3 ± 3.2		7.1 ± 2.9	
Infection < 1 month post-LT	Yes	9.4 ± 4.0	0.81						
	No	9.1 ± 3.6							
Infection [1–6] month post-LT	Yes	8.9 ± 3.7	0.73	8.0 ± 2.7	0.83				
	No	9.1 ± 3.6		8.2 ± 3.2					
Infection > 6 month post-LT	Yes	8.9 ± 3.3	0.7	8.3 ± 3.3	0.61	8.3 ± 3.1	0.86		
	No	9.2 ± 3.7		8.2 ± 3.1		8.5 ± 3.4			
Metabolic disorders									
Dyslipidemia	Yes	8.9 ± 3.6	0.91	8.3 ± 3.3	0.73	8.4 ± 3.4	0.65	7.0 ± 2.8	0.28
	No	9.2 ± 3.8		8.1 ± 3.1		8.6 ± 3.4		7.4 ± 3.1	
De novo dyslipidemia	Yes	8.9 ± 3.5	0.88	8.3 ± 3.4	0.7	8.5 ± 3.4	0.9	7.0 ± 2.9	0.27
	No	9.1 ± 3.9		8.1 ± 3.1		8.5 ± 3.3		7.4 ± 3.1	
Hypertension	Yes	9.0 ± 3.8	0.61	8.3 ± 3.2	0.36	8.4 ± 3.3	0.32	7.0 ± 2.9	0.27
	No	9.2 ± 3.4		8.0 ± 3.2		8.8 ± 3.6		7.6 ± 3.1	
De novo hypertension	Yes	9.0 ± 3.8	0.63	8.6 ± 3.2	0.049	8.5 ± 3.3	0.95	6.9 ± 2.9	0.24
	No	9.2 ± 3.6		7.8 ± 3.2		8.5 ± 3.4		7.5 ± 3.1	
Severe hypertension	Yes	9.0 ± 3.2	0.89	8.7 ± 3.2	0.21	8.2 ± 3.2	0.42	7.6 ± 3.4	0.38
	No	9.1 ± 3.8		8.1 ± 3.2		8.6 ± 3.4		7.1 ± 2.8	
Diabetes	Yes	8.8 ± 3.9	0.54	8.3 ± 3.4	0.98	8.6 ± 3.6	0.91	7.0 ± 2.7	0.29
	No	9.2 ± 3.6		8.2 ± 3.1		8.5 ± 3.2		7.3 ± 3.1	
De novo diabetes	Yes	9.4 ± 4.0	0.6	8.6 ± 3.5	0.41	8.8 ± 3.2	0.31	7.5 ± 3.2	0.75
	No	9.0 ± 3.6		8.1 ± 3.1		8.4 ± 3.4		7.2 ± 2.9	
Diabetes treated with insulin	Yes	9.3 ± 4.2	0.78	8.3 ± 3.2	0.71	8.7 ± 3.9	0.92	6.7 ± 2.8	0.22
	No	9.0 ± 3.6		8.2 ± 3.2		8.5 ± 3.2		7.3 ± 3.0	
De novo diabetes treated with insulin	Yes	9.7 ± 4.5	0.53	8.2 ± 3.3	0.84	8.3 ± 3.1	0.58	6.9 ± 3.0	0.58
	No	8.9 ± 3.6		8.2 ± 3.2		8.5 ± 3.4		7.3 ± 3.0	
Malignancies									
De novo malignancy or HCC recurrence	Yes	9.3 ± 3.4	0.18	8.5 ± 3.3	0.21	8.7 ± 3.3	0.61	7.5 ± 3.1	0.31
	No	8.8 ± 3.9		8.0 ± 3.1		8.4 ± 3.4		7.0 ± 2.8	
De novo malignancy	Yes	9.4 ± 3.5	0.2	8.5 ± 3.3	0.28	8.6 ± 3.3	0.73	7.4 ± 3.0	0.27
	No	8.9 ± 3.8		8.0 ± 3.1		8.5 ± 3.5		7.0 ± 2.9	
De novo malignancy (excl. Skin cancer)	Yes	9.5 ± 3.6	0.22	8.1 ± 3.0	0.92	8.4 ± 3.3	0.76	7.4 ± 3.1	0.58
	No	8.9 ± 3.7		8.2 ± 3.3		8.6 ± 3.4		7.1 ± 2.9	
Non-melanoma skin cancer	Yes	9.0 ± 2.8	0.6	10.3 ± 4.0	0.01	9.0 ± 3.3	0.45	7.8 ± 2.7	0.24
	No	9.1 ± 3.8		8.0 ± 3.0		8.5 ± 3.4		7.1 ± 3.0	
PTLD	Yes	10.4 ± 3.2	0.19	7.5 ± 3.2	0.66	7.4 ± 2.2	0.38	6.2 ± 1.5	0.41
	No	9.0 ± 3.7		8.2 ± 3.2		8.6 ± 3.4		7.2 ± 3.0	
Upper aerodigestive tract cancer	Yes	9.0 ± 3.3	0.79	8.1 ± 2.8	0.92	8.5 ± 3.1	0.93	7.7 ± 3.3	0.22
	No	9.1 ± 3.8		8.2 ± 3.3		8.5 ± 3.5		7.1 ± 2.9	
HCC recurrence	Yes	9.1 ± 1.7	0.55	9.1 ± 2.8	0.19	10.6 ± 5.0	0.04	7.8 ± 4.5	0.60
	No	8.9 ± 3.7		7.6 ± 2.9		8.0 ± 3.0		7.2 ± 2.8	
Renal failure									
1 month after LT									
eGFR < 30	Yes	8.6 ± 4.1	0.27						
	No	9.2 ± 3.6							
eGFR < 60	Yes	9.1 ± 3.5	0.92						
	No	9.2 ± 3.7							
6 months after LT									
eGFR < 30	Yes	8.1 ± 4.4	0.37	8.7 ± 3.5	0.77	8.7 ± 3.6	0.75		
	No	9.2 ± 3.6		8.2 ± 3.2		8.5 ± 3.4			
eGFR < 60	Yes	8.3 ± 3.2	0.01	7.9 ± 3.4	0.16	7.6 ± 3.1	0.01		
	No	9.6 ± 3.8		8.4 ± 3.0		9.1 ± 3.4			
1 year after LT									
eGFR < 30	Yes	9.8 ± 2.6	0.52	8.1 ± 4.3	0.67	8.9 ± 3.2	0.79	6.5 ± 2.4	0.42
	No	9.1 ± 3.6		8.2 ± 3.2		8.5 ± 3.4		7.2 ± 3.0	
eGFR < 60	Yes	8.8 ± 3.6	0.48	7.9 ± 3.2	0.07	8.1 ± 3.5	0.07	7.1 ± 3.0	0.4
	No	9.2 ± 3.7		8.4 ± 3.2		8.8 ± 3.3		7.3 ± 2.9	
5 years after LT									
eGFR < 30	Yes	10.1 ± 3.2	0.41	9.8 ± 2.8	0.08	7.6 ± 3.7	0.44	7.6 ± 3.2	0.55
	No	9.1 ± 3.6		8.2 ± 3.2		8.6 ± 3.4		7.2 ± 3.0	
eGFR < 60	Yes	8.8 ± 3.7	0.27	8.2 ± 3.2	0.81	7.8 ± 3.3	0.01	6.6 ± 2.7	0.02
	No	9.3 ± 3.6		8.2 ± 3.2		8.9 ± 3.4		7.5 ± 3.0	
10 years after LT									
eGFR < 30	Yes	7.1 ± 3.4	0.07	8.5 ± 2.9	0.51	7.1 ± 2.1	0.1	7.8 ± 4.4	0.88
	No	9.2 ± 3.6		8.2 ± 3.2		8.6 ± 3.4		7.2 ± 2.9	
eGFR < 60	Yes	9.2 ± 3.8	0.94	8.8 ± 3.2	0.09	8.3 ± 3.4	0.3	7.0 ± 3.4	0.25
	No	9.1 ± 3.5		8.0 ± 3.2		8.6 ± 3.4		7.3 ± 2.8	

