ORIGINAL ARTICLE

Impact of anti-HCV direct antiviral agents on graft function and immunosuppressive drug levels in kidney transplant recipients: a call to attention in the mid-term follow-up in a single-center cohort study

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SUMMARY

The medium-term impact on graft function and immunosuppressive drug pharmacokinetics of direct antiviral agents (DAAs) among hepatitis C virus (HCV)-infected kidney transplant (KT) recipients remain unclear. We compared pre- and post-treatment 12-month trajectories of estimated glomerular filtration rate (ΔeGFR) and 24-h proteinuria (Δ24-h proteinuria) in 49 recipients treated with DAAs (mostly sofosbuvir plus ledipasvir). Among evaluable patients, 66.7% and 100.0% had undetectable viral load by week 4 and end of therapy (EoT). The sustained virologic response rate at 12 weeks was 95.8%. Overall, 80.6% of patients receiving tacrolimus required dose escalation while on DAA-based therapy (median increase of 66.7%) to maintain target levels. Tacrolimus levels resulted to be higher at 12 months compared to EoT (7.8 ± 2.1 vs. 6.7 ± 2.0 ng/ml; *P*-value = 0.002). No changes in graft function during the course of therapy were observed. However, eGFR significantly decreased (*P*-value <0.001) throughout the first 12 months after EoT. Median ΔeGFR and Δ24-h over pre- and post-treatment periods were 3.9% and −6.1% (*P*-value = 0.002) and −5.3% and 26.2% (*P*-value = 0.057). Caution should be exercised when adjusting immunosuppression in HCV-infected KT recipients upon initiation of DAAs, followed by mid-term monitoring of immunosuppressive drug levels and graft function.

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Key words
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Introduction

Hepatitis C virus (HCV) infection has historically represented a poor prognostic factor after kidney transplantation (KT) [1,2]. Apart from the mortality directly attributable to liver injury, the increased incidence of chronic transplant glomerulopathy and other complications explains such a worse outcome [2–4].

Traditional approaches to HCV infection among KT recipients have been hindered by low sustained virologic response (SVR) rates and poor tolerance [1]. Interferon-α (IFN-α)-based regimens are further limited by its potential triggering effect on alloreactivity that may potentially increase the risk of acute graft rejection. On the other hand, the concomitant use of ribavirin (RBV) entails the additional risk of hemolytic anemia [1,5].

This dismal scenario has dramatically changed with the advent of direct antiviral agents (DAAs), allowing the design of all-oral IFN-α-free regimens [6]. Sofosbuvir (SOF) is a pan-genotypic nucleotide analog with a potent inhibitory effect on the viral NS5B polymerase [7]. In combination with other agents targeting the nonstructural NS5A protein [i.e., ledipasvir (LDV) or daclatasvir (DCV)] or the NS3/4A protease, SOF-based regimens have been shown to achieve SVR rates exceeding 95% across pivotal trials in nontransplant patients [8–10].

Although such evidence is more limited in the setting of solid organ transplantation and mainly restricted to liver transplant (LT) recipients [11–13], a number of small-sample-size studies have reported favorable outcomes among HCV-infected KT recipients treated with DAAs [14–18]. A recent retrospective report from the Spanish National Registry (HEPA-C Registry) showed a rate of SVR of 98% among KT recipients. No changes in graft function were observed between baseline and end of therapy (EoT), although the follow-up period was short [19].

No previous studies have focused on the effect that HCV clearance would exert on the medium-term renal graft function. In addition, we aimed at gaining insight into the impact of DAA-based regimens on dose requirements for immunosuppressive drugs by describing our experience with a large cohort of KT recipients prospectively followed up for more than 12 months after completion of therapy.

Materials and methods

Study population and setting

This study was conducted at the University Hospital "12 de Octubre" (Madrid, Spain), a tertiary care center in which the prevalence of HCV infection among new KT recipients has been approximately 8.0% over the last decade [20]. We prospectively reviewed all HCV-infected adult (≥18 years) KT recipients that initiated an IFN-α-free DAA regimen between November 2014 and January 2016. The research was performed in accordance with the ethical standards laid down in the Declarations of Helsinki and Istanbul. The study was performed according to the STROBE statement. The local Ethics Committee approved the study protocol, and written informed consent was obtained from all participants.

Study design and therapeutic regimens

All HCV-infected adult KT recipients with detectable serum HCV-RNA by nucleic acid amplification test [polymerase chain reaction (PCR)], functioning graft and active follow-up at our center were offered DAA-based therapy. Patients with acceptable graft function [estimated glomerular filtration rate (eGFR) ≥30 ml/min] were considered for a SOF-based regimen regardless of the degree of liver disease. With the aim of minimizing the risk of drug-to-drug interactions, non-NS3/4A protease inhibitor-containing regimens were preferentially used when possible [21]. At the time of study design, data on the safety of SOF in patients with severe renal impairment were lacking [22]. Therefore, in the presence of eGFR <30 ml/min, we decided to prioritize patients with advanced liver disease by offering the only SOF-free, protease inhibitor-based combo EMA-approved by that time. Decision on treatment for patients with eGFR <30 ml/min/1.73 m^2 but only mild-to-moderate liver injury was delayed until newer non-protease inhibitor agents with favorable renal safety profile became available [23].

All patients were referred to the Department of Hepatology for anti-HCV treatment. After discussion with the attending nephrologist, one of the following regimens was chosen according to the above-mentioned criteria: (i) SOF (400 mg once daily) plus LDV (90 mg once daily) for patients with eGFR ≥30 ml/min and HCV genotypes 1 or 4; (ii) SOF plus DCV (60 mg once daily) for those with eGFR ≥30 ml/min and genotype 3; or (iii) dasabuvir (DSV) (250 mg once daily) plus ombitasvir/paritaprevir/ritonavir (OBV/PTV/r) (12.5/75/50 mg twice daily) for those with eGFR <30 ml/min, genotype 1 and advanced liver disease (F3–F4 in the METAVIR score). Weight-based RBV (with further adjustment for eGFR and hemoglobin levels) was added in selected patients at the discretion of the treating

hepatologist. Details on immunosuppressive regimens and clinical and virologic follow-up are provided as Supporting Information.

