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PHARMACOKINETICS AND DISPOSITION

Comparison of the effect of direct-acting antiviral with and without ribavirin on cyclosporine and tacrolimus clearance values: results from the ANRS CO23 CUPILT cohort

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Abstract

Purpose Direct-acting antiviral agents have demonstrated their efficacy in treating HCV recurrence after liver transplantation and particularly the sofosbuvir/daclatasvir combination. Pharmacokinetic data on both calcineurin inhibitors and direct-acting antiviral exposure in liver transplant recipients remain sparse.

Methods Patients were enrolled from the ANRS CO23 CUPILT cohort. All patients treated with sofosbuvir/daclatasvir with or without ribavirin were included in this study when blood samples were available to estimate the clearance of immunosuppressive therapy before direct-acting antiviral initiation and during follow-up. Apparent tacrolimus and cyclosporine clearances were estimated from trough concentrations measured using validated quality control assays.

Results Sixty-seven mainly male patients (79%) were included, with a mean age of 57 years and mean MELD score of 8.2; 50 were on tacrolimus, 17 on cyclosporine. Ribavirin was combined with sofosbuvir/daclatasvir in 52% of patients. Cyclosporine clearance remained unchanged as well as tacrolimus clearance under the ribavirin-free regimen. Tacrolimus clearance increased 4 weeks after direct-acting antivirals and ribavirin initiation versus baseline (geometric mean ratio 1.81; 90% CI 1.30–2.52). Patients under ribavirin had a significantly higher fibrosis stage (> 2) ($p = 0.02$) and lower haemoglobin during direct-acting antiviral treatment ($p = 0.02$) which impacted tacrolimus measurements. Direct-acting antiviral exposure was within the expected range.

Conclusion Our study demonstrated that liver transplant patients with a recurrence of hepatitis C who are initiating ribavirin combined with a sofosbuvir-daclatasvir direct-acting antiviral regimen may be at risk of lower tacrolimus concentrations because of probable ribavirin-induced anaemia and higher fibrosis score, although there are no effects on cyclosporine levels.

Trial registration NCT 01944527

Keywords Tacrolimus · Ribavirin · Anaemia · Liver fibrosis

Introduction

Liver transplantation (LT) remains the only curative option for patients who develop end-stage liver disease or hepatocellular carcinoma. In France, more than 20% of LT candidates are infected with hepatitis C virus (HCV) [1]. Clearing this HCV is the only way to improve outcomes in these patients. The first second-generation direct-acting antiviral agents (DAA) to become available were the nucleotide analogue

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NS5B polymerase inhibitor sofosbuvir (SOF) and the nucleotide analogue NS5A polymerase inhibitor daclatasvir (DCV). Thanks to the high and sustained virological response (SVR) rates achieved (> 90%) and the minimal side effects experienced by non-transplant patients, this combination was then made available to patients undergoing a liver transplant [2–4].

Recently, the multicentre experience of the ANRS CO23 CUPILT cohort, which included a large number of patients who were receiving SOF and DCV (with or without ribavirin (RBV)) to treat HCV recurrences of varying degrees of severity following LT, was reported [2]. In brief, in 137 patients with HCV recurrence receiving SOF and DCV, the primary efficacy endpoint (an SVR 12 weeks after the end of treatment) reached 96% under an intention-to-treat analysis and 99% when excluding non-virological failures. Anaemia was the most common adverse event (AE), with significantly more cases in the RBV group (56% versus 18%; $p < 0.0001$). A slight but significant reduction in creatinine clearance was also reported. No clinically relevant drug-drug interactions were noted, but 52% of the patients required a change to the dosage of their immunosuppressive drugs. Between baseline and week 4 (W4), tacrolimus and cyclosporine dosages needed to be increased by 19% and 4%, respectively [2]. The authors explained that most changes were necessary 4 weeks after treatment initiation, probably because of an improvement to hepatic function under therapy, and concluded as to the need to closely monitor trough blood concentrations of immunosuppressive drugs during antiviral therapy in liver transplant recipients. During the present study, our aim was to further investigate changes to blood concentrations of cyclosporine or tacrolimus and to rule out any drug-drug interactions during SOF/DCV-based antiviral therapy in liver transplant recipients. We also wanted to assess exposure to SOF, its metabolite and DCV after liver transplantation.