Table 4
Overall mean tacrolimus concentration and complications after LT.

Overall mean Tac concentration									
		1 month	p	3 months	p	6 months	p	1 year	p
Cardiovascular event	Yes	9.6 ± 3.1	0.99	9.0 ± 2.9	0.35	9.0 ± 3.0	0.21	8.2 ± 2.6	0.68
	No	9.6 ± 3.1		8.6 ± 2.5		8.5 ± 2.2		8.0 ± 2.0	
Infections									
Severe infection	Yes	9.4 ± 3.2	0.33	8.6 ± 2.6	0.46	8.4 ± 2.3	0.28	7.9 ± 2.0	0.63
	No	9.8 ± 3.1		8.8 ± 2.5		8.7 ± 2.4		8.1 ± 2.2	
CMV infection	Yes	9.0 ± 3.0	0.26	8.0 ± 2.5	0.08	8.4 ± 2.5	0.7	8.1 ± 2.3	0.83
	No	9.7 ± 3.1		8.8 ± 2.5		8.6 ± 2.4		8.0 ± 2.1	
Infection < 1 month post-LT	Yes	9.7 ± 3.4	0.73						
	No	9.7 ± 3							
Infection [1–6] month post-LT	Yes	9.7 ± 3.1	0.88	8.6 ± 2.9	0.88				
	No	9.7 ± 3.0		8.7 ± 2.5					
Infection > 6 month post-LT	Yes	9.4 ± 2.7	0.34	8.7 ± 2.5	0.95	8.5 ± 2.1	0.89		
	No	9.8 ± 3.1		8.7 ± 2.5		8.6 ± 2.4			
Metabolic disorders									
Dyslipidemia	Yes	9.6 ± 3.2	0.83	8.6 ± 2.6	0.72	8.5 ± 2.5	0.36	8.0 ± 2.1	0.69
	No	9.6 ± 3.1		8.8 ± 2.5		8.6 ± 2.3		8.1 ± 2.1	
De novo dyslipidemia	Yes	9.6 ± 3.1	0.97	8.7 ± 2.6	0.93	8.5 ± 2.5	0.51	8.0 ± 2.1	1
	No	9.6 ± 3.2		8.7 ± 2.5		8.6 ± 2.3		8.0 ± 2.0	
Hypertension	Yes	9.5 ± 3.2	0.38	8.7 ± 2.5	0.73	8.5 ± 2.3	0.57	8.0 ± 2.0	0.66
	No	9.9 ± 3.0		8.8 ± 2.7		8.7 ± 2.6		8.1 ± 2.2	
De novo hypertension	Yes	9.5 ± 3.2	0.75	8.8 ± 2.5	0.55	8.7 ± 2.3	0.52	8.0 ± 2.1	0.99
	No	9.7 ± 3.1		8.6 ± 2.6		8.5 ± 2.4		8.0 ± 2.1	
Severe hypertension	Yes	9.5 ± 2.9	0.8	8.8 ± 2.6	0.66	8.7 ± 2.5	0.73	8.2 ± 2.2	0.54
	No	9.6 ± 3.2		8.7 ± 2.5		8.5 ± 2.3		8.0 ± 2.0	
Diabetes	Yes	9.3 ± 3.2	0.33	8.5 ± 2.6	0.36	8.5 ± 2.5	0.85	7.9 ± 2.1	0.68
	No	9.8 ± 3.1		8.8 ± 2.5		8.6 ± 2.3		8.1 ± 2.1	
De novo diabetes	Yes	9.8 ± 2.8	0.62	8.9 ± 2.4	0.59	8.8 ± 2.2	0.35	8.4 ± 2.0	0.27
	No	9.6 ± 3.2		8.7 ± 2.6		8.5 ± 2.4		7.9 ± 2.1	
Diabetes treated with insulin	Yes	9.9 ± 3.1	0.42	8.8 ± 2.5	0.83	8.7 ± 2.5	0.63	8.0 ± 2.1	0.95
	No	9.5 ± 3.1		8.7 ± 2.6		8.5 ± 2.3		8.0 ± 2.1	
De novo diabetes treated with insulin	Yes	10.0 ± 2.8	0.44	8.8 ± 2.4	0.89	8.5 ± 2.0	0.85	8.1 ± 1.8	0.91
	No	9.5 ± 3.2		8.7 ± 2.6		8.6 ± 2.4		8.0 ± 2.1	
Malignancies									
