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ORIGINAL ARTICLE

The Tor Vergata weaning of immunosuppression protocols in stable hepatitis C virus liver transplant patients: the 10-year follow-up

Tommaso Maria Manzia,[1] Roberta Angelico,[1] Leonardo Baiocchi,[2] Luca Toti,[1] Paolo Ciano,[1] Giampiero Palmieri,[3] Mario Angelico,[2] Giuseppe Orlando[4] and Giuseppe Tisone[1]

1 UOC Trapianti d'Organo, Tor Vergata University, Rome, Italy
2 Hepatology Unit, Department of Internal Medicine, Tor Vergata University, Rome, Italy
3 Histopathology Department, Tor Vergata University, Rome, Italy
4 Department of General Surgery, Section of Transplantation, Wake Forest University School of Medicine, Winston Salem, NC, USA

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Correspondence
Tommaso Maria Manzia MD, U.O.C. Chirurgia dei Trapianti, Tor Vergata University, Viale Oxford n.81., Rome 00144, Italy.
Tel.: 00390620902498;
e-mail: tomanzia@libero.it

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Summary

We report herein the 10-year outcome of the Tor Vergata weaning off immunosuppression protocol in hepatitis C virus (HCV) liver transplant patients. Thirty-four patients who had received a liver graft for HCV-related cirrhosis were enrolled in a prospective study in which they were progressively weaned off immunosuppression. The primary endpoints were feasibility and safety of the weaning; the second aim was to assess fibrosis progression. At the 10-year follow-up, of the eight original tolerant patients, six remained IS-free. Of the 26 individuals who could not be weaned, 22 were alive. When the baseline biopsies were compared with the 10-year biopsies, the tolerant group showed no differences in staging, whereas the nontolerant group showed a significant increase in staging. The fibrosis progression rates calculated for the tolerant and the nontolerant groups were -0.06 ± 0.12 and 0.1 ± 0.2, respectively ($P = 0.04$). Furthermore, with the last taken biopsies, nine nontolerant patients were showing frank cirrhosis versus no cirrhosis among the tolerant patients. After a 10-year follow-up of a Tor Vergata weaning protocol, 6/34 patients completed follow-up without reinstitution of immunosuppression and this appeared beneficial regarding a reduction in fibrosis progression.

Introduction

The net state of immunosuppression (IS) is one of the main variables responsible for the degree of progression of hepatitis C virus (HCV) disease recurrence after liver transplantation (LT) [1–4]. With a higher impairment of the host immune response stemming from a strong immunosuppressive regimen, there is a more aggressive advancement of HCV disease recurrence and a faster evolution toward liver cirrhosis; therefore, we hypothesized that the onset of an IS-free state (IFS) may be beneficial to HCV LT recipients [5–8]. To test this hypothesis, we designed and implemented a prospective study where 34 such patients were enrolled and subjected to IS weaning [5,6]. At the 4-year and 6-year follow-ups, the attempt of IS weaning was feasible in 23% of patients. In the eight patients who were tolerant (TOL), IS could be completely withdrawn within the first 5 months from the start of the tapering. In this group, the onset of IFS also strikingly improved graft fibrosis and necro-inflammation and liver function tests [5]. On the contrary, the 26 individuals who were nontolerant (non-TOL) showed rejection during the weaning process; therefore, IS was resumed safely without major adverse events and without corticosteroids treatment [5]. This group was also followed prospectively. At 6 years, while the impact on graft histology was less discernable, TOL patients showed significant reduction in IS-related morbidity [6].

In this article, we report the results regarding the 10-year follow-up of this cohort of patients.

Materials and methods

Patients

Thirty-four patients (25 M/9 F, 62 ± 6.4 years) transplanted for HCV end-stage liver disease were enrolled in the study starting in 1999 [2]. All patients were under cyclosporine monotherapy. As a part of a published pilot study [9], 20 patients were receiving low-dose (median 400 mg/day, range 200–800) maintenance ribavirin monotherapy until December 2001. Seventeen patients were treated with PEG-interferon plus Ribavirin (48 weeks) as they were included in a study on antiviral therapy in HCV liver recipients [10]. The study design is shown in Fig. 1. The rationale, baseline demographic characteristics of all enrolled individuals, and the endpoints have all been extensively illustrated previously [5,6] and are also summarized in Table 1. Inclusion criteria were as follows: HCV-RNA serum positivity; at least 12 months follow-up from LT; biopsy proven disease recurrence with normal graft function; and treatment compliance. Patients with deteriorated liver function, cirrhosis, or other hepatic or nonhepatic disease were excluded [5].