Methods

Patients and study design

All patients included in the pharmacokinetic study gave their informed consent to be included in the ANRS CO23 "Compassionate Use of Protease Inhibitors in viral C Liver Transplantation" (CUPILT) study, which is a multicentre prospective cohort being implemented in 24 French and one Belgian LT centre (ClinicalTrials.gov number NCT01944527). It is being funded and sponsored by ANRS (France REcherche Nord&Sud Sida-hiv Hépatites). In brief, to be enrolled in this cohort, patients must have (i) received a liver transplant, (ii) experienced an HCV recurrence whatever the stage of fibrosis, (iii) been treated with a second-generation DAA and (iv) given their written informed consent. The protocol has been implemented in accordance with the Declaration of Helsinki and French laws on biomedical research and was approved by the "South Mediterranean Ethics Committee" (France). Patients below the age of 18 years, and those who were pregnant, were excluded from this study. The CUPILT cohort is observational so that the type of treatment, dosing of drugs and duration of treatment are at the discretion of each investigator. For the present pharmacokinetic study, we selected patients who were receiving calcineurin inhibitor (CNI)-based immunosuppressive therapy (cyclosporine or tacrolimus) and an SOF/DCV combination as antiviral therapy.

Drug assays

Immunosuppressive drugs

CNI were assayed in terms of trough blood concentrations which were assayed in laboratories linked to each clinical centre. The assays used were immunoassay or liquid chromatography-tandem mass spectrometry (LC-MS/MS). All laboratories participated in internal and external quality control tests. The timing of CNI intakes was recorded by questioning the patients. Trough CNI blood concentrations were monitored at regular intervals during treatment as part of standard follow-up. The concentration targeted depended on the past medical history of the patients. Changes to the doses of immunosuppressive drugs were made accordingly, at the investigators' discretion.

Antiviral drugs

Patients received SOF/DCV at the recommended dose of 400 mg and 60 mg, once daily, respectively. When used, the RBV dose is adjusted by considering body weight, potential RBV-related haematological toxicity and renal function in the LT recipients. Treatment duration was initially planned for 12 or 24 weeks. According to the CUPILT protocol, blood or plasma samples were collected at regular intervals. Samples were thus collected before the initiation of SOF/DCV, 4 weeks after initiation, at the end of SOF/DCV treatment and 4 weeks after SOF/DCV discontinuation. The timing of DAA intakes was recorded from questioning the patients.

SOF, its metabolite GS331007 and DCV were quantified in plasma samples collected at week 4 post-treatment initiation using liquid chromatography coupled with tandem mass spectrometry. Details are given in Supplementary material.

Pharmacokinetic and statistical analysis

Only blood concentrations of immunosuppressive drugs at steady state, measured at least 48 h after any dosing change, were used to estimate apparent drug clearance (Cl/F). The apparent clearance of CNI was estimated from

the rate of input (the dose per intake, D) over the time interval between 2 doses (Δt) which is equal to the rate of elimination, clearance by the average concentration at steady state (Cl×Css). Consequently, assuming that the bioavailability remained unchanged and that trough concentration could be a surrogate of Css, Cl/F was estimated from the ratio of D over the trough or pre-dose concentration (C0), multiplied by the time interval between two doses (Δt), according to the equation $\mathrm{Cl/F} = D/(\Delta t \times \mathrm{C0})$. Cl/F was estimated at baseline (D0), W4 after DAA initiation (W4), at the end of DAA treatment (EoT) and 4 weeks after that (FUW4). To assess the effect of DAA on the Cl/F of the immunosuppressive drugs, two comparisons were made: W4 versus D0 and FUW4 versus EoT. The Cl/F values of cyclosporine or tacrolimus off or on antiviral therapy were compared using the geometric mean ratio (GMR) and the two-sided 90% confidence interval (CI90) and compared with the 0.80–1.25 bioequivalence range. The concentrations of DAA are presented graphically as a function of the time of sampling. Plasma concentrations measured before next drug intake, 24 ± 2 h, were then compared with data in the literature.