De novo malignancy or HCC recurrence	Yes	9.9 ± 2.8	0.26	8.9 ± 2.3	0.38	8.7 ± 2.3	0.4	8.2 ± 2.0	0.16
	No	9.4 ± 3.3		8.6 ± 2.7		8.5 ± 2.4		7.9 ± 2.1	
De novo malignancy	Yes	9.9 ± 2.9	0.27	8.8 ± 2.4	0.51	8.7 ± 2.4	0.5	8.2 ± 2.0	0.23
	No	9.4 ± 3.3		8.6 ± 2.6		8.5 ± 2.4		7.9 ± 2.1	
De novo malignancy (excl. Skin cancer)	Yes	10.0 ± 3.0	0.19	8.7 ± 2.4	0.92	8.5 ± 2.3	0.8	8.1 ± 2.0	0.84
	No	9.4 ± 3.2		8.7 ± 2.6		8.6 ± 2.4		8.0 ± 2.1	
Non-melanoma skin cancer	Yes	9.6 ± 1.4	0.97	9.5 ± 2.3	0.14	9.4 ± 2.4	0.15	8.8 ± 1.9	0.12
	No	9.6 ± 3.2		8.6 ± 2.6		8.5 ± 2.4		7.9 ± 2.1	
PTLD	Yes	10.2 ± 1.9	0.6	8.6 ± 2.4	0.95	8.3 ± 2.1	0.75	7.9 ± 1.6	0.91
	No	9.6 ± 3.2		8.7 ± 2.5		8.6 ± 2.4		8.0 ± 2.1	
Upper aerodigestive tract cancer	Yes	9.5 ± 2.8	0.86	8.5 ± 2.4	0.6	8.5 ± 2.4	0.87	8.1 ± 2.1	0.61
	No	9.6 ± 3.2		8.7 ± 2.6		8.6 ± 2.4		8.0 ± 2.1	
HCC recurrence	Yes	9.8 ± 2.4	0.78	9.7 ± 1.7	0.78	9.6 ± 2.1	0.06	9.0 ± 2.2	0.07
	No	9.5 ± 3.0		8.3 ± 2.5		8.0 ± 2.1		7.6 ± 1.8	
Renal failure									
1 month after LT									
eGFR < 30	Yes	8.8 ± 4.0	0.22						
	No	9.8 ± 2.9							
eGFR < 60	Yes	9.5 ± 3.1	2.94						
	No	9.9 ± 2.9							
6 months after LT									
eGFR < 30	Yes	8.3 ± 3.4	0.25	8.1 ± 3.2	0.53	8.0 ± 2.4	0.53		
	No	9.7 ± 3.1		8.7 ± 2.5		8.6 ± 2.4			
eGFR < 60	Yes	8.7 ± 3.0	0.01	8.1 ± 2.6	0.01	8.0 ± 2.6	0.01		
	No	10.3 ± 2.9		9.1 ± 2.4		9.0 ± 2.3			
1 year after LT									
eGFR < 30	Yes	10.0 ± 1.0	0.61	8.2 ± 2.1	0.60	8.3 ± 1.5	0.76	7.7 ± 1.2	0.67
	No	9.7 ± 3.1		8.7 ± 2.5		8.6 ± 2.4		8.0 ± 2.1	
eGFR < 60	Yes	9.4 ± 3.2	0.32	8.4 ± 2.5	0.18	8.2 ± 2.3	0.02	7.7 ± 2.1	0.09
	No	9.8 ± 3.1		8.9 ± 2.5		8.8 ± 2.4		8.2 ± 2.0	
5 years after LT									
eGFR < 30	Yes	10.0 ± 2.3	0.77	9.5 ± 1.4	0.39	9.1 ± 2.6	0.56	7.9 ± 2.3	0.89
	No	9.6 ± 3.2		8.7 ± 2.6		8.6 ± 2.4		8.0 ± 2.1	
eGFR < 60	Yes	9.3 ± 3.2	0.29	8.4 ± 2.3	0.13	8.2 ± 2.3	0.03	7.6 ± 2.0	0.01
	No	9.8 ± 3.2		9.0 ± 2.7		8.8 ± 2.4		8.3 ± 2.1	
10 years after LT									
eGFR < 30	Yes	10.0 ± 3.1	0.1	8.7 ± 3.0	0.59	8.6 ± 2.2	0.95	8.1 ± 2.0	0.89
	No	8.6 ± 3.3		9.1 ± 2.5		8.9 ± 2.4		8.2 ± 2.1	
eGFR < 60	Yes	9.8 ± 3.1	0.74	9.1 ± 2.5	0.86	8.7 ± 2.4	0.3	8.1 ± 2.2	0.45
	No	10.0 ± 3.1		9.0 ± 2.6		8.9 ± 2.3		8.3 ± 1.9	