Liver histology

The type of treatment and the clinical status of the patients were all unknown to the pathologist (G.P.), who performed all the histologic examinations. Liver biopsies were taken at the baseline and the 1-month endpoint after completion of weaning to exclude any silent feature of acute or chronic rejection [2]. Protocol biopsies were repeated yearly throughout the study, as well as when it was clinically indicated by fluctuation of liver function tests. Liver specimens were obtained percutaneously using 1.6 mm modified Menghini needles. To minimize sampling errors, only specimens that were longer than 1.5 cm and wider than 1.4 mm were considered, including ≥8 portal tracts [11]. Specimens were formalin-fixed and paraffin-embedded for histologic analysis. 5-μm sections were stained with hematoxylin and eosin, as well as the Masson's trichrome stain of collagen and cytokeratins for the assessment of ductopenia. Grading and staging were assessed according to the Ishak score [12]. The yearly fibrosis progression rate was calculated as the difference between the *staging* score in the last and the baseline liver biopsies, divided by the years of follow-up [13]. Acute and chronic rejection was defined according to standard criteria described below [2,14].

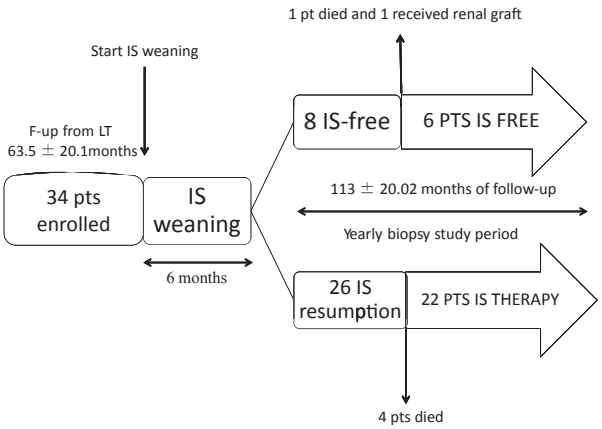

Figure 1 Study design: 34 LT recipients were enrolled in the IS weaning study; eight were successfully weaned off IS while 26 required IS readmission as a result of acute rejection. After more than 10 years of follow-up, six recipients are still off IS.

Table 1. Median baseline clinical features of patients alive > 10 years who achieved IFS and of those who required immunosuppression resumption.

Variable	Tolerant	Nontolerant	*P* value
Number of patients	6	22	–
Age at enrollment	61 (IQR 12)	61 (IQR 9)	NS
Gender (M/F)	5/6	16/6	NS
Serum creatinine (mg/dl)	1.33 (IQR 0.53)	1.30 (IQR 0.41)	NS
HCV genotype 1	4 (66%)	17 (77%)	NS
HBcAb positive	3 (50%)	11 (50%)	NS
Cholestatic hepatitis	None	None	–
HIV positive	None	None	–
ACE-inhibitors treated	2 (33%)	3 (13%)	NS
IDDM	1 (16%)	5 (22%)	NS
Azathioprine use (<12 months post-LT)	6 (100%)	19 (86%)	NS
OKT3/ATG use	None	None	–
Steroid bolus use	None	None	–
Treated with ribavirin	7 (87%)	13 (60%)	NS
Patients who achieved SVR	1 (16%)	4 (18%)	NS
HCC at transplant	0 (0%)	4 (18%)	NS
CIT	510 (IQR:144)	375 (IQR 65)	NS
WIT	47.5 (IQR: 6)	45 (IQR 12)	NS
Donor age	35 (IQR: 17)	38 (IQR 28)	NS
Donor gender (M/F)	5/1	16/6	NS
Donor HCV positive	None	None	–
Donor HBcAb positive	None	None	–

IFS, immunosuppression free status; HCV, hepatitis C virus; HBcAb, anti-hepatitis B core; CIT, cold ischemic time; WIT, warm ischemic time; HIV, human immunodeficiency virus; ACE-inhibitors, angiotensin-converting enzyme inhibitors; IDDM, insulin-dependent diabetes mellitus; ATG, anti-thymocyte globulin; SVR, sustained viral response after antiviral treatment; NS, difference not statistically significant.

Serum HCV-RNA titers

Viral load was measured at the baseline, at month 6, and yearly, using the AMPLICOR HCV MONITOR (Roche Diagnostics, Indianapolis, IN, USA).

Serum biochemistry

Liver function tests, including total bilirubin, direct bilirubin, AST, ALT, GGT, AP, and albumin, were assessed at least every 3 months, unless otherwise indicated by the patient's clinical conditions.

IS-related morbidity

Cardiovascular, tumoral, infective, and metabolic complications were recorded during the outpatient visit in all subjects together with the prescribed number of medications [6].

The study protocol was approved by our Ethical Committee and conducted in compliance with the Helsinki Declaration. All patients gave written informed consent.

Statistical analysis

Data were collected from a prospective database (Microsoft Access 2.0; Microsoft Corporation, Redmond, WA, USA) and categorical variables were analyzed using nonparametric testing (Fisher exact test). For normal distribution, the continuous data parametric test (Student t-test) was used. Statistical results were expressed as mean ± standard error. A P-value of <0.05 was considered statistically significant. Survival rates were calculated using the Kaplan–Meier method. The program used for statistical analysis was SPSS® 13.0 (SPSS, Chicago, IL, USA) for Windows.

Results

Patient and graft survival

After a median time follow-up of 113 ± 20.02 months, the eight original TOL patients, one died as a result of biopsy proven severe cholestatic HCV recurrence, which occurred 10 years after the transplant and 6 years after the complete withdrawal of cyclosporine; in the yearly biopsies, no signs of acute or chronic rejection were recorded [6]. One other TOL patient who required dialysis treatment after 5 years from LT because of renal failure CNI-related received a renal graft with reinstitution of IS 7 years after its complete withdrawal and 13 years after LT. At the last follow-up visit, his liver function tests were normal and the liver biopsy showed no signs of rejection.

Of the 26 non-TOL recipients, four died after a mean follow-up time of 115 (range 100–124) months from LT. Of these patients, two died following HCV recurrence both 8 years after LT and 4 and 3 years after the attempt at weaning, one died from lung carcinoma, and one died from acute myocardial infarction [6]. In addition, one non-TOL patient received a second LT for severe HCV recurrence, 10 years after her first transplant and 5 years after the attempt at weaning. At the last follow-up, she was on micophenolate mofetil with preserved graft function.

Overall, the 10-year patient and graft survival rates for TOL versus non-TOL patients were 87.5% vs. 84.6% (Log Rank: 0.648) and 87.5% vs. 80.8%, respectively (Log Rank: 0.482) (Fig. 2). Participation in the study did not cause any death and/or graft loss owing to acute or chronic rejection.

Liver histology

At least 10 consecutive yearly biopsies were available for each patient, including the baseline. When baseline biopsies were compared with 10-year biopsies, the TOL patients showed an improvement in grading (from 4.7 ± 2.0 at the baseline to 3 ± 1.6 at 10 years, $P = 0.01$) (Fig. 3b) and no differences in staging (3.0 ± 0.8 at baseline vs. 2 ± 1.5 at 10 years, $P = 0.6$) (Fig. 3a); thus, confirming data previously reported after the 6-year follow-up [6]. In the non-TOL patients, staging increased from 2.1 ± 0.9 at the baseline to 3.1 ± 1.8 after 10 years ($P = 0.03$) (Fig. 3a), while grading decreased from 4.8 ± 1.5 at the baseline to 2.5 ± 1.2 10 years after enrollment ($P = 0.0003$) (Fig. 3b). In terms of the fibrosis progression rate at 10 years, the TOL patients showed a slower progression of tissue damage than the non-TOL patients (−0.06 ± 0.12 vs. 0.1 ± 0.2, respectively, $P = 0.04$). At the last biopsy taken, 14/22 (63%)

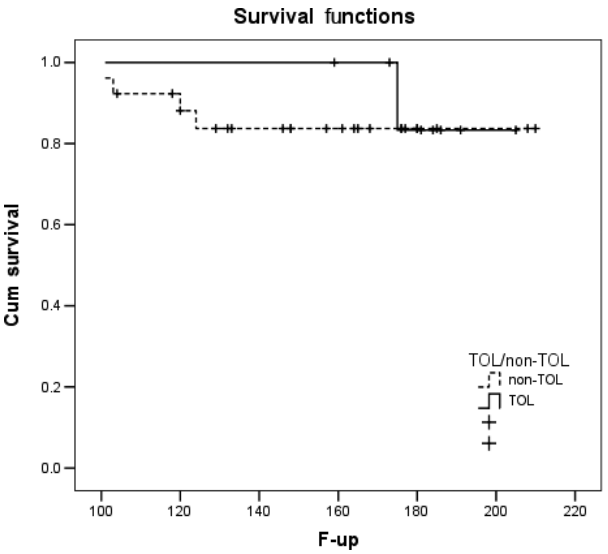

Figure 2 Actuarial patient survival. Both TOL and non-TOL show similar survival.

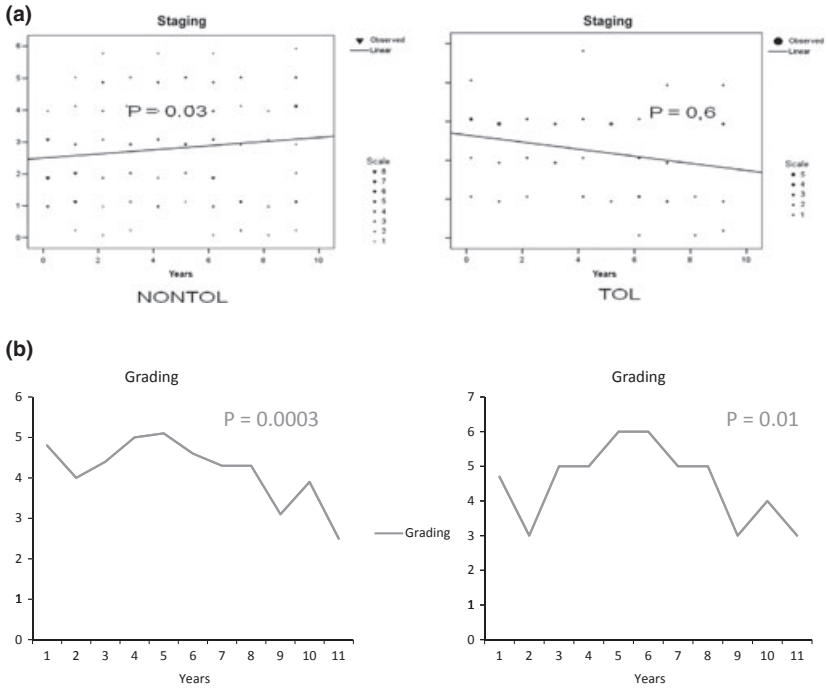

Figure 3 Progression of grading and staging score. When the baseline biopsies are compared with the 10-year biopsies, TOL patients showed no differences in terms of staging, whereas in the non-TOL patients, the staging increased significantly to the point that 40% were showing frank cirrhosis at the last follow-up biopsy (a). In terms of fibrosis progression rate, TOL patients showed a much slower progression of irreversible tissue damage than non-TOL. Both groups showed a fluctuation of the necro-inflammatory index throughout the study period, that ultimately did not correlate with the progression of the HCV disease (b).

non-TOL patients showed features of advanced fibrosis (defined as a fibrosis >4 according to the Ishak score) [15] with 9 of these 14 patients (40% of the whole group) even showing frank cirrhosis. On the contrary, none of the patients in the TOL group presented either advanced fibrosis or cirrhosis ($P = 0.006$). Two non-TOL patients died as a result of end-stage liver disease while on the waiting list for a new graft. No evidence of early or late chronic rejection was ever observed during the follow-ups (Table 2).

Serum HCV–RNA titers

At 10 years, we failed to observe any difference in viremia between the two patient groups. In the TOL group, high levels of HCV replication (HCV-RNA levels $>500 \times 10^3$ IU/ml) were observed in 3/6 (50%) patients, whereas only 9/22 (40%) patients among the non-TOL group showed high levels of HCV replication (Table 3). All recipients in the TOL group were infected by HCV genotypes 1 or 4 vs. 19/22 (87%) in the non-TOL group ($P = 1.00$).

Serum biochemistry

At 10 years, both groups showed a slight increase in liver enzyme values (Table 4).

IS-related morbidity

At 10 years, the beneficial effects on the patients' general conditions derived from the onset of IFS observed at 4 and 6 years were confirmed. In fact, among the non-TOL patients, 16 suffer from recurrent urinary and/or pulmonary infections that require appropriate treatments (versus none among the TOL group), