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ORIGINAL ARTICLE

Long-term, maintenance MMF monotherapy improves the fibrosis progression in liver transplant recipients with recurrent hepatitis C

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Conflicts of Interest
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Summary

Hepatitis C virus (HCV) recurrence after orthotopic liver transplantation (LT) is universal. We designed a retrospective case–control study to evaluate the effect of mycophenolate mofetil (MMF) monotherapy in patients with recurrent hepatitis C. Fifteen patients with histologically proven hepatitis C recurrence after LT were switched from calcineurin inhibitors (CNIs) to MMF monotherapy because of impairment of kidney function and/or metabolic side effects, and treated for 48 months (MMF group). Fifteen well-matched LT recipients who continued to receive CNIs therapy over the same period served as control group. Demographics, clinical data, time after LT, and baseline liver biopsies were similar in the two groups. There was no worsening of hepatic fibrosis during the study in the MMF group [2.6 ± 1.5 (baseline) Ishak Units vs. 2.7 ± 1.8 (after 48 months of MMF treatment), $P = 0.6$]. In contrast, a significant increase in the fibrosis score [2 ± 1.1 (baseline) vs. 3.2 ± 1.7 (after 48 months of CNI treatment), $P = 0.0002$] was observed in the control group. The yearly fibrosis progression rate was of 0.05 ± 0.44 in the MMF group and 0.33 ± 0.24 in the CNI group ($P = 0.04$). MMF monotherapy is associated with a favourable effect on hepatic fibrosis progression in HCV liver transplant recipients.

Introduction

Hepatitis C virus (HCV)-related end-stage liver disease is the most common indication for liver transplantation (LT) in adults [1]. Recurrent HCV infection of the allograft is universal and immediate at reperfusion [2,3]. The natural history of chronic hepatitis C in liver transplant recipients is characterized by progression to cirrhosis in 8–30% within 5 years of follow-up [4]. Fibrosis progression is accelerated compared to immunocompetent patients, with a progressive increase in patients who have recently undergone LT [5]. The most significant factors associated with rapid progression to cirrhosis include donor-related factors, as donor age, steatosis and gender mismatch; viral-related factors such as HCV genotype and pre-transplant viral load and external factors such as immuno-

suppression therapy, year of LT and post-transplant alcohol use [6].

Mycophenolate mofetil (MMF), a reversible inhibitor of the inosine-monophosphate-dehydrogenase, is commonly used as a standard immunosuppressant after LT [7]. It's main immunological effect is the induction of a relatively selective inhibition of B and T lymphocyte proliferation [8]. MMF is most frequently introduced as part of multidrug regimens, together with low doses of calcineurin inhibitors (CNI), with the aim to reduce CNI-related adverse events and severe chronic toxicity [9–14].

However, several authors have shown that maintenance MMF monotherapy in LT recipients is feasible and safe. Only few patients may develop mild side effects, such as leucocytopenia or gastrointestinal disorders, which can be generally managed by dosage adjustment, while development of acute rejection has been rarely reported [15–22].

In vitro studies have also shown that MMF reduces the capacity of fibroblast collagen production, extracellular matrix contraction and cell migration [23,24]; these favourable findings have been recently confirmed to occur also *in vivo*, in LT recipients with recurrent HCV infection, showing significant reduction, in short term results, of inflammation and fibrosis progression associated with MMF treatment [25].

The aim of our study was therefore to evaluate the effect of long-term MMF monotherapy on fibrosis progression rate in LT recipients with recurrent HCV infection.

Patients and methods

Study design

We designed a retrospective case–control study to evaluate the effects of long-term MMF monotherapy in patients who underwent LT for cirrhosis HCV-related at our institution between December 1993 and June 2004. We retrieved from a prospectively collected data base the records of 15 LT recipients with recurrent hepatitis C who had been switched from CNI to MMF monotherapy (MMF group), with special attention to the long-term histological course. In all patients liver biopsies had been obtained yearly, either before the start of MMF (baseline) as during 4 years of treatment, until the end of the study, and were scored blindly for necroinflammatory activity and rate of fibrosis. Histological findings were then compared with those observed in a group of 15 well-matched patients with hepatitis C recurrence who had been maintained under CNI monotherapy throughout the entire study period (control group) (Fig. 1).

All liver biopsies were from the same post-transplant period and all patients showed histologically proven graft hepatitis. The two groups were matched for age, gender, histological grading and staging, mean time after LT, donor age, liver function tests (LFTs), HCV genotype and RNA copies (Table 1). A diagnosis of acute rejection was based on elevated liver enzymes and biopsy proven.

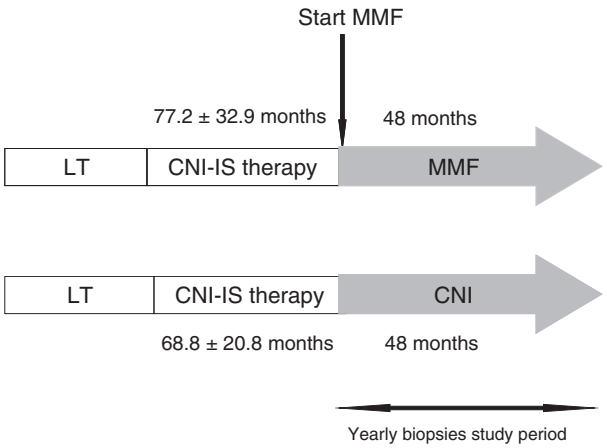

Figure 1 Study design. One group was switched to mycophenolate mofetil (MMF) monotherapy after approximately 6 years from liver transplantation (LT) and followed for the following 4 years. The control group was maintained under the same immunosuppression (calcineurin inhibitor) from the time of LT.

The study was approved by the Institutional ethics committee. Written informed consent was obtained from each patient before the start of MMF therapy.

MMF group

The MMF group included 15 adult HCV-infected LT recipients with a minimum LT follow-up of 48 months at entry, who were switched from CNI monotherapy to MMF monotherapy between November 2001 and October 2006. These patients were part of a cohort of 42 recipients previously enrolled in a prospective trial performed at our institution to assess the effects of MMF monotherapy on patients' metabolic profile and renal function [15]. Switch to MMF monotherapy was chosen when the patients first developed signs of renal impairment (defined as an estimated glomerular filtration rate <60 ml/min/1.73 m^2) or hyperlipidaemia (serum cholesterol >240 mg/dl and/or triglycerides >150 mg/dl). Twelve people in the MMF group were male patients and three were female patients, with a mean age at enrolment of 58.8 ± 6.1 years. No one had either cirrhosis or had experienced episodes of acute rejection at time of study entry. MMF treatment was started at a median time of 77 months after LT (range: 17–127 months). The mean duration of MMF monotherapy was 48 ± 2.5 months and the average MMF dosage taken throughout the study was 1500 mg/day in all patients. Six (40%) patients received low dose ribavirin (usually 400 mg/day) before and during the study period.

Table 1. Baseline characteristics of the two groups.

Variable	MMF group	Control	*P*-value
Number of patients	15	15	NS
Age (years)	53 ± 6.1 (55–75)	54 ± 8.3 (46–75)	NS
Gender (M/F)	12/3	11/4	NS
BMI*	26.4 ± 3.7	27.8 ± 5.3	NS
Donor age (years)	32.4 ± 18.8	40.4 ± 13.3	NS
Months from LT to baseline (median)	77 (range: 17–127)	62 (range: 24–120)	NS
HCV genotype 1–4 (%)	11 (73.3)	11 (73.3)	NS
HCV genotype 2–3 (%)	4 (26.6)	4 (26.6)	NS
HCV-RNA >500 000 IU/ml (%)	46.6	66.6	NS
Anti-HBc positive (%)	5 (33.3)	6 (40)	NS
Cholestatic hepatitis	None	None	NS
HIV positive	None	None	NS
Rejection episode	None	None	NS
ACE-inhibitors treated (%)	2 (13.3)	3 (20)	NS
IDDM (%)	5 (33.3)	4 (26.7)	NS
Fibrosis score (Ishak)	2.6 ± 1.5	2.0 ± 1.1	NS
Grading score (Ishak)	4.15 ± 1.2	4.08 ± 2.02	NS
Treated with ribavirin (%)	6 (40)	8 (53.3)	NS
Patients who achieved SVR (%)	2/6 (33.3)	3/8 (37.5)	NS
Azathioprine treated (%)	10/15 (66)	11/15 (73)	NS
Mean ALT (IU/l)	74 ± 40.5	87.3 ± 73.6	NS

MMF, mycophenolate mofetil; LT, liver transplantation; HCV, hepatitis C virus; Anti-HBc, anti hepatitis B core; HIV, human immunodeficiency virus; ACE-inhibitors, angiotensin-converting enzyme inhibitors; IDDM, insulin-dependent diabetes mellitus; ALT, alanine aminotransferase; SVR, sustained viral response after antiviral treatment.
*BMI no changed at 48 months.

CNIs tapering and start of MMF protocol

At the tapering start, 12 patients were under cyclosporine (CsA) and three under tacrolimus (Tac) therapy; the mean daily CsA/Tac dose were 102.3 ± 36.1 and 2 ± 0.8 mg, respectively. The mean daily dose of CsA was reduced by 25 mg every month. While the CsA dose was being reduced, MMF treatment was started at a daily dose of 500 mg. The step-by-step CsA reduction and the concomitant increase in MMF dose was interrupted if serum alanine transaminase levels increased or side effects due to MMF appeared, and then restarted upon normalization of liver enzymes. CsA was completely tapered off in all cases within 4 months, when the target MMF maintenance dose was reached (1500 mg daily). In those patients receiving Tac regimen, the dose was tapered by 0.5 mg monthly, using the same schedule described above for MMF introduction.

Control group

Fifteen well-matched adult HCV-infected LT recipients with a minimum LT follow-up of 48 months, initially treated with CNI following LT and who continued unchanged the same immunosuppression at entry in this study, were studied as a control group (Fig. 2). They included 11 male patients and four female patients, with a mean age of 54 ± 8.3 years. At entry the median time elapsed after LT was 68 (range: 24–120) months. No patient in the control group had cirrhosis at the start of the study, and none had experienced episodes of acute rejection. Maintenance immunosuppressive therapy at baseline consisted of CsA monotherapy in all patients, with a mean daily dose of 103.3 ± 31.1 mg and CsA trough levels of 91 ± 8.7 ng/ml. This immunosuppression remained unchanged throughout the study periods, without significant changes in trough levels and dosing was not needed to be tapered due to the absence of relevant CNI-related side effects. Eight (53.3%) patients received low dose ribavirin before and during the study period.

Histology

All histological examinations were carried out by one experienced pathologist (G.P) who was blinded to the patients' clinical status and the assigned therapy, except for the knowledge of a previous transplantation due to HCV-related end-stage liver disease. Protocol liver biopsies were obtained yearly since time of LT, including a period of up to 10 years before the switch of immunosuppression to MMF (baseline) and a period of 4 years during MMF monotherapy. Similarly, yearly liver biopsies were

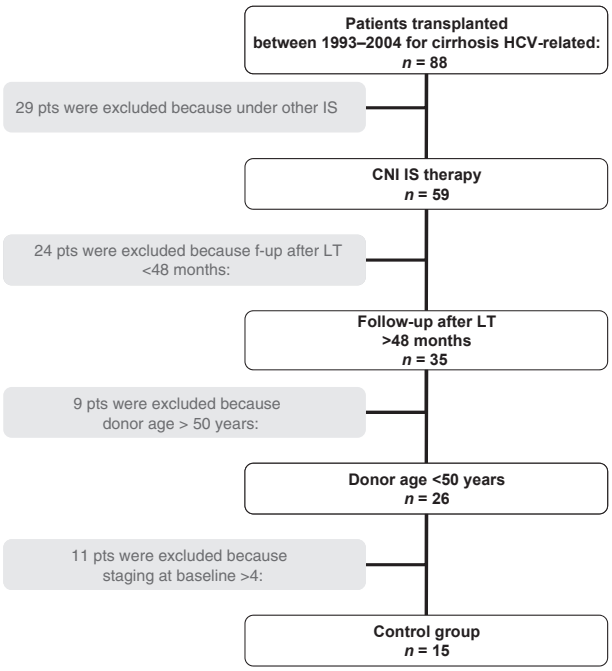

Figure 2 Selection process of the matched control group. Exclusion criteria were: immunosuppression with drugs other than calcineurin inhibitor, follow-up <48 months, donor age >50 years old, staging at baseline >4 units.

available in all control patients for the whole study period. Biopsies were scored for the grade of necroinflammatory activity and stage of fibrosis according to Ishak *et al.* [26] and also examined to exclude features of acute or chronic rejection or other relevant findings. Additional biopsies were eventually taken whenever an acute rejection was suspected. Liver specimens were obtained percutaneously, using 1.6 mm modified Menghini needles. To minimize sampling errors, only specimens longer than 1.5 cm and wider than 1.4 mm, including at least eight portal tracts were considered. Specimens were formalin-fixed and paraffin-embedded. Five-micrometre sections were stained with haematoxylin and eosin, Masson's trichrome for collagen and cytokeratins for the assessment of ductopenia. The yearly fibrosis progression rate was calculated as the difference between the staging score in the last liver biopsy and the baseline biopsy divided by the years of follow-up. Specimens were also examined for the presence of acute or chronic rejection and other relevant findings. Acute rejection was defined according to standard criteria. Chronic rejection was assessed according to Banff classification rejection activity index (RAI) [27].

Virological assays

Levels of serum HCV-RNA were quantified using a competitive RT-PCR analysis (Amplicor, Roche Molecular Systems, Inc., Branchburg, NJ, USA) at the Laboratory of Molecular Virology of our University. HCV-RNA titres $>500 \times 10^3$ IU/l were considered as high viral replication. HCV genotypes were assessed using the Inno-Lipa HCV (Immunogenetics, Zwijnaarde, Belgium).

Recurrent hepatitis C was defined by the concomitance of detectable serum HCV-RNA and histological signs of recurrent disease.

Statistical analysis

Data were recruited from a prospectively collected consecutive database (Microsoft Access 2.0; Microsoft Corporation, Redmond, WA, USA). Categorical data were analysed using the Fisher's exact test. Normal distribution continuous data were analysed by parametric test (Student's *t*-test). Statistical results were expressed as mean ± standard deviation or median values and ranges. A *P*-value of <0.05 was considered significant. The programme used for statistical analysis was SPSS® 13.0 (SPSS, Chicago, IL, USA) for Windows.

Results

All patients complied successfully with the treatment protocol. None of the patients in the two groups died during the study period. CsA and Tac were completely weaned off within an average time of 4 months, during which period all patients in the MMF group achieved the target dose of 1500 mg/day, which was then maintained as monotherapy throughout the study. Using this strategy none of the patients in the MMF group developed acute rejection episodes either during the weaning or the maintenance phase and did not require additional immunosuppression therapy. None of the patients in the control group experienced acute rejection episodes during the study.

Histological findings

Five consecutive yearly liver biopsies were available for examination for each patient, including one at baseline. At baseline there were no differences between the MMF group and the control both in terms of fibrosis (2.6 ± 1.5 Ishak staging units vs. 2 ± 1.1, respectively; *P* = NS) and necroinflammatory activity (4.15 ± 1.2 Ishak grading units vs. 4.08 ± 2.02, respectively, *P* = NS). After 48 months a significant worsening of fibrosis occurred in the control group (from 2 ± 1.1 to 3.2 ± 1.7 (*P* = 0.0002)] while the in MMF group the staging score remained unchanged [from 2.6 ± 1.5 to 2.7 ± 1.8; (*P* = NS)] (Fig. 3).

Conversely, a significant decrease of the grading score was observed in the MMF group (from 4.15 ± 1.2 to 2.7 ± 1.7 (*P* = 0.026) after 48 months, while no changes

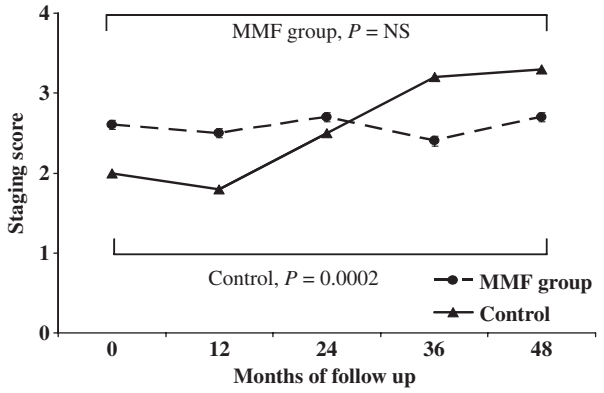

Figure 3 Changes in hepatic fibrosis in the two groups during the 48 months of study period.

were seen in the control group [from 4.08 ± 2.02 to 3.72 ± 1.64 (P = NS)].

In the MMF group, 5/15 (33%) recipients showed an improvement of at least one unit of fibrosis after 48 months versus none in the CNI group (P = 0.042). CNI group, instead, showed a fibrosis worsening in 13/15 (86.7%) vs. 6/15 (40%) in MMF group (P = 0.021).

The 4-year fibrosis progression rate in MMF group was 0.05 ± 0.44 vs. 0.33 ± 0.24 in control (P = 0.04) (Fig. 4).

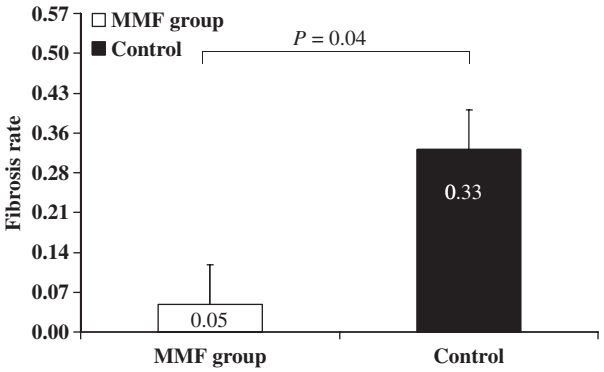

Figure 4 Four-year fibrosis progression rate in the two groups.

Biochemical findings

No significant differences were observed between the MMF and the control group in the biochemical findings throughout the study. Changes over time of serum transaminases are shown in Table 2.

Virological findings

Eleven patients (73.3%) in both groups were infected by HCV genotype 1 or 4. In the MMF group high levels of HCV replication (HCV-RNA levels $>500 \times 10^3$ IU/ml) were observed in 54.5% patients at baseline and in 72% at the study end (P = NS). In the control group, HCV-RNA levels $>500 \times 10^3$ IU/ml were observed in 60% at entry and 40% at last follow-up (P = NS). No differences were seen in HCV-RNA levels between the two groups throughout the study (Table 3).

Side effects of MMF treatment

The MMF-associated mild side effects were observed in eight (53%) of 15 patients. Gastrointestinal disorders, such as nausea and other dyspeptic symptoms were seen in four patients. Five patients developed leukopenia (defined as a white blood cell count of <3000/μl). In all cases it was sufficient to change the administration of

Table 3. HCV-RNA serum levels (mean ± standard error) throughout the study period.

Follow-up (months)	HCV-RNA (IU/ml) (MMF group, n = 13)	HCV-RNA (IU/ml) (Controls, n = 15)	P
Baseline	$1.2 \pm 0.39 \times 10^6$	$1.4 \pm 0.44 \times 10^6$	NS
12	$1.4 \pm 0.44 \times 10^6$	$1.3 \pm 0.39 \times 10^6$	NS
24	$1.3 \pm 0.42 \times 10^6$	$1 \pm 0.36 \times 10^6$	NS
36	$1.3 + 0.44 \times 10^6$	$1.1 \pm 0.39 \times 10^6$	NS
48	$1.2 \pm 0.53 \times 10^6$	$1 \pm 0.39 \times 10^6$	NS

MMF, mycophenolate mofetil.

Table 2. Histological and biochemical finding of the two groups after 48 months of follow-up.

	MMF group			Control		
Variables	Baseline	48 months	P	Baseline	48 months	P
Grading score	4.15 ± 1.2	2.7 ± 1.7	0.026	4.08 ± 2.02	3.72 ± 1.6	NS
Staging score	2.6 ± 1.5	2.7 ± 1.8	NS	2 ± 1.1	3.2 ± 1.7	0.0002
ALT (IU/l)	57.5 ± 19.12	40.3 ± 22	NS	61.13 ± 43.4	46.4 ± 19.6	NS
eGFR (ml/min/1.73 m^2)	39.13 ± 10.9	51.28 ± 6.2	0.001	52.86 ± 9.54	53.23 ± 8.99	NS
Cholesterol (mg/dl)	162.9 ± 39.3	143 ± 34.3	0.01	175.2 ± 45.02	178.3 ± 50.6	NS
Triglycerides (mg/dl)	148.9 ± 57.9	116 ± 44.8	0.02	146.7 ± 96.9	215.9 ± 145	NS
HCV-RNA >500 000 IU/ml (%)	6/11 (54.5)	8/11 (72)	NS	9/15 (60)	6/15 (40)	NS

ALT, alanino aminotrasferase; eGFR, estimated glomerular filtration rate; MMF, mycophenolate mofetil.

MMF from twice daily to three times daily, without changing the total dose to recover from the gastrointestinal symptoms and to return the white blood cell count to normal values.

Discussion

Hepatitis C recurrence after transplant represents the greatest problem for HCV-infected LT recipients. Post-transplant allograft re-infection, which occurs universally, causes cirrhosis after only 5 years in 30–40% of LT recipients [1]. Fibrosis progression in these patients is typically accelerated compared with the immune-competent host [1–3]. Several variables can predict the severity of hepatitis C recurrence. These include old donor age, liver steatosis and other donor-related factors (as type of donor death, high vasopressor drug requirement), which are recognized as risk factors predictive of progression to severe disease [1,3,6,28]. Unfortunately, due to the current organ shortage and long waiting list time, donor-dependent factors are difficult to modify. This has forced the transplant community to expand the criteria for organ donation, in order to compensate for the increasing request of LT. However, this results in less than optimal matching between donors and recipients, which is reflected by a 5 years-graft survival rate lower than 60% (according to the European Liver Transplant Registry data, http://www.eltr.org, 12/2008).

High HCV viraemia levels, prior and/or following transplantation and HCV genotype 1b are also associated with a more aggressive post-transplantation disease, but these viral-related factors difficulty may be modified [1–6].

In this scenario, the choice of immunosuppression is intuitively an important matter, being one of the few variables that can be manipulated after LT. Several studies have, in fact, suggested that the type of immunosuppression plays an important role in the severity of HCV recurrence [1–6]. However, the optimal immunosuppression to be used in this setting is still under discussion [29]. The ideal immunosuppression should be able on one hand to prevent acute rejection and other transplant-related complications and on the other hand also be able to inhibit viral replication and decrease the natural progression of disease [30]. Several authors, for example, reported that corticosteroid boluses, antilymphocyte therapies (OKT3) and the use of high doses of CNIs are associated with reduced graft survival and impaired the prognosis [2,4,31] and should be preferably avoided in LT recipients with HCV infection.

On the other hand, *in vitro* studies have suggested that MMF exerts antifibrotic properties by inhibiting type I collagen expression and the migratory and contractile functions of fibroblasts [23,24]. These favourable effects have been also recently confirmed to occur also *in vivo*, in LT recipients with HCV receiving MMF therapy, who showed significant reduction of inflammation and fibrosis progression [25].

In the present study we evaluated the progression of liver fibrosis in a group of LT recipients who had been switched from CNIs to MMF immunosuppression due to the development of renal or metabolic impairment. The histological findings data were compared with those observed in a group of well-matched LT recipients who were continued on CNI monotherapy. We found that after 4 years of follow-up, patients receiving MMF monotherapy showed neither changes of necro-inflammation nor evidence of fibrosis progression. In contrast, patients in continued CNI monotherapy reported a significant worsening of both necro-inflammation and fibrosis. These data are in agreement with those of Bahra *et al.* [25], who also found no progression of necroinflammatory activity and fibrosis in 40 recipients with a mean time from LT of 2 years, who were treated with MMF and low dose CNIs for 24 months. Conversely, Kornberg *et al.* [32], who analysed 19 LT recipients (nine under MMF and low CsA dose versus 10 under CsA given at standard dose), showed a significant progression of fibrosis in those receiving the MMF and CsA combination after 12 months of follow-up. A possible explanation for this apparent contradiction may be due to the different times after LT between our study (77 months, range: 17–127) and that of Kornberg *et al.* (48 months, range: 12–60). The duration of post-transplant follow-up is, in fact, an important factor to be considered in this setting, as fibrosis progression has been shown to be faster in the early years after LT [4].

We believe that one of the strengths of our study is given by the long-term follow-up and the availability, in all patients of both groups, of yearly protocol liver biopsies. We examined, in fact, about 150 biopsies during the study period, which allowed us to closely monitor the histological evolution of recurrent HCV-related disease and to minimize potential biases attributable to sampling errors.

Another strength of the present study is that we obtained a complete switch to MMF monotherapy from CNIs. To our knowledge, this is the first report on the effect of CNIs-free MMF monotherapy on fibrosis progression, with a histology-based follow-up as long as 4 years. We could achieve this goal in the context of the local policy at our Transplant Center over the last decade, attempting to minimize as much as possible the level of maintenance immunosuppression after LT, particularly in HCV-infected patients.

We are aware that potential weaknesses of our study are represented by its retrospective nature and the limited

number of patients in the two groups and the possibility of selection biases. In other words, it is possible that our data cannot be extrapolated to other cohorts of patients and clearly our findings need to be confirmed by further prospective studies. Yet, the two groups of patients compared in this study were transplanted over the same time period, had similar demographics and baseline clinical data and received similar initial treatment after LT. However, patients switched to MMF developed CNI-related initial renal dysfunction and/or metabolic complications, which did not occur in the control group. Therefore, we cannot exclude that the more favourable course of HCV-related recurrent disease might be a consequence of these complications, although this possibility seems unrealistic. It should also be noted that our findings are entirely based on histological data and no hard clinical end-points were considered.

On the other hand, although an antiviral effect of MMF antiviral had been demonstrated *in vitro* [33], we did not observe significant changes in viral replication throughout the study, as reflected by comparable levels of HCV-RNA in the two groups during the entire study period (Table 3). Thus, the relevance of a direct antiviral effect of MMF remains uncertain and further studies are necessary to verify whether the reported *in vitro* antiviral effect translates into reduced viral replication also *in vivo*.

The observed improvement in histological findings in the MMF monotherapy group therefore possibly reflects the reported antifibrotic proprieties of MMF [23,24], or alternatively, is favoured by CNIs suspension, possibly allowing the immune system to better control HCV infection, which is thought to have a more aggressive course with stronger immunosuppressive regimens [34]. It is also conceivable, however, that previous reports on MMF in HCV-infected LT recipients failed to show a favourable effect because of the concomitant CNI therapy which may have mitigated the antifibrotic effect of MMF.

Finally, it is important to note that we did not observe in the present study episodes of acute rejection, either during CNIs tapering as in the long-run, underlying that MMF monotherapy is indeed a safe and effective maintenance immunosuppressive regimen in LT. The latter finding is also in keeping with Kamphues *et al.* [35], who retrospectively reviewed 123 liver recipients converted to MMF monotherapy, who did not experience any acute rejection in the first 3 months.

In conclusion, our study suggests that in LT recipients with recurrent HCV-related disease a permanent switch of IS from CNI to MMF monotherapy has a favourable effect on fibrosis progression compared to continuing CNI monotherapy. This therapeutic strategy is safe and well tolerated, is not associated with the emergence of clinical or histological signs of rejection and permits a complete durable suspension of CNIs and consequently a reduction of their renal and metabolic toxicity. These findings suggest that MMF monotherapy may currently represent the preferred immunosuppressive alternative for the long-term management of LT recipients with recurrent HCV infection, particularly in those who do not respond to, or cannot be treated with antiviral drugs. They also suggest that well-designed prospective randomized studies should be conducted to confirm these results.

Authorship

TMM: designed research, performed research, analysed data and wrote the paper. RA and LT: collected and analysed data. LT, MIB and DS: performed research, analysed data and wrote the paper. GP: performed research. GO: analysed data and reviewed the paper. GT: designed the study and reviewed the paper.

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