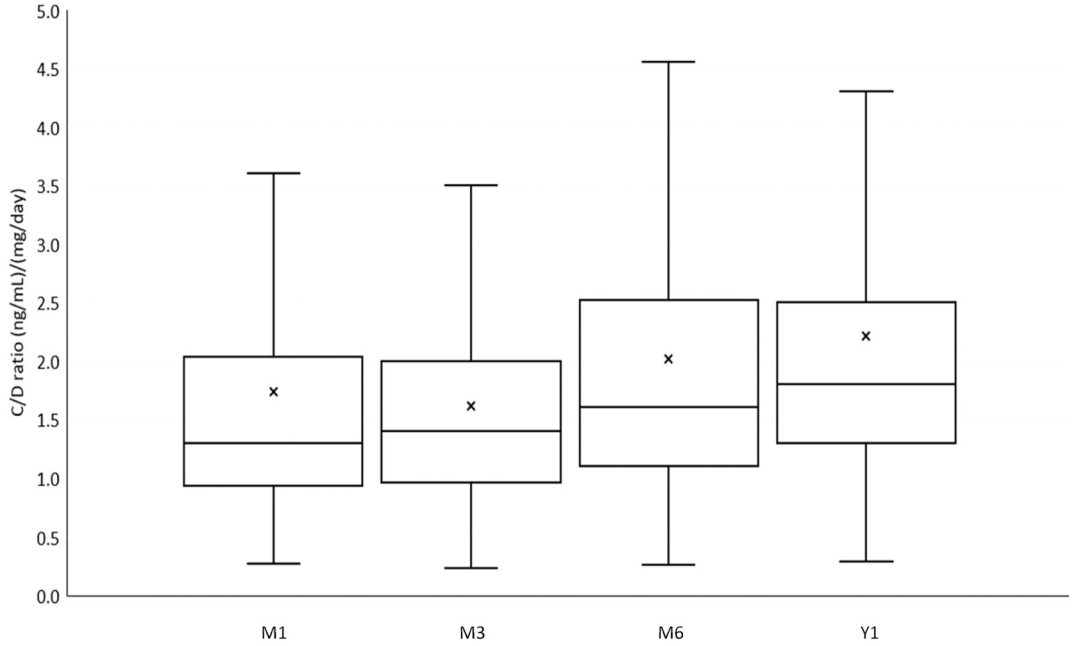


Fig. 3. Evolution of Tac C/D ratio after LT.
At 1 month, mean + /-SD (cross) Tac C/D ratio was at 1.7 ± 1.4, then 1.6 ± 1.0 at 3 months, 2.0 ± 1.4 at 6 months and 2.2 ± 1.8 at 12 months. Median Tac C/D ratio (straight line) was 1.3 (IQR = 1.1), 1.4 (IQR = 1.0), 1.6 (IQR = 1.4) and 1.8 (IQR = 1.2) at 1, 3, 6 and 12 months respectively.

mean eGFR before LT was 91.2 mL/min/1.73m^2, and declined to 61.6 ± 25.9 the first month after LT, and 63.2 ± 24.7 mL/min/1.73m^2 10 years after LT.

3.2. Exposure to tacrolimus and association with post-LT complications

3.2.1. Tac trough blood concentration

Fig. 2 describe the patients' exposure to Tac using Tac trough blood concentrations and overall mean Tac concentrations. The mean Tac trough blood concentration was 9.1 ± 3.7 (median at 8.7) 1 month after LT and decreased to 7.2 ± 3.0 (median at 6.8) one year after LT. The overall mean Tac concentration was 9.6 ± 3.1 during the first month and 8.0 ± 2.1 during the first year.

Tables 3 and 4 describes the association between the mean Tac trough blood concentrations or overall mean Tac concentrations and the occurrence of clinical complications after LT.

Patients with moderate and severe renal impairment (eGFR under 60 mL/min) at 6 months had significantly lower overall mean Tac concentrations and Tac trough blood concentrations at 1, 3 and 6 months. Patients with moderate and severe renal impairment at 1 and 5 year had also at 6 and 12 months a lower overall mean Tac concentrations and Tac trough blood concentrations. Tac trough blood concentration at 3 months was significantly higher in patients who developed skin cancer, cardiovascular event, or de novo hypertension. Patients who presented CMV infection or HCC recurrence had significantly higher Tac trough blood concentration at 6 months.

The Tac trough blood concentrations and the overall mean Tac concentrations were not significantly different according to survival and according to the occurrence of de novo dyslipidemia, de novo diabetes (or diabetes treated with insulin), de novo hypertension (or severe hypertension), severe infectious events (or CMV infection), cardiovascular events, and de novo malignancies.