The *primary study outcome* was SVR, defined as an undetectable serum HCV-RNA level [i.e., below the lower limit of detection (LLoD)] at the end of follow-up (at least 12 weeks after completion of therapy). The occurrence of any adverse event during anti-HCV therapy, including biopsy-proven acute graft rejection (BPAR) or development of *de novo* anti-human leukocyte antigen (HLA) antibodies, was considered as *secondary outcomes*. We also specifically assessed the impact of DAA-based anti-HCV therapy on medium-term graft function in terms of eGFR (by means of the MDRD-4 formula) and 24-h proteinuria. To this end, we compared the variation (Δ) in both parameters throughout the 12 months immediately preceding the initiation of therapy [from −M12 to baseline ("pre-treatment year")] with that observed within the first 12 months after its completion [from baseline to M12 after EoT ("post-treatment year")]. By means of such a within-patient controlled design, we attempted to overcome the confounding effect in terms of clinical characteristics (i.e., immunological risks or concomitant medications) or post-transplant care practices that would result from the use of an historical external control group of untreated patients. Participants were followed up until August 2017 (unless death or graft loss occurred earlier). Therefore, all of them had a minimum follow-up of 12 months from the completion of therapy.

Definitions

Virological relapse was defined as the subsequent reappearance of serum HCV-RNA at any time after having achieved undetectable HCV-RNA at the end of therapy. Variations in eGFR (ΔeGFR) throughout pre- and post-treatment years were calculated as the difference between the value at the index point (T_0) and that measured 12 months earlier (T_{-12}) [(eGFR at T_0 − eGFR at T_{-12}) × 100/eGFR at T_{-12}]. An analogous method was used to calculate Δ24-h proteinuria.

Statistical analysis

Quantitative data were shown as the mean ± standard deviation (SD) or the median with interquartile ranges (IQR). Qualitative variables were expressed as absolute and relative frequencies. Variations in graft function (ΔeGFR and Δ24-h proteinuria) between T_0 and T_{-12} were expressed as relative values (%) with 95% confidence intervals (CIs). Categorical variables were compared using the χ^2 test or Fisher's exact test, whereas Student's *t*-test or Mann–Whitney *U* test was used to compare continuous variables, as appropriate. Repeated measurements (i.e., changes overtime in graft function or drug levels during the course of therapy) were compared using paired parametric or nonparametric tests (Student's *t*-test for paired samples, Wilcoxon signed-rank test or Friedman test) as dictated by data distribution. Linear and nonlinear correlations between continuous variables were assessed using the Pearson's correlation coefficient (*R*) or Spearman's correlation coefficient (Rho), respectively. Statistical analysis was performed using SPSS version 20.0 (IBM Corporation, Armonk, NY, USA).

Results

Clinical characteristics and anti-HCV regimens

Overall, 84 KT recipients were deemed eligible for treatment. Fourteen patients were excluded due to undetectable serum HCV-RNA levels and six due to severe graft function impairment (eGFR <30 ml/min) in the absence of advanced liver disease. Five further patients declined treatment. Of the remaining 59 patients, 49 had initiated anti-HCV therapy as of January 2016 and constituted the study population of the present report.

Their clinical characteristics are detailed in Table 1. Genotype 1 was predominant [79.6% (39/49)], and most of them had mild degree of fibrosis [F1 in 40.8% (20/49)]. There were no cases of coinfection with human immunodeficiency virus (HIV). The median interval between KT and initiation of anti-HCV therapy was 115.5 months (IQR: 76.5–191.7), and exceeded 10 years in 22 patients (44.9%). The median HCV viral load at the time of treatment initiation was 6.15 $\log_{10}$ IU/ml (IQR: 5.73–6.58). Baseline anti-HLA antibodies were found in 19 patients (41.3%), although none of them were donor-specific. The composition of maintenance immunosuppression regimens remained stable during therapy and the subsequent 12 months.

The most common DAA-based anti-HCV regimen was SOF plus LDV [89.8% (44/49)] (Table 2). Most patients [81.6% (40/49)] were scheduled to receive a 12-week course, and RBV was added in 28 of them (57.1%). The initial RBV dose ranged from 100 to 1000 mg daily (median: 400 mg), and 39.3% (11/28) of

Table 1. Clinical characteristics of KT recipients treated with DAA-based regimens (n = 49).

Variable	
Age of recipient, years [mean ± SD]	45.5 ± 12.1
Gender (male) [*n* (%)]	34 (69.4)
Type of transplant [*n* (%)]	
Kidney	38 (77.6)
Liver–kidney	8 (16.3)
Pancreas–kidney	3 (6.1)
Time interval between transplantation and initiation of anti-HCV therapy, months [median (IQR)]	115.5 (76.5–191.7)
Time ranges per 5-year intervals [*n* (%)]	
<5 years	8 (16.3)
5–10 years	19 (38.8)
10–15 years	11 (22.4)
15–20 years	2 (4.1)
20–25 years	4 (8.2)
25–30 years	5 (10.2)
Pretransplant comorbidities [*n* (%)]	
Hypertension	36 (73.5)
Diabetes mellitus	19 (38.8)
Dyslipidemia	9 (18.4)
Coronary heart disease	6 (12.2)
Other chronic heart disease	8 (16.3)
Chronic pulmonary disease	3 (6.1)
Stroke	2 (4.1)
Peripheral arterial disease	2 (4.1)
History of intravenous drug use [*n* (%)]	3 (6.1)
Underlying end-stage renal disease [*n* (%)]	
Glomerulonephritis*	19 (38.8)
Chronic interstitial nephropathy	8 (16.3)
Diabetic nephropathy	7 (14.3)
Polycystosis	3 (6.1)
Nephrocalcinosis	2 (4.1)
Obstructive nephropathy	1 (2.0)
Renal hypoplasia	1 (2.0)
Unknown	8 (16.3)
Use of renin-angiotensin system inhibitors [*n* (%)]†	
At baseline	19 (38.8)
At EoT	20 (40.8)
At 12 months after EoT	21 (42.9)
Previous BPAR [*n* (%)]	12 (24.5)
Peak panel reactive antibody >50.0% [*n* (%)]‡	9 (19.6)
Anti-HLA antibodies at baseline [*n* (%)]‡	19 (41.3)
Immunosuppression regimen [*n* (%)]	
Tacrolimus, MMF/MPA and steroids	19 (38.8)
Tacrolimus and MMF/MPA	8 (16.3)
Tacrolimus and steroids	5 (10.2)
Monotherapy with tacrolimus or cyclosporine A	4 (8.2)
MMF/MPA and steroids	3 (6.1)
Tacrolimus, everolimus and steroids	2 (4.1)
MMF/MPA, everolimus and steroids	2 (4.1)
Other	6 (12.2)
HCV genotype [*n* (%)]	
1b	34 (69.4)
1a	5 (10.2)
5	4 (8.2)
3	3 (6.1)
4	3 (6.1)
Fibrosis stage (METAVIR) [*n* (%)]	
F1	20 (40.8)
F2	7 (14.3)
F3	8 (16.3)
F4	14 (28.6)
Prior IFN-α-based anti-HCV therapy [*n* (%)]	12 (24.5)

BPAR, biopsy-proven acute graft rejection; EoT, end of therapy; HCV, hepatitis C virus; HLA, human leukocyte antigen; IFN, interferon; IQR, interquartile range; KT, kidney transplant; MMF/MPA, mycophenolate mofetil/mycophenolic acid; SD, standard deviation.

*Includes membranoproliferative glomerulonephritis (n = 9), IgA nephropathy (n = 4), focal segmental glomerulosclerosis (n = 3), Goodpasture syndrome (n = 1), membranous glomerulonephritis (n = 1) and lupus glomerulonephritis (n = 1).

†Includes angiotensin-converting-enzyme inhibitors and angiotensin II receptor blockers.

‡Data not available for three patients.

patients required a dose modification during treatment. The two patients treated with DSV plus OBV/PTV/r were infected with genotype 1b.

Virological outcomes

The median follow-up periods from the initiation (baseline) and completion of anti-HCV therapy (EoT) were 16.3 (IQR: 14.9–19.3) and 12.7 months (IQR: 11.4–15.9), respectively. One patient (2.0%) required discontinuation of therapy (SOF/LDV plus RBV) after 1 month due to the persistent decline in her eGFR attributed to an intercurrent infectious complication. DAAs could not be subsequently reintroduced and the patient died due to a complicated skin and soft tissue infection 9 months later. The remaining patients completed the planned therapy duration. As depicted in Fig. 1, 66.7% (32 of 48) and 100.0% (48 of 48) of evaluable patients had cleared the virus (i.e., serum HCV-RNA levels below the LLoD) by week 4 of treatment and EoT, respectively. Two patients (4.2%) experienced virological relapse. One of them was infected

Table 2. Anti-HCV regimens administered.

DAA regimen	RBV	Weeks	*N* (%)
SOF plus LDV*	Yes	12	25 (51.0)
	Yes	24	1 (2.0)
	No	12	10 (20.4)
	No	24	8 (16.3)
SOF plus DCV	Yes	12	2 (4.1)
	No	24	1 (2.0)
DSV plus OBV/PTV/r	No	12	2 (4.1)

DAA, direct antiviral agent; DCV, daclatasvir; DSV, dasabuvir; HCV, hepatitis C virus; OBV/PTV/r, ombitasvir/paritaprevir/ritonavir; RBV, ribavirin; SOF, sofosbuvir.

*One patient was initially treated with DSV plus OBV/PTV/r, although the regimen was discontinued 3 weeks later due to the development of heart failure and the subsequent risk of interaction with antiarrhythmic drugs. Anti-HCV therapy was reinitiated 2 months later with SOF plus LDV.

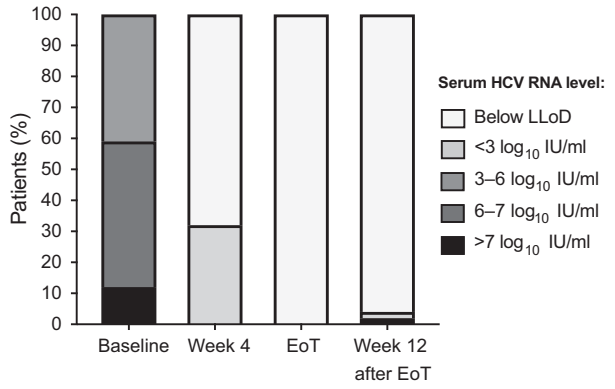

Figure 1 Kinetics of HCV viral loads at different points, including baseline and 12 weeks after completion of therapy (i.e., time for assessment of sustained virologic response).

with genotype 1b and relapsed 10 weeks after completion of a 12-week course of SOF, LDV and RBV. Genotypic resistance testing revealed the L31M mutation in NS5A, which is known to confer *in vitro* low-level resistance to both LDV and DCV [24,25]. The other patient, in whom poor drug adherence was suspected, was infected with genotype 4 and had virological relapse by week 12 after EoT. No genotypic resistance was detected. Thus, the rate of SVR was 95.8% (46 of 48).

Tolerability and evolution of liver function

Overall, the DAA-based regimen was well tolerated, with no cases of treatment discontinuation due to adverse events. Regarding liver function tests, serum transaminases and bilirubin levels showed a significant decrease from baseline to EoT, whereas the platelet count significantly increased over the same period (Fig. 2a–d). Seventeen patients (34.7%) required erythropoietin at some time during treatment, with no significant differences between those that received or not a RBV-containing regimen [39.3% (11 of 28) vs. 28.6% (6 of 21); *P*-value = 0.436]. Despite this therapeutic intervention, serum hemoglobin overall decreased during therapy (Fig. 2e), although such trend was mainly driven by those recipients treated with RBV (13.7 ± 1.7 g/dl at baseline vs. 12.5 ± 1.2 g/dl and EoT; *P*-value <0.0001), as no differences were found for the remaining patients (13.0 ± 1.5 g/dl vs. 13.2 ± 1.6 g/dl, respectively; *P*-value = 0.409). There were no cases of severe infection (i.e., requiring hospital admission) during the course of therapy, and 3 patients (6.1%) developed this complication after a median interval of 329 days from EoT.

Graft function and immunosuppression levels during therapy

There were no significant changes in graft function during the course of anti-HCV therapy, either as assessed by eGFR (*P*-value for trend = 0.134; Fig. 2f) or 24-h proteinuria (0.4 ± 0.5 g/day at baseline vs. 0.6 ± 1.2 g/day at EoT; *P*-value = 0.421). No patients developed *de novo* anti-HLA antibodies at the 12-week evaluation. There were two episodes of BPAR (T-cell-mediated rejection type IB with histological signs of mild chronic allograft nephropathy and negative C4d staining, and borderline T-cell-mediated rejection with focal C4d deposition not fulfilling diagnostic criteria for antibody-mediated rejection) at 6 and 11 months after completion of therapy, with favorable response to steroid boluses. Both patients were receiving tacrolimus and mycophenolic acid at the time of rejection, and tacrolimus levels had maintained stable at the lower limit of the target range during the preceding months. One of them experienced a progressive increase in the titers of nondonor-specific anti-class II HLA antibodies (already present at the pretransplant evaluation) during the previous months, with the development of anti-MHC class I polypeptide-related sequence A (MICA) antibodies (Fig. S1 in Supporting Information). We found no differences in lipid control between the baseline evaluation and EoT. However, glycemic control among diabetic recipients improved in terms of hemoglobin A1c (6.5 ± 1.2% at baseline vs. 5.9 ± 0.9% at EoT; *P*-value = 0.011) (Fig. S2).

We analyzed the effect of anti-HCV therapy on the pharmacokinetics of immunosuppressive drugs. Despite

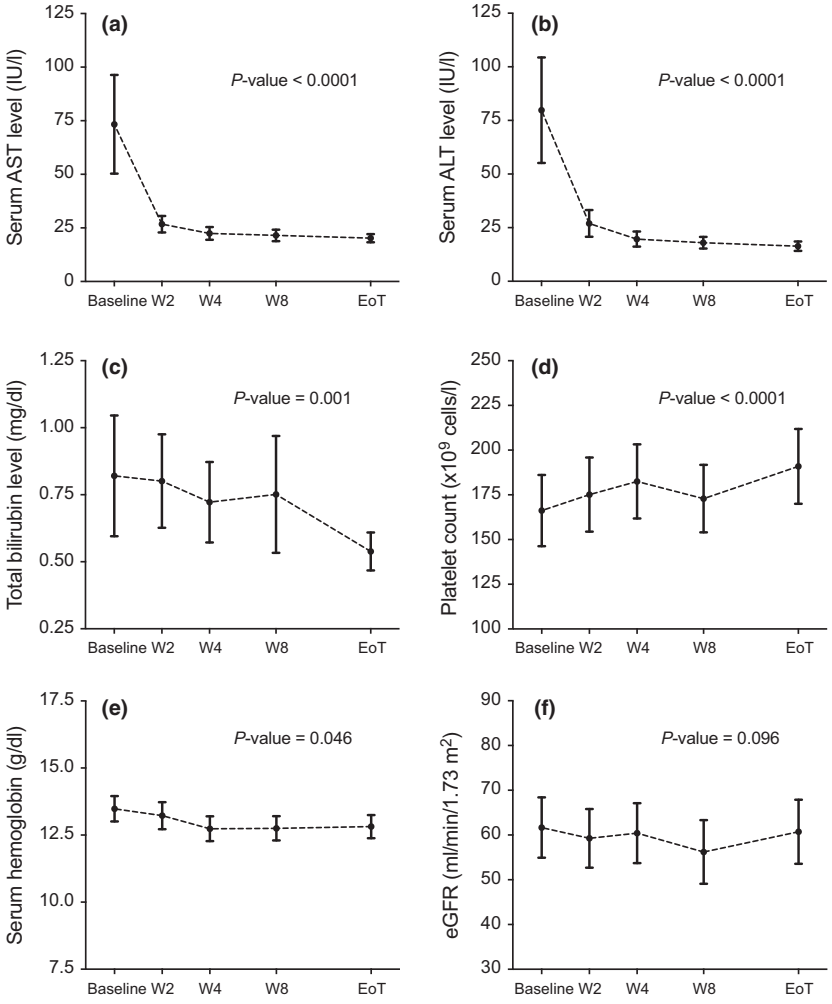

Figure 2 Evolution of laboratory parameters at baseline and during therapy: liver function tests (a–d), serum hemoglobin (e), and graft function (f) (all *P*-values were estimated by tests for repeated measures). Circles represent median values and bars indicate 95% confidence intervals. eGFR, estimated glomerular filtration rate; EoT, end of therapy.

repeated dose adjustments aimed at maintaining blood concentrations within the target ranges, trough levels of tacrolimus, cyclosporine A and everolimus decrease during therapy, with this trend reaching statistical significance for tacrolimus (*P*-value for trend = 0.043). Decrease in tacrolimus levels was particularly evident by the first month of treatment (7.6 ± 2.2 ng/ml at baseline vs. 6.2 ± 2.0 ng/ml at week 4; *P*-value = 0.017). Accordingly, mean daily doses of these drugs had to be increased during this period, although again the difference was only significant for tacrolimus (2.6 ± 1.9 mg at baseline vs. 3.5 ± 1.7 mg at EoT; *P*-value for trend <0.0001) (Fig. 3a). In detail, 80.6% (29 of 36) of patients receiving tacrolimus required escalation of daily dose for a median increase of 66.7% (IQR: 41.4–100.0). This intervention was more common in the absence of advanced liver disease [90.5% (19 of 21) for F1–F2 vs. 66.7% (10 of 15) for F3–F4; *P*-value = 0.103]. The increase in daily dose of tacrolimus was also higher among patients with lower degrees of fibrosis (median: 60.0% for F1–F2 vs. 33.3% for F3–F4; *P*-value = 0.176), although none of these differences reached statistical significance. Such findings remained unchanged when the two patients treated with protease inhibitor-containing regimens were excluded (data not shown).

Medium-term evolution of graft function

Graft function maintained stable during the pretreatment year, with no significant differences between −M12 and baseline either for eGFR (56.4 ± 19.1 vs. 57.9 ± 22.0 ml/min; *P*-value = 0.306) or for 24-h proteinuria (0.4 ± 0.5 vs. 0.4 ± 0.5 g/day; *P*-value = 0.658). However, eGFR significantly decreased during the first 12 months after therapy (57.3 ± 22.7 ml/min at EoT vs. 52.9 ± 21.1 ml/min at M12; *P*-value for trend <0.001), whereas there was a near significant increase in 24-h proteinuria (0.4 ± 0.3 g/day at EoT vs. 0.5 ± 0.4 g/day at M12; *P*-value for trend = 0.059) (Fig. 4a,c). The decrease in

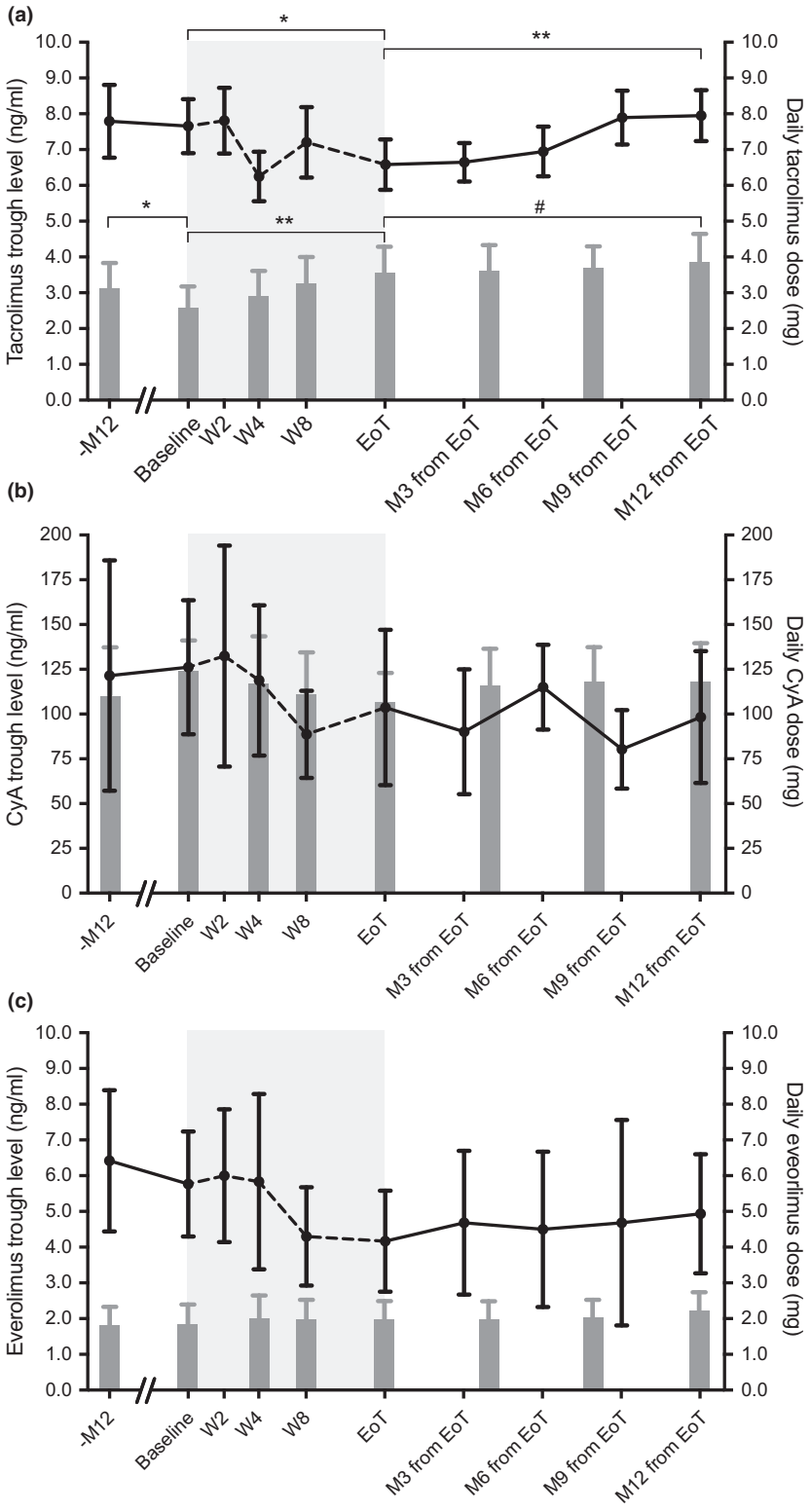

Figure 3 Evolution of trough blood concentrations (line) and daily doses (columns) of immunosuppressive drugs during anti-HCV therapy (clear gray area) and pre- and post-treatment years: tacrolimus (n = 36) (a), cyclosporine A (n = 6) (b), and everolimus (n = 6) (c) Circles and columns represent mean values and bars indicate 95% confidence intervals. *P-value <0.05; **P-value <0.005; #P-value <0.06 (all P-values were estimated by tests for repeated measures). CyA, cyclosporine A; EoT, end of therapy; M, month; W, week.

eGFR was already evident within the first 4 post-treatment months (P-value = 0.003). To confirm these findings, we performed an extension of the follow-up until the last available contact, accounting for a median interval of 576 days (IQR: 525–670) from the completion of therapy (M18). Compared to EoT, both the decrease in eGFR (53.1 ± 22.0 ml/min; P-value = 0.003) and the increase in 24-h proteinuria (0.5 ± 0.6 g/day; P-value = 0.024) remained significant at this extended follow-up assessment. In addition, we also observed

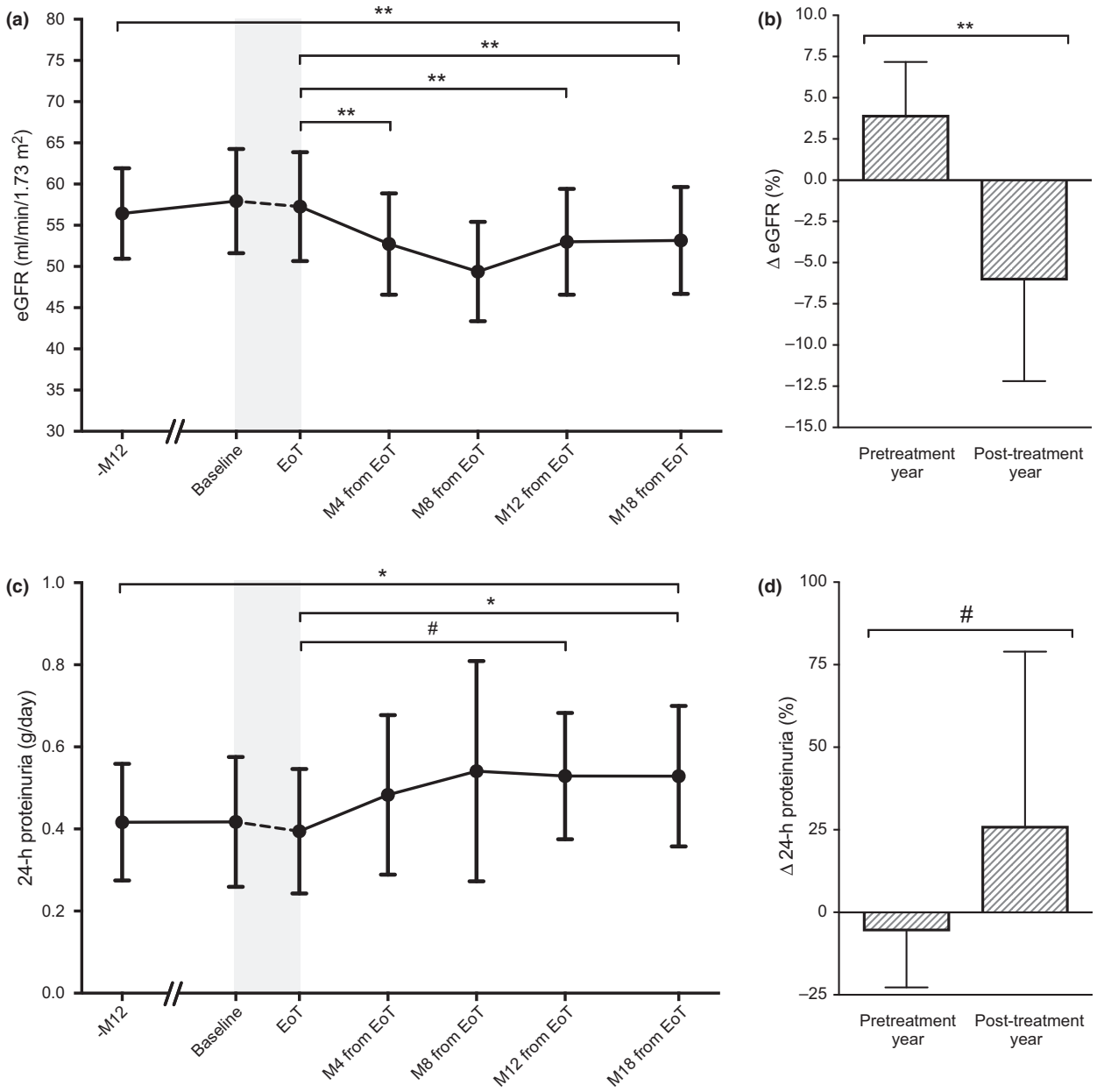

Figure 4 Medium-term evolution of renal graft function: mean values (circles) and 95% confidence intervals (bars) for eGFR (a) and 24-h proteinuria (c), median variations (Δ) with 95% confidence intervals for eGFR (b) and 24-h proteinuria (d) during the 12 months preceding the initiation of therapy [−M12 to baseline (pretreatment year)] and the 12 [EoT to M12 (post-treatment year)] and 18 months following EoT (M18). **P*-value <0.05; ***P*-value <0.005; #*P*-value <0.06 (all *P*-values were estimated by tests for repeated measures). eGFR, estimated glomerular filtration rate; EoT, end of therapy; M, month; W, week.

significant differences throughout all the time points encompassed from −M12 to M18 after EoT, both for eGFR (56.4 ± 19.1 vs. 53.1 ± 22.0 ml/min, respectively; *P*-value for trend = 0.002) and 24-h proteinuria (0.4 ± 0.5 vs. 0.5 ± 0.6 g/day; *P*-value for trend = 0.013) (Fig. 4).

When these trajectories were expressed in terms of variations (Δ) of both parameters during each 12-month period, median ΔeGFR was 3.9% (95% CI: −1.6% to 7.9%) and −6.1% (95% CI: −12.7% to −2.1%) during pre- and post-treatment years (*P*-value = 0.002), whereas median Δ24-h proteinuria was −5.3% (95% CI: −15.2% to 135.6%) and 26.2% (95% CI: 39.5–110.9%), respectively (*P*-value = 0.057) (Fig. 4b,d). Such differences remained unchanged when the two patients that developed BPAR were excluded

from the analysis (data not shown). To assess the confounding effect on the latter finding of changes in the use of antiproteinuric agents, we excluded those patients that initiated renin–angiotensin system inhibitors during the 12 months following EoT ($n = 4$). In this sensitivity analysis, the difference in median Δ24-h proteinuria between pretreatment [−6.8% (95% CI: −21.7% to 139.0%)] and post-treatment years [35.4% (95% CI: 46.1–120.3%)] achieved statistical significance (*P*-value = 0.025).

Graft biopsy was performed in one patient with worsening eGFR and proteinuria (a 30-year-old man that had undergone transplantation 14 years before due to renal hypoplasia), revealing histopathological changes compatible with transplant glomerulopathy, without demonstration of C4d deposition or glomerulitis. There was a significant increase in tacrolimus levels between EoT and M12 (6.7 ± 2.0 vs. 7.8 ± 2.1 ng/ml; *P*-value for trend = 0.002), with no differences for the remaining immunosuppressive drugs. In addition, tacrolimus mean daily doses also increased, although the difference did not attain statistical significance (3.5 ± 1.7 vs. 3.9 ± 1.8 mg at EoT and M12, respectively; *P*-value = 0.059) (Fig. 3a). No drugs with potential interactions with immunosuppressive agents were initiated during this period. We found no significant differences in ΔeGFR during post-treatment year according to the use of tacrolimus, the evolution of tacrolimus levels, the type of underlying renal disease (HCV-associated glomerulonephritis versus others) or the degree of liver fibrosis (Fig. 5).

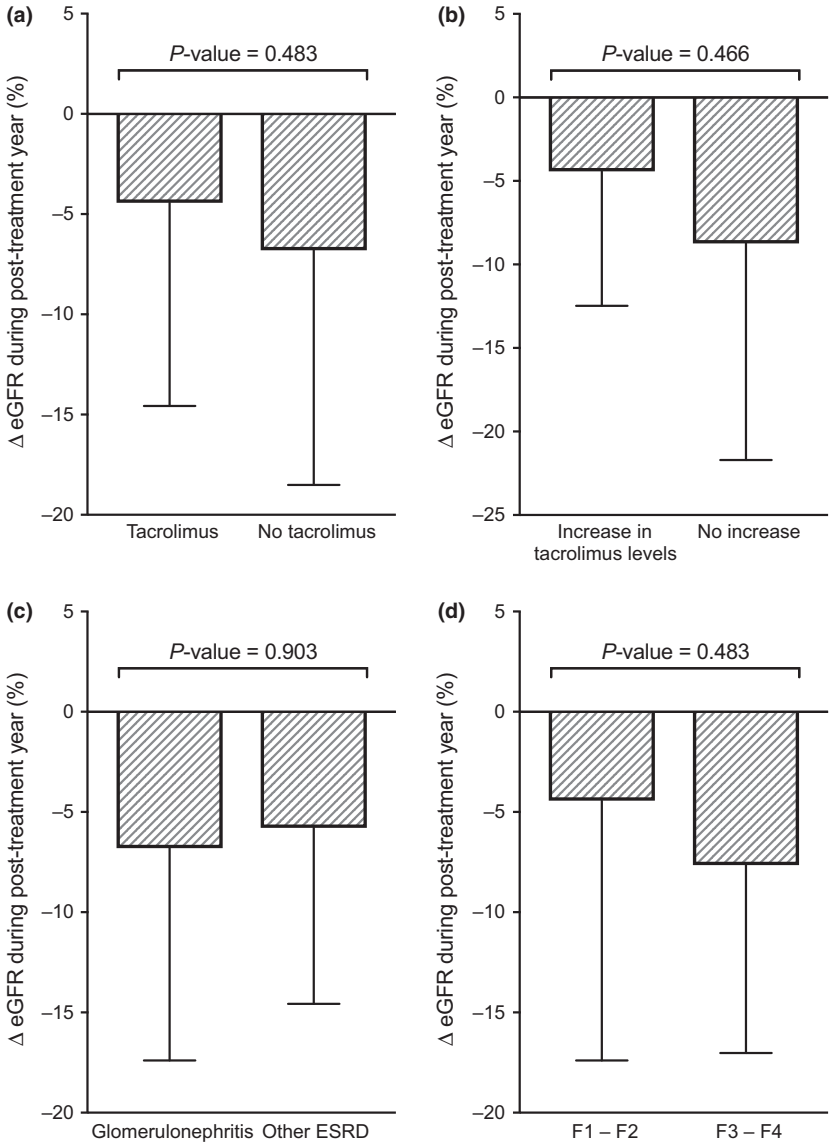

Figure 5 Median variations (Δ) with 95% confidence intervals for eGFR during the 12 months following EoT according to: use of tacrolimus (a), increase in tacrolimus levels throughout post-treatment year (only for patients receiving this drug) (b), type of ESRD (c), and degree of liver fibrosis (d). eGFR, estimated glomerular filtration rate; EoT, end of therapy; ESRD, end-stage renal disease.

Discussion

The present study supports the high virologic efficacy and short-term safety of newer IFN-α-free regimens among HCV-infected KT recipients. Accordingly, there were no cases of adverse event-related drug discontinuation. Nevertheless, our experience also highlights the impact of DAAs on the pharmacokinetics of immunosuppressive drugs. As a call of attention to be confirmed in future, we observed a statistically significant decrease in eGFR during the first year following EoT.

Previous studies have reported rates of SVR for KT recipients ranging from 91% to 100% [14–17], comparable to those observed in LT recipients [11] and the nontransplant population [8–10] and suggesting that post-transplant immunosuppression does not meaningfully affect the odds of achieving viral clearance. The SVR observed in the present cohort (95.8%) was, therefore, in line with these experiences as well as the HEPA-C Registry [19]. Two patients experienced virological relapse at 12 weeks from EoT, similarly to other studies that found relapse rates between 0% [14–16] and 8.3% [17]. Of note, only in one of them genotypic resistance to DCV was documented.

We observed a marked increase in dose requirements of calcineurin inhibitors and everolimus to maintain concentrations within target ranges once DAAs were initiated. Despite close therapeutic drug monitoring, trough tacrolimus levels significantly decreased throughout the course of therapy, particularly during the first month. An increase in tacrolimus daily dose was required in 72% of patients included in the HEPA-C Registry, although no association with a particular DAA-based regimen was demonstrated [19]. Both tacrolimus and cyclosporine are substrates of liver cytochrome (CYP) P450 isoforms 3A4 and the multidrug transporter P-glycoprotein (P-gp), as are mTOR inhibitors. Therefore, drug-to-drug interactions are expected to occur between post-transplant immunosuppression and NS3/4A protease inhibitors [11]. However, only 2 of our patients were treated with OBV/PTV/r, and there is no evidence that SOF or NS5A inhibitors interact with CYP3A4 or P-gp [7]. Such findings are in line with single case reports and case series of LT [26,27] and KT recipients [16,28], and demand explanations other than direct drug-to-drug interactions. It has been proposed a role for the repression of certain drug-metabolizing enzymes, such as CYP3A4, due to the sustained inflammatory stimulus associated with chronic HCV infection [28]. For instance, plasma levels of midazolam or HCV or HIV protease inhibitors (all of them CYP3A4 subtracts) have been shown to be higher in HCV patients than healthy volunteers [29,30]. The rapid viral clearance upon initiation of DAA-based therapy, mirrored by the prompt normalization of liver function tests, would abrogate the HCV-driven inflammatory inhibition of CYP3A4 and lead to an enhanced metabolism of immunosuppressive drugs. Interestingly, we found that escalation in tacrolimus dose was more commonly needed for patients with lower degrees of fibrosis as compared to those with more severe fibrosis or established cirrhosis, an observation that could reflect a higher DAA-induced reversibility of the drug-metabolizing function among the former group.

Our study provides original data on the medium-term evolution of graft function among KT recipients treated with DAA-based regimens, as the follow-up in most of previous cohorts was restricted to the first 12–24 weeks upon completion of therapy [14,15,17]. Given the well-demonstrated deleterious effect exerted by HCV infection in the setting of KT and the lack of significant on-therapy changes in eGFR or 24-h proteinuria, it could be hypothesized that viral clearance would result in the subsequent improvement of graft function. Our experience confirms the safety of IFN-α-free regimens in terms of alloreactivity, with only two cases of BPAR occurring late after completion of therapy and not associated with *de novo* anti-HLA antibodies. However, the comparison of separate 12-month trajectories revealed that while the graft function remained stable over the pretreatment year (from −M12 to baseline), there was a slight decrease in eGFR during the first year following EoT (mean absolute difference of ~5 ml/min) that reached statistical significance. Extended follow-up (until M18 after EoT) seems to confirm this finding as well as a significant worsening in 24-h proteinuria, although again the clinical relevance in absolute terms (mean absolute difference of ~0.1 g/day) is uncertain.

Beyond the need for replication in further cohorts, it should be stressed that we lack a definitive explanation for these results. Various mechanisms, not necessarily mutually exclusive, may be evoked. First, DAAs might have a direct nephrotoxic effect, although it seems unlikely in view of the lack of on-therapy changes in graft function. In this sense, Lubetzky *et al.* [18] reported that one-fifth of KT recipients treated with SOF-based regimens developed worsening 24-h proteinuria during or shortly after therapy, and that this complication was more common than among historical controls (i.e., untreated HCV-infected recipients with comparable follow-up). Alternatively, we could speculate on the alleged intrinsic immunosuppressive effect exerted by long-term

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HCV infection, as suggested by the lower rate of acute rejection and reduced *naïve* CD45RA T-cell counts found in some studies among HCV-infected KT recipients [31]. HCV clearance would paradoxically favor alloimmune-mediated injury. Recent evidence points to the restoration of innate and adaptive immune cell populations after DAA-based therapy in patients with chronic HCV infection. Unexpectedly, some studies have reported increased rates of hepatocellular carcinoma recurrence among cirrhotic patients clearing the virus [32], an observation that has been attributed to the disruption of immunological balances governing hepatocarcinogenesis [33]. Increase in body weight and muscle mass after infection resolution could have impacted on the estimation of creatinine clearance. Finally, it should be noted that adjustments in maintenance immunosuppression were not managed according to a pre-established protocol. Attending nephrologists might have been somewhat reluctant to decrease again tacrolimus doses after EoT. Thus, some degree of mid-term therapeutic inertia resulting from repeated dose adjustments during the course of anti-HCV therapy could have contributed to the observed increases in tacrolimus levels and daily doses throughout the following months, with the subsequent nephrotoxicity. In summary, no direct conclusions on the eventual interaction between DAAs and immunosuppressive drugs can be derived from the present research.

Our study is limited by its single-center nature, small sample size and, in particular, the lack of a control group composed of untreated HCV-infected recipients to accurately delineate the impact of DAA-based therapy on graft outcomes. With the exception of one single case, histological examination in patients experiencing worsening proteinuria or eGFR was not carried out, as the decision of performing graft biopsy was left to the judgment of the attending nephrologist. It is likely that most physicians judged as questionable the clinical relevance of the changes observed in graft function. In addition, protocol graft biopsies are not routinely performed in our center. In addition, it is unclear whether the observed trajectories in graft function will persist in the longer run. Taking into account such limitations, our study must be regarded as merely hypothesis-generating rather than conclusive.

Its strengths, although, include the detailed assessment of the medium-term graft evolution and within-patient controlled comparisons of trajectories of eGFR and proteinuria throughout pre- and post-treatment years. Practical and methodological difficulties prevented us from incorporating a control group. However, within-patient comparisons may replace to some extent such an approach in a predictably progressive disorder (i.e., decline in renal graft function) and normalize for baseline and clinical differences across patients.

The present study confirms that IFN-α-free DAA-based regimens have excellent virologic efficacy and favorable short-term safety profile in the KT setting. However, the expected improvement in graft function resulting from HCV clearance may be potentially confounded by unfavorable mid-term outcomes in eGFR and 24-h proteinuria, whose cause and real extent remain to be established. We propose the conduction of prospective studies formally focused on the mid- and long-term evolution of graft function following anti-HCV therapy. Such studies should ideally include larger sample sizes, a control group of untreated HCV-infected recipients, validated assays for monitoring of alloreactive responses and per-protocol biopsies, to gain further insight into the complex interplay between HCV clearance, direct drug-induced nephrotoxicity and potential immune reconstitution phenomena.

In the meantime, we strongly suggest that clinicians taking care of HCV-infected KT recipients treated with SOF-containing regimens should pay particular attention in adjusting the doses of tacrolimus (and, likely, cyclosporine and mTOR inhibitors), as a meaningful increase in daily dose requirements to achieve therapeutic target ranges may be evident already at the first month of therapy. Close monitoring of eGFR, proteinuria, and immunosuppressive drug levels should be maintained not only during the course of anti-HCV therapy, but also at least over the first year after EoT to promptly detect relevant deviations in such parameters. In the long-run, and until further evidence emerges, it seems reasonable to extreme caution with the use of CYP450 inhibitors that could impact the pharmacokinetics of immunosuppressive drugs and to avoid the implementation of tacrolimus minimization strategies in these patients.

Authorship

M.F.R., N.P., I.F., J.M.A., M.P. and A.A. participated in research design. M.F.R., N.P., A.G.S., R.M., A.M.H., V.R.M., I.F. and A.A. participated in collecting clinical data. M.F.R., N.P., A.G.S., R.M., A.M.H. and I.F. participated in chart review. M.F.R. and N.P. participated in data analysis. M.F.R. and N.P. participated in the writing of the paper. R.M., I.F., J.M.A., M.P. and A.A critically revised and completed the submitted version of the manuscript.

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Conflict of interest

All the authors declare they have no conflict of interest.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1. Clinical and histological characteristics of the two episodes of acute graft rejection diagnosed after completion of anti-HCV therapy in the study cohort.

Figure S2. Evolution of lipid and glycemic control at baseline and EoT: (a) serum total cholesterol, (b) serum triglycerides, and (c) hemoglobin A1c (only diabetic patients).

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