Statistical modelling was performed under SAS 9.4 (SAS Institute, Cary, NC, USA). All numerical variables were expressed as a mean ± standard deviation. Student's approximation for the Wilcoxon two-sample test was used to compare variations in biological parameters in the two groups of treatments. A mixed model approach was used to account for the dependence among repeated measurements performed in the same subject. Assuming $y_{i,\mathrm{time}}$ represented the response (clearance of tacrolimus) for the observation at a given time point in a particular subject, the model could be written as follows:

$$Y_{i,\mathrm{time}} = \beta_0 + \eta_i + \beta_{\mathrm{group}} \times \mathrm{Group} + \beta_{\mathrm{time}} \times \mathrm{Time} + \beta_{\mathrm{interaction}} \times \mathrm{Time} \times \mathrm{Group} + \Sigma\beta_j \times \mathrm{Factor_j} + e_{i,\mathrm{time}},$$

with β_0 being the basal clearance of the population, η_i random effect for inter-individual variability and $e_{i,\mathrm{time}}$ the random error, time regressor and time-treatment interaction and all possible risk factors. The results obtained using this model are presented in the section on multivariate analysis in order to quantify the adjusted effects of factors on the clearance of tacrolimus. Non-adjusted effects of time and treatment factors were obtained using mixed models with a time regressor and random effect on the intercept with treatment factor and time-treatment interaction and are presented in the section on univariate analysis. Only fixed-effects coefficients and the corresponding statistics are reported in this article.

Results

Characteristics of the population

Among the 137 patients in the CUPILT cohort who were receiving SOF/DCV, CNI trough blood concentrations were available for 67 of them (49%) (sampled within ± 2 h of the actual dosing interval) and were included in the pharmacokinetic study. Selection of patients included in the CUPILT cohort is shown in the flow chart (Fig. 1). Baseline characteristics are summarised in Table 1. The mean period elapsing between LT and the initiation of DAA was 64.8 (± 53.7) months. Fifty-two (78%) patients were receiving RBV. The mean RBV dosage at baseline was 731.4 (± 237.4) mg/day. Seven patients (10%) were co-infected with HIV. Antiretroviral therapies remained unchanged throughout the study, as detailed in Table 1.

Among the 67 patients concerned, the SVR12 rate was 98.5% as only one patient experienced a relapse.

Clearance of CNI during treatment with DAA

Variations in apparent individual clearances of tacrolimus and cyclosporine at DAA initiation (W4 versus baseline) and discontinuation (EoT versus FUW4) are shown in Figs. 2 and 3, respectively. No changes to cyclosporine clearance were observed during the study. There was a trend for an increase in the daily dose of tacrolimus between W4 and baseline (2.3 versus 1.9 mg/day) in patients receiving RBV (Supplementary Table 1). The increase in Cl/F was 23 versus 15 L/h, at W4 and baseline, respectively (Table 2). The corresponding GMR of tacrolimus Cl/F was 1.81 (1.30; 2.52) in patients receiving

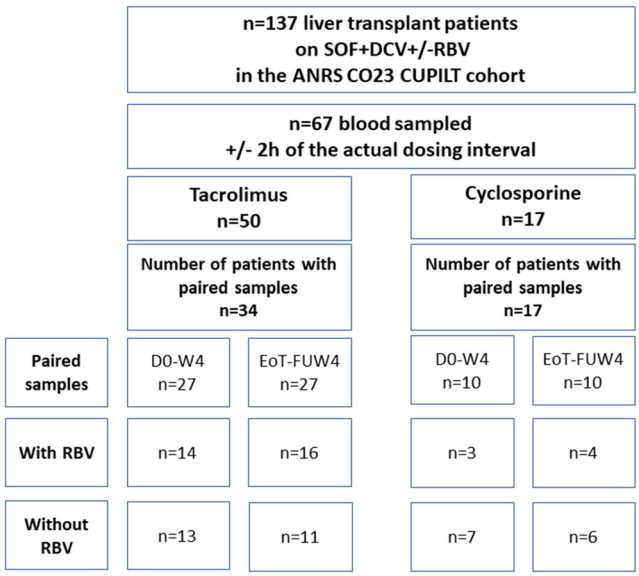

Fig. 1 Flow chart

Table 1 Demographic, clinical, laboratory and therapeutic characteristics of patients at baseline. Results were expressed as mean ± SD unless otherwise indicated

	All patients	Tacrolimus	Cyclosporine
Number of patients	67	50	17
Age (years)	57.4 (± 7.7)	58.2 (± 8.2)	55.2 (± 5.8)
Male gender—*n* (%)	53 (79)	40 (80)	13 (77)
Body mass index (kg/m^2)	24.2 (± 4.0) (*n* = 57)	24.0 (± 4.0)	24.7 (± 4.3)
Delay between LT and therapy (months)	64.8 (± 53.7)	66.7 (± 54.7)	59.3 (± 52.0)
Genotype 1a/1b/1—*n* (%)	23 (34)/25 (37)/3 (4)	17 (34)/16 (32)/3 (6)	6 (35)/9 (53)/0
Genotype 3/4—*n* (%)	9 (13)/7 (10)	8 (16)/6 (12)	1 (6)/1 (6)
Total bilirubin (μmol/L)	27.2 (± 48.5)	19.3 (± 26.7)	50.6 (± 82.0)
MELD Score	8.2 (± 5.0) (*n* = 62)	7.5 (± 4.8)	10.3 (± 5.3)
Fibrosis stage—*n* (%)			
≤ F2	29 (43)	26 (52)	3 (18)
F3	13 (19)	7 (14)	6 (35)
F4	17 (25)	13 (26)	4 (23)
Not determined	8 (12)	4 (8)	4 (23)
Immunosuppressive drugs (other than CNI)—*n* (%)			
Corticosteroids	14 (21)	12 (24)	2 (12)
Everolimus	1 (2)	0	1 (6)
Mycophenolate mofetil	37 (55)	29 (58)	8 (47)
RBV use—*n* (%)	35 (52)	24 (48)	11 (65)
RBV dosage (mg)	731.4 (± 237.4)	708.3 (± 256.9)	781.8 (± 188.8)
HIV co-infection[a]—*n* (%)	7 (10)	3 (6)	4 (24)
Creatinine clearance (mL/min)	73.3 (± 30.7) (*n* = 66)	74.9 (± 30.2)	68.8 (± 32.7)
Haemoglobin (g/dL)	13.4 (± 2.2)	13.8 (± 2.1)	12.4 (± 2.0)
12/24 weeks of therapy—*n* (%)	5 (7.5)/62 (92.5)	3 (6)/47 (94)	2 (12)/15 (88)

LT, liver transplantation; *RBV*, ribavirin; *CNI* calcineurin inhibitors

[a] Antiviral therapy was as follows: raltegravir, emtricitabine and tenofovir (*n* = 3); raltegravir, lamivudine and abacavir (*n* = 1); raltegravir, efavirenz, lamivudine and abacavir (*n* = 1); ritonavir-boosted darunavir and etravirine (*n* = 1); atazanavir, lamivudine and abacavir (*n* = 1)

SOF/DCV and RBV and 1.18 (0.96; 1.44) in patients receiving SOF/DCV alone. Most biological parameters remained unchanged throughout the study, but haemoglobin parameters varied (Supplementary Figure 1). Overall, a fall in haemoglobin levels of 1.5 g/dL (11%) was observed between baseline and W4; this was significantly more marked in patients receiving RBV (18% versus 3%; $p = 0.001$).

The univariate analysis also demonstrated the increase in tacrolimus Cl/F after 4 weeks of antiviral therapy (Table 3). The model which included a time-treatment interaction

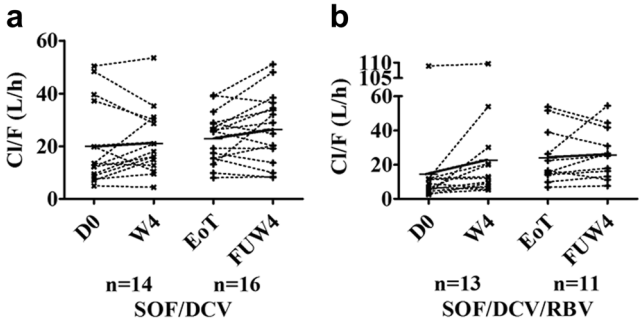

Fig. 2 Changes in apparent clearance of tacrolimus (Cl/F) between D0 and W4 and between EoT and FUW4 are shown in patients off ribavirin (**a**) or on ribavirin (**b**)

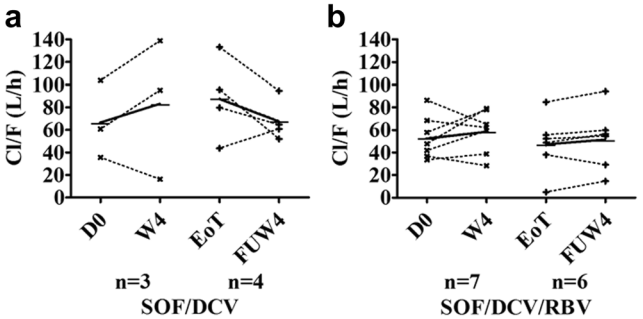

Fig. 3 Changes in apparent clearance of cyclosporine (Cl/F) between D0 and W4 and between EoT and FUW4 are shown in patients off ribavirin (**a**) or on ribavirin (**b**)

Table 2 Changes to apparent clearance (Cl/F) of tacrolimus and cyclosporine during antiviral treatment depending on the use of ribavirin

	n	Period		GMR (CI90%)
		D0	W4	
Apparent clearance of tacrolimus L/h				
All	27	11.3 (126.3)	16.4 (97.7)	
Without RBV	14	15.4 (79.5)	18.1 (60.0)	1.18 (0.96; 1.44)
With RBV	13	8.1 (186.7)	14.6 (125.4)	1.81 (1.30; 2.52)
	EoT	FUW4		
All	27	20.8 (52.6)	22.8 (52.2)	
Without RBV	16	21.2 (41.3)	23.1 (50.1)	0.91 (0.80; 1.04)
With RBV	11	20.2 (66.5)	22.3 (57.6)	0.91 (0.77; 1.07)
Apparent clearance of cyclosporine L/h				
All	10	53.5 (40.8)	56.8 (53.3)	
Without RBV	3	60.8 (51.7)	59.9 (74.5)	0.99 (0.52; 1.86)
With RBV	7	50.6 (35.7)	55.6 (32.4)	1.10 (0.91; 1.33)
	EoT	FUW4		
All	10	50.2 (56.1)	52.0 (42.5)	
Without RBV	4	81.7 (42.1)	66.4 (27.2)	1.23 (0.89; 1.70)
With RBV	6	36.2 (54.8)	44.1 (53.5)	0.82 (0.60; 1.12)

Results are expressed as geometric mean (% coefficient of variation)

D0 DAA initiation, *EoT* end of DAA, *FUW4* week 4 after DAA discontinuation, *GMR* geometric mean ratio, *RBV* ribavirin, *W4* week 4 after DAA initiation

showed a time effect ($p = 0.035$). The interaction was non-significant ($p = 0.11$) but allowed estimation of the increase in tacrolimus Cl/F in the RBV group at 8 L/h. Factors influencing tacrolimus Cl/F are change to haemoglobin between D0 and W4 ($p = 0.02$), initial MELD score ($p = 0.05$) and initial fibrosis grade (0.02) (Table 3). Change to haemoglobin is mostly negative and results show that a greater decrease in haemoglobin is associated with an increased tacrolimus Cl/F. As a consequence of these adjustments, the time effect was no longer significant ($p = 0.069$).

Exposure to sofosbuvir and daclatasvir in liver transplant recipients

The mean plasma concentrations of SOF, its metabolite and DCV measured at 24 ± 2 h were 1.0 ± 1.5 ng/mL, 643.4 ± 406.9 ng/mL and 460.4 ± 508.3 ng/mL, respectively. No differences were seen whether the patients were receiving tacrolimus or cyclosporine.

Discussion

This is the first study to have investigated the pharmacokinetic profiles of both CNI and DAA in liver transplant recipients. It was conducted in the CUPILT cohort in order to determine whether pharmacokinetic drug-drug interactions could explain the DAA assay results previously described [2]. Our findings suggest that liver transplant recipients on tacrolimus-based immunosuppressive therapy are at a higher risk of reduction in tacrolimus blood concentrations following the initiation of SOF/DCV combined with RBV, this being the consequence of an average 80% increase in the apparent oral clearance of tacrolimus. This finding corroborates recent case reports which described a decrease in tacrolimus levels in two patients who were receiving tacrolimus concomitantly with DCV, SOF and RBV and in one patient who received tacrolimus and ribavirin [5, 6]. During our study, two factors were identified as significantly impacting tacrolimus clearance: a fall in haemoglobin levels and liver fibrosis stage > 2. Both factors are related to treatment with RBV, the first as a consequence and the second as a reason for its use. The lack of clinically significant changes in patients receiving SOF/DCV without RBV ruled out any involvement of these two DAA in tacrolimus clearance. Indeed, no effect inducing the metabolism of these two DAA has been reported to date [7]. Interestingly, an impairment of tacrolimus clearance in patients receiving RBV was observed shortly after the initiation of antiviral therapy but not after their discontinuation; this could be related to a correction of anaemia and an improvement in liver function. No clinically significant changes in cyclosporine assay results and its apparent clearance were observed, although only a few patients were receiving cyclosporine-based immunosuppressive therapy during this pharmacokinetic study.

The pharmacokinetic characteristics of tacrolimus and cyclosporine are well established and were recently summarised alongside a number of factors explaining the huge intra- and inter-individual variabilities which we indeed observed during this study [8–10]. Interestingly, the mean apparent clearance rates of tacrolimus and cyclosporine at steady state estimated in these liver transplant recipients were within the same range as those recently reported in healthy volunteers, at 18 L/h and 42 L/h, respectively [11]. These two drugs share certain pharmacokinetic properties, such as being mainly eliminated by the liver and their gut biotransformation as substrates of CYP3A and P-glycoprotein which are one of the sources of differences between patients. In addition, a number of drug-drug interactions have been described with potent inducers or inhibitors of CYP3A which further increase variability in clearance, as noted among patients on efavirenz or ritonavir-based antiretroviral therapy [12, 13]. Presumably, our data could give valuable information about the mechanism behind the drug-drug interaction. For example an increased clearance due to enzyme induction would presumably have remained stable between W4 and EoT and then returned to baseline at FUW4. This does not fit the observed pattern, which is more compatible with, e.g. transient anaemia. Another key factor reported to impair the pharmacokinetics of CNI is anaemia, related to their high

Table 3 Factors associated with changes to the apparent clearance of tacrolimus after 4 weeks of antiviral therapy

	Factors	Fixed effect (slope for continuous factor or coefficient for a given modality)	*p* value
Univariate analysis (without adjustment)[a]	Treatment (ref = without ribavirin)	Ribavirin, − 5.13	0.84
	Time (ref = day 0)	W4 in the ref group, 1.22 W4 in the ribavirin group, *8.09*	Time, *0.035* Interaction, 0.11
Multivariate analysis (with adjustment by possible risk factors)[b]	Treatment (ref = without ribavirin)	Ribavirin, 12.78	0.10
	Time (ref = day 0)	W4 in the ref group, − 0.13 W4 in the ribavirin group, *8.11*	Time, 0.069 Interaction, 0.06
	Change in haemoglobin	*9.37*	*0.02*
	Change in creatinine clearance	− 0.30	0.34
	Meld score	*− 1.19*	*0.05*
	Fibrosis (ref = F0–F2)	HFC-F3-F4, *− 20.82*	*0.02*
	Post-transplantation delay	− 0.06	0.33

Italic numbers are for $p <$ or = 0.05

[a] Mixed model with time regressor and random effect on intercept and time-treatment interaction

[b] Mixed model with time regressor, time-treatment interaction and random effect on intercept with all the possible risk factors (clearance of tacrolimus of subject is predicted by $Y_i = \beta_0 + \eta_i + \beta_{group} \times$ Group $+ \beta_{time} \times$ Time $+ \beta_{interaction} \times$ Time × Group $+ \Sigma\beta_j \times$ Factor j, with β_0 the basal clearance of population and η_I random effect for the inter-individual variability)

concentration in red blood cells when compared with plasma. However, there is a difference between tacrolimus and cyclosporine as the red blood cell to plasma ratio ranges from 13 to 114:1 for tacrolimus and is much lower at around 2–3:1 for cyclosporine [8]. Consequently, anaemia can reduce tacrolimus blood concentrations and increase the blood clearance of tacrolimus, with little or no effect on cyclosporine clearance. Of note, such reduction in blood concentrations should not lead to an increase dosing. The intracellular concentration in leukocytes should be proportional to the unbound plasma concentration likely to be unaltered in a situation where the total blood concentration is lowered due to anaemia (since anaemia has no effect on unbound clearance). Indeed, our results showing that a change to the haemoglobin level was a significant factor impacting tacrolimus clearance were in line with previous findings that identified haematocrit as a covariate impacting tacrolimus clearance [14, 15] but not that of cyclosporine [16]. Haemolytic anaemia is the major dose-limiting toxicity of RBV, possibly as a result of an accumulation of phosphorylated forms in red blood cells which lack dephosphorylation enzymes [17]. Indeed, anaemia was more frequently observed in our patients on RBV when compared with those who were not ($p = 0.001$). In contrast, liver fibrosis was identified as a factor negatively impacting tacrolimus clearance because of a possible decrease in CYP3A content. This was in keeping with a recent study which highlighted a lowering of tacrolimus concentrations in liver transplant recipients receiving asunaprevir/DCV which was explained by their improved liver function [18].

To our knowledge, this is the first report of plasma concentrations of SOF and DCV in liver transplant recipients [19–21]. Although this was not the primary objective of our study, the concentrations measured were within the range of those previously reported, despite some patients having liver fibrosis at treatment initiation [19, 20]. This was in line with the lack of dose adjustment required in patients with liver impairment [7]. It had previously been demonstrated that the co-administration of cyclosporine resulted in a 40% increase in the area under the concentration-time curve of DCV but this did not affect its maximum observed concentration [22]. Unfortunately, the number of patients on cyclosporine in our cohort was too small to detect any minor and irrelevant clinical differences affecting DCV concentrations when compared with patients receiving tacrolimus.

This study had certain limitations. First, this pharmacokinetic study was conducted in liver transplant recipients included in an observational cohort and not in a clinical trial. For example the timing of drug intakes was recorded from patient questioning. In order to overcome this issue, we performed a drastic selection among patients in the CUPILT cohort who were receiving SOF/DCV, based on exhaustive reports of the timing of their drug intakes. In addition, unfortunately, not all patients had the four blood concentrations available which decreases the statistical power of the comparisons. Second, the blood assays of tacrolimus and cyclosporine in each centre were potentially performed using different methods which might have enhanced inter-patient and inter-centre variabilities in the Cl/F estimates, although this was limited by

participation in quality control tests. Third, the apparent clearance of both tacrolimus and cyclosporine was probably somewhat over-estimated when calculated from trough concentrations rather than the area under the concentration versus time curve or average concentration at steady state. However, our aim was not to estimate these clearances precisely, but rather to determine whether there were any clinically significant changes between two periods. Our study clearly demonstrated that tacrolimus Cl/F rose between baseline and the initiation of RBV concomitantly to DAA, leading to an increase in the tacrolimus dose to maintain tacrolimus blood concentrations within the target range. However, more subjects would have been necessary to allow an analysis by treatment group, with or without RBV, as the time-treatment interaction is close to statistical significance.

Fourth, plasma concentrations of RBV were not measured; the average RBV serum concentration at steady state and RBV-monophosphate levels in red blood cells were found to be inversely correlated with the nadir of haemoglobin levels [17]. However, analysing this situation was beyond the scope of our study. Finally, only few samples per patient were collected for DAA assays which did not enable an estimation of pharmacokinetic parameters for these drugs.

In conclusion, our findings showed that in patients treated for HCV recurrence and receiving RBV, the apparent clearance of tacrolimus is significantly increased during the first month of treatment, leading to a fall in tacrolimus concentrations. Among other factors, anaemia induced by RBV was a likely explanation that all physicians caring for these patients should be aware of especially as any reduction in tacrolimus concentration due to anaemia should not prompt a dose increase but rather an acceptance of concentrations below the target. Indeed, whenever possible, RBV should not be used in liver transplant recipients receiving tacrolimus-based immunosuppressive therapy, a practice which may be feasible with some newly marketed DAA. Plasma concentrations of DAA were within the range reported in previous studies; combined with their impressive efficacy and satisfactory tolerance, as previously demonstrated by our group [2] and others [4, 23, 24], this led us to the conclusion that there are no clinically significant drug-drug interactions that require the optimisation of DAA doses when added to a CNI-based immunosuppressive regimen.

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Compliance with ethical standards

The protocol has been implemented in accordance with the Declaration of Helsinki and French laws on biomedical research and was approved by the "South Mediterranean Ethics Committee" (France).

Conflict of interest Didier Samuel has been a clinical investigator, speaker and/or consultant for Astellas, Bristol-Myers Squibb, Gilead Sciences, Janssen Pharmaceuticals, Abbvie, Novartis, Merck Sharp & Dohme and Roche. Georges-Philippe Pageaux has been a clinical investigator, speaker and/or consultant for Astellas, Bristol-Myers Squibb, Gilead Sciences, Janssen Pharmaceuticals, Merck Sharp & Dohme, Novartis and Roche. Jean-Charles Duclos-Vallée has been a clinical investigator, speaker and/or consultant for Astellas, Bristol-Myers Squibb, Gilead Sciences, Janssen Pharmaceuticals, Abbvie, Novartis and Roche. Audrey Coilly has been a clinical investigator, speaker and/or consultant for Astellas, Bristol-Myers Squibb, Gilead Sciences, Janssen Pharmaceuticals, Novartis, Merck Sharp & Dohme and Abbvie. Christophe Moreno was paid as speaker or adviser from MSD, Janssen,

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