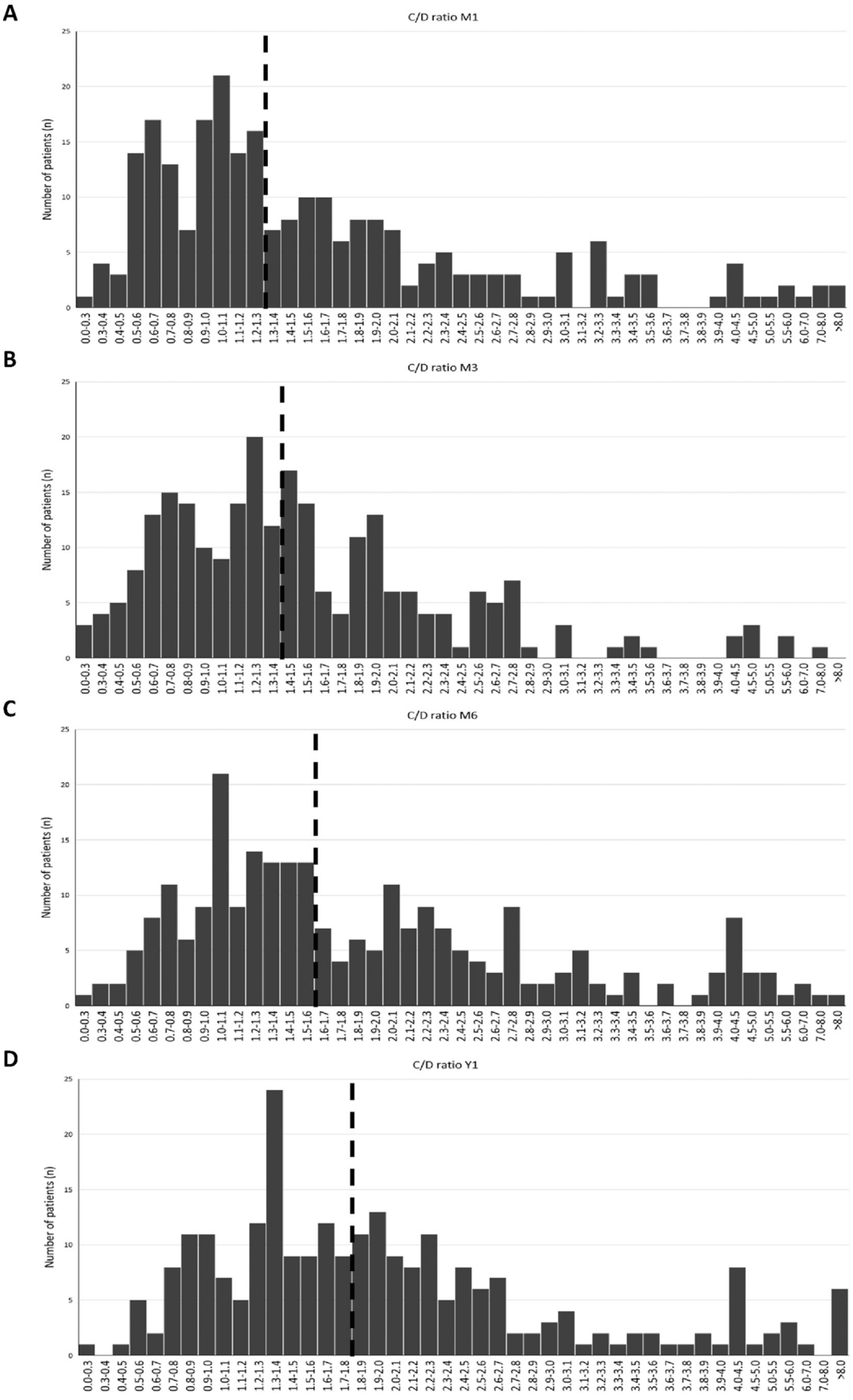
Finally, there was a trend between recurrence of HCC and a higher overall mean Tac concentrations at 6 and 12 months.

3.2.2. Tac metabolism and C/D ratio

Figs. 3 and 4 describe the time course of the median C/D ratio after LT and the distribution of C/D ratio after LT. The median C/D ratio increased with time, from 1.3 at one month to 1.8 at one year after LT. We therefore defined fast and slow metabolizers in our cohort, depending on the median C/D ratio, at each time point. Thus, fast metabolizers were patients with C/D ratio under 1.3, 1.4, 1.6 and 1.8 at 1, 3, 6 and 12 months after LT, respectively.

Using fast/slow metabolizer status based on the median rate of C/D ratio for each time point, we evaluate the stability of that status with time: 126 patients (50.2%) kept the same fast/slow metabolizers status all along the 1st year after LT. Between the 1st and 3rd month, 186 (74.1%) patients kept the same status, between the 1st and 6th month, 153 (61.0%) patients, between the 3rd and 6th month, 192 (76.5%) patients, and between the 6th month and 12th month, 189 (75.3%) patients were classified in the same group. We then evaluated the stability of the fast/slow metabolizer status if the median at 6 or 12 months was used. If C/D ratio of 1.6 was taken as the cut-off for fast/slow metabolizer, 193 (76.9%) kept the same status between 6 and 12 months vs. 190 (75.7%) with a C/D ratio of 1.8. We also evaluated the 1.09 cut-off of C/D separating fast and slow metabolizers as previously defined by Thölking and coll. [13,14,21,22]. Using this cut-off, C/D was not stable over time: between one month and one year after LT, 147 (58.6%) patients kept the same fast/slow metabolizer status. Between the 1st and 3rd month, 199 (79.3%) patients kept the same status, between the 1st and 6th month 162 (64.5%) patients, between the 3rd and 6th month 197 (78.5%) patients and between the 6th month and 12th month, 214 (85.3%) patients were classified in the same group.

Table 5 describes the association between fast/slow metabolizer status (depending on 3 different cut-offs of C/D ratio: 1.6, 1.8 and 1.09) and the occurrence of complications after LT. Table 6 describes the mean eGFR depending of fast/slow metabolizer status. Using the 1.6 cut-off of C/D, at 6 and 12 months after LT, fast metabolizers at 6 months had a significantly lower eGFR at 1, 5 and 10 year and had more moderate and severe renal impairment at 5 year and more severe renal impairment at 10 year than slow metabolizer. There was no other significant association between fast/slow metabolizer status defined with C/D ratio < 1.6 at 6 or 12 months. Using the 1.8 cut-off of C/D, at 6 and 12 months after LT, fast metabolizers at 6 months had a significantly lower eGFR at 1, 5 and 10 year and had more moderate and



(caption on next page)

Fig. 4. Distribution of Tac C/D ratio after LT.
At 1 month the median Tac C/D ratio (dotted line) was 1.3. Tac C/D ratio range from 0.3 to 12.9.
At 3 month the median Tac C/D ratio (dotted line) was 1.5. Tac C/D ratio range from 0.2 to 7.3.
At 6 month the median Tac C/D ratio (dotted line) was 1.6. Tac C/D ratio range from 0.3 to 12.2.
At 1 year the median Tac C/D ratio (dotted line) was 1.8. Tac C/D ratio range from 0.3 to 15.6.

Table 5
Fast/slow metabolizers status and complications after LT.

	C/D < 1.6 at M6			C/D < 1.6 at M12			C/D < 1.8 at M6			C/D < 1.8 at M12		
	Yes	No	p	Yes	No	p	Yes	No	p	Yes	No	p
Cardiovascular event	16.9% (21/124)	11.8% (15/127)	0.1	17.6% (18/102)	14.1% (21/149)	0.45	19.7% (27/137)	17.2% (12/114)	0.046	17.2% (21/122)	14.0% (18/129)	0.48
Infections												
Severe infectious event	48.4% (60/124)	40.9% (52/127)	0.26	43.1% (44/102)	45.6% (68/149)	0.66	49.6% (68/137)	44.3% (44/114)	0.09	44.3% (54/122)	45.0% (58/129)	0.87
CMV infection	10.5% (13/124)	16.5% (21/127)	0.16	8.8% (9/102)	16.8% (25/149)	0.07	12.4% (17/137)	9.8% (17/114)	0.56	9.8% (12/122)	17.1% (22/129)	0.1
Metabolic disorder												
Dyslipidemia	50.0% (62/124)	44.1% (56/127)	0.35	50.0% (51/102)	45.0% (67/149)	0.43	51.1% (70/137)	52.5% (48/114)	0.16	52.5% (64/122)	41.9% (54/129)	0.09
De novo dyslipidemia	49.2% (61/124)	39.4% (50/127)	0.12	49.0% (50/102)	40.9% (61/149)	0.21	49.6% (68/137)	51.6% (43/114)	0.06	51.6% (63/122)	37.2% (48/129)	0.02
Hypertension	74.2% (92/124)	64.6% (82/127)	0.1	71.6% (73/102)	67.8% (101/149)	0.52	74.5% (102/137)	73.0% (72/114)	0.05	73.0% (89/122)	65.9% (85/129)	0.23
De novo hypertension	54.0% (67/124)	44.9% (57/127)	0.12	68.6% (59/86)	58.6% (65/111)	0.15	52.6% (72/137)	56.6% (52/114)	0.27	56.6% (69/122)	42.6% (55/129)	0.03
Severe hypertension	22.6% (28/124)	17.3% (22/127)	0.3	17.6% (18/102)	21.5% (32/149)	0.46	24.1% (33/137)	22.1% (17/114)	0.07	22.1% (27/122)	17.8% (23/129)	0.39
Diabetes	36.3% (45/124)	39.4% (50/127)	0.62	36.3% (37/102)	38.9% (58/149)	0.69	38.7% (53/137)	39.3% (42/114)	0.76	39.3% (48/122)	36.4% (47/129)	0.64
De novo diabetes	17.7% (22/124)	19.7% (25/127)	0.69	17.6% (18/102)	19.5% (29/149)	0.72	18.2% (25/137)	19.7% (22/114)	0.83	19.7% (24/122)	17.8% (23/129)	0.71
Diabetes treated with insulin	21.0% (26/124)	21.3% (27/127)	0.93	24.5% (25/102)	18.8% (28/149)	0.29	21.9% (30/137)	26.2% (23/114)	0.77	26.2% (32/122)	16.3% (21/129)	0.049
De novo diabetes treated with Insulin	12.9% (16/124)	13.4% (17/127)	0.91	14.7% (15/102)	12.1% (18/149)	0.55	13.9% (19/137)	16.4% (14/114)	0.71	16.4% (20/122)	10.1% (13/129)	0.14
Malignancies												
De novo malignancy or HCC recurrence	45.2% (56/124)	37.8% (48/127)	0.24	38.2% (39/102)	43.6% (65/149)	0.4	44.5% (61/137)	38.5% (43/114)	0.28	38.5% (47/122)	44.2% (57/129)	0.36
De novo malignancy	43.5% (54/124)	34.6% (44/127)	0.15	37.3% (38/102)	40.3% (60/149)	0.63	43.1% (59/137)	37.7% (39/114)	0.15	37.7% (46/122)	40.3% (52/129)	0.67
De novo malignancy (exc. Skin cancer)	32.3% (40/124)	29.9% (38/127)	0.69	28.4% (29/102)	32.9% (49/149)	0.45	32.1% (44/137)	28.7% (34/114)	0.7	28.7% (35/122)	33.3% (43/129)	0.43
Non-melanoma skin cancer	12.1% (15/124)	5.5% (7/127)	0.07	8.8% (9/102)	8.7% (13/149)	0.98	11.7% (16/137)	9.0% (6/114)	0.7	9.0% (11/122)	8.5% (11/129)	0.89
PTLD	4.0% (5/124)	2.4% (3/127)	0.5	5.9% (6/102)	1.3% (2/149)	0.07	4.4% (6/137)	5.7% (2/114)	0.3	5.7% (7/122)	0.8% (1/129)	0.04
Upper aerodigestive tract cancer	24.2% (30/124)	19.7% (25/127)	0.39	20.6% (21/102)	22.8% (34/149)	0.68	23.4% (32/137)	19.7% (23/114)	0.54	19.7% (24/122)	24.0% (31/129)	0.4
HCC recurrence	8.6% (3/35)	10.0% (4/40)	1	7.1% (2/28)	10.6% (5/47)	0.71	2.2% (3/137)	1.6% (4/114)	0.71	1.6% (2/122)	3.9% (5/129)	0.45
Renal failure												
1 year after LT												
eGFR < 30	2.4% (3/124)	2.4% (3/127)	1	2.0% (2/102)	2.7% (4/149)	1	2.2% (3/137)	2.5% (3/114)	1	2.5% (3/122)	2.3% (3/129)	1
eGFR < 60	42.7% (53/124)	34.6% (44/127)	0.19	34.3% (35/102)	41.6% (62/149)	0.25	43.1% (59/137)	35.2% (38/114)	0.12	35.2% (43/122)	41.9% (54/129)	0.28
5 years after LT												
eGFR < 30	5.3% (6/114)	1.9% (2/107)	0.28	4.3% (4/92)	3.1% (4/129)	0.72	4.7% (6/127)	4.5% (2/94)	0.47	4.5% (5/110)	2.7% (3/111)	0.5
eGFR < 60	46.5% (53/114)	32.7% (35/107)	0.04	45.7% (42/92)	35.7% (46/129)	0.14	47.2% (60/127)	45.5% (28/94)	0.01	45.5% (50/110)	34.2% (38/111)	0.09
10 years after LT												
eGFR < 30	13.1% (11/84)	4.1% (3/73)	0.049	8.6% (6/70)	9.2% (8/87)	0.89	13.0% (12/92)	9.8% (2/65)	0.03	9.8% (8/82)	8.0% (6/75)	0.7
eGFR < 60	45.2% (38/84)	38.4% (28/73)	0.38	42.9% (30/70)	41.4% (36/87)	0.85	46.7% (43/92)	43.9% (23/65)	0.16	43.9% (36/82)	40.0% (30/75)	0.62

Table 6
Fast/slow metabolizers status and renal function after LT.

		eGFR					
		Y1	p	Y5	p	Y10	p
C/D < 1.6 at M6	Yes	59.9 ± 14.2	0.01	59.3 ± 19.23	0.02	59.23 ± 2.61	0.04
	No	68.5 ± 22.7		68.72 ± 20.63		67.58 ± 24.34	
C/D < 1.6 at M12	Yes	64 ± 17.3	0.76	62.46 ± 20.15	0.64	62.6 ± 22.47	0.82
	No	63.9 ± 20.6		64.7 ± 20.62		63.56 ± 26.55	
C/D < 1.8 at M6	Yes	60 ± 14.4	0.01	59.35 ± 18.9	0.01	59.04 ± 24.71	0.01
	No	69.5 ± 23.2		69.82 ± 20.95		68.9 ± 23.85	
C/D < 1.8 at M12	Yes	64.8 ± 18.0	0.9	62.69 ± 20.16	0.38	61.93 ± 22.93	0.54
	No	63.1 ± 20.4		64.81 ± 20.7		64.44 ± 26.69	
C/D < 1.09 at M1	Yes	62.3 ± 15.7	0.36	62.24 ± 19.14	0.29	62.07 ± 22.04	0.67
	No	65.1 ± 21.1		64.71 ± 21.22		63.86 ± 26.53	
C/D < 1.09 at M3	Yes	63.4 ± 16.7	0.77	61.55 ± 17.61	0.46	60.55 ± 23.87	0.36
	No	64.2 ± 20.3		64.76 ± 21.6		64.39 ± 25.19	
C/D < 1.09 at M6	Yes	60.9 ± 16.4	0.15	57.91 ± 19.22	0.07	59.36 ± 25	0.27
	No	65.1 ± 19.9		65.78 ± 20.46		64.48 ± 24.64	
C/D < 1.09 at M12	Yes	64.4 ± 16.2	0.69	61.87 ± 17.83	0.33	65.05 ± 23.01	0.60
	No	63.8 ± 19.9		64.2 ± 21.05		62.62 ± 25.27	

severe renal impairment at 5 year, more severe renal impairment at 10 year and developed also more cardiovascular events after LT. Fast metabolizers at 12 months developed more PTLD, de novo dyslipidemia, de novo hypertension, and insulin-treated diabetes than slow metabolizers.

Finally, using the 1.09 cut-off of C/D from Thölking, there was no significant association between fast/slow metabolizer status with the mean eGFR at 1, 5 and 10 year after LT. There was a trend for fast metabolizers, defined at M6, to have lower eGFR 5 years after LT.

4. Discussion

Due to significant improvement in both surgical techniques and immunosuppressive therapy during the past decades, LT recipients are expected to live longer. Nevertheless, with time, complications of immunosuppression and immunosuppressive drugs themselves are frequent and can impact survival of patients. Tac became the cornerstone of immunosuppression after LT in the mid 90's and close Tac monitoring is needed because of a high intra- and inter-variability of its metabolism. In addition to general complications of immunosuppression, mainly infections and malignancies, side-effects of Tac include hypertension, diarrhea, hyperglycemia, hyperlipemia, neurological symptom such as tremor, and renal impairment [3–6]. Tac nephrotoxicity could manifest either as acute kidney injury, which is a dose-dependent and reversible, or as a chronic kidney disease (usually defined as GFR < 60 mL/min/1.73m^2) that appears usually after 6 months use of Tac and is irreversible (ranging from 4 to 73% depending on the length of follow up) [23]. Tac use can also lead to diabetes (up to 15.9%), arterial hypertension (up to 70%) and hyperlipidemia (up to 69%), well known cardiovascular risk factors [24,25], and cardiovascular events range from 9 to 25% in LT recipients [26,27]. Therefore, the observed rates of all these complications of Tac in our cohort with long follow-up were as expected, and we report that the occurrence of a significant part of complications of Tac-based immunosuppression is associated with Tac exposure (trough blood concentrations and overall mean Tac concentrations) and Tac metabolism (C/D ratio).

We first focused our attention on trough blood concentrations and overall mean Tac concentrations, which are used in daily practice. It has already been demonstrated that high Tac blood concentration, especially during the first year after LT leads to renal impairment [3,4]. In our study, the most of renal function loss occurred during the first month after LT confirming that long-term renal impairment might be determined by early exposition of Tac. Several studies reported that the use of lower dose of Tac during the first month after LT could therefore decrease the number of patient with eGFR < 60 mL/min/1.73m^2 [4,11]. In our population patients with moderate and severe renal impairment (eGFR under 60 mL/min/1.73m^2) at 6 months had significantly lower Tac concentrations during the first year after LT; it can be hypothesized that clinicians adapted (lowered) Tac doses in patients with impaired renal function in the early post-operative period.

Although different factor contributes as well such as history of smoking or genetic predisposition, Tac is also a well-known factor of cancer progression [28]. The risk of solid cancer after LT is two to three times higher compared to the general population, and 10 to 30 times higher for skin cancer (16% to 22.5% of patients) and PTLD (reported up to 2.8% of patients) [7,29]. We initially chose a LT population of patients with ALD because of the high risk of malignancies. Carenco and coll. reported [6] that the mean Tac concentration during the first year after LT was significantly higher in patients who developed non-skin solid tumors (10.3 and 7.9 ng/mL, respectively, $p < 0.0001$). In our population, we failed to find such association; except for non-melanoma skin cancers and HCC recurrence (patients had a higher Tac trough blood concentration at 3 or 6 months). One possible explanation is that the mean blood concentration of Tac in our study was lower during the first 6 months following LT compared to the population of the Carenco and coll. study [12]. For example, the mean Tac trough blood concentration at 3 months was 8.2 in our population vs. 9.2 in Carenco study, and the overall mean Tac concentration at 3 months for patients with de novo malignancy was in our population 8.8 (± 2.4) vs. 8.6 (± 2.6) for patient without de novo cancer (10.8 vs. 8.9 in Carenco study). Regarding HCC recurrence, our results confirm that a reduced exposure to CNIs (for instance by using sirolimus) within the first months after LT could be associated with decreased risk of HCC recurrence [30,31]. Similarly, conversion from CNI to sirolimus is able to prevent squamous-cell carcinoma recurrence in kidney transplant recipients [32].

Finally, we report here that patients who presented CMV infection had significantly higher Tac trough blood concentration at 6 months. Since the impact of Tac exposure on CMV infection has not been previously reported, this probably needs further evaluation.

Until now, only Tac trough blood concentration is used to adjust the dosage of Tac, and other tools, easy to use in daily practice, to determine patient at risks to developed long-term complications related to Tac therapy are needed. Several factors (outside patients' adherence) are known to influence and explained the high intra- and inter-variability of Tac metabolism. Genetic polymorphisms of cytochrome P450 enzymes such as CYP3A4 or CYP3A5 and P-glycoprotein, of both recipient and donor are involved, but this cannot be used routinely [33,34]. Some drugs, such as corticosteroids or antibiotics, or food, are

able to modify the activity of those enzymes, leading to modification in Tac bioavailability [35]. Some studies found contradictory results as to the influence of age or sex on Tac metabolism: some reported that females required higher dosage of Tac compared to male [36]. Tac metabolism rate, defined as C/D [13,14,22] ratio, has been recently proposed as an easy tool to predict patient at risk to develop complications after liver or kidney transplantation. C/D ratio is usually calculated at 6 months, in order to prevent the interaction of corticosteroids or antibiotics with Tac metabolism. The C/D ratio are calculated whether directly the 6th month or using the mean C/D ratio at 6 months (using the data at 1, 3 and 6 months). Interestingly, we demonstrated here that C/D ratio progressively increases with time during the first year after LT, as previously reported after kidney transplantation [37].

Using the median calculated C/D ratio, patients can be separated in 2 groups, patients with a C/D ratio over the median (slow-metabolizers) and those with a C/D ratio under the median (fast-metabolizers). Fast-metabolizers are thought to be exposed to higher risk of Tac adverse effect because of the need of higher doses of Tac to achieve target, increasing thus the risk of higher (and deleterious) Tac peak. After kidney transplantation, fast-metabolizers could have more BK viremia and significantly lower eGFR [37]. In the study of Thölking and coll. [14], LT recipients considered as fast-metabolizers (defined with a C/D ratio < 1.09), had significantly lower eGFR than slow-metabolizers. We did find similar result in our study, using different cut-offs because of significant differences in C/D ratio (and median) calculated in our study, which were much higher (for instance, 1.6 vs. 1.09 at 6 months). This difference of C/D ratio might reside in the difference in the populations: for example, there were less women in our study than in Thölking (23.1% vs. 49.4%) and gender had already been associated with difference in Tac metabolism (women probably require higher doses of Tac). In addition, our study focused only on patients with ALD whereas Thölking included all indications for LT: there might exist differences between Tac metabolism depending on the clinical profile of LT recipients.

In the present study, we evaluated 2 cut-offs of C/D, derived from median at 6- and 12 months (1.6 and 1.8). We consider 1.8 as the most efficient cut-off rather than 1.6, because it was more powerful and more associated with long term complications. Defining fast metabolizers patient with C/D ratio under 1.8, we found similar results than Thölking and coll.: fast-metabolizers had lower eGFR and more renal impairment (eGFR < 60 mL/min/1.73m^2). Moreover, in our population, C/D ratio was more efficient than trough blood concentrations to identify patients with renal failure. In addition, we report here for the first time that fast-metabolizers also developed more de novo dyslipidemia, more de novo hypertension, more diabetes treated with insulin and presented more cardiovascular events while Tac trough blood concentration was only associated with the onset of de novo hypertension and cardiovascular events. Interestingly, it has been recently reported that kidney transplant fast-metabolizers develop more cardiovascular events [21]. Finally, we report that fast-metabolizers had a higher risk of developing PTLD, and this also needs further evaluation.

In conclusion, our results support that, in addition to blood trough concentrations, Tac metabolism, as estimated by the simple C/D ratio, could be an efficient parameter in daily practice to identify LT patients at risk to develop long term general complications of Tac, such as renal impairment, but also de novo dyslipidemia, diabetes and hypertension. Limitations of our study include its retrospective and monocentric nature, the characteristics of the French study population (Caucasian men (both ethnicity and gender influence genetic polymorphism on Tac metabolism), ALD LT recipients), variation of immunosuppressive regimen all along follow-up (long-term Tac withdrawal in almost half of our patients). Further studies are therefore needed to evaluate our findings in larger cohorts, including non-ALD LT recipients, and with a specific focus on other than Tac immunosuppressants, especially mTOR inhibitors which emerged in the recent years.

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This study was not funded from any grant. Authors have no conflict of interest to declare.

Authors' contribution

Jérôme Dumortier had the idea of the project and participated in analysis and interpretation of data.

Franck-Nicolas Bardou, Domitille Erard-Poinsot, Christine Chambon-Augoyard, Elsa Thimonier, Mélanie Vallin and Jérôme Dumortier collected the data.

Olivier Guillaud performed statistical analysis of data and participated in interpretation of data.

Franck-Nicolas Bardou and Jérôme Dumortier participated in writing of the manuscript.

Olivier Guillaud, Christine Chambon-Augoyard, Olivier Boillot and Jérôme Dumortier were involved in clinical management and selection of the patients.

All authors approved the final version of the manuscript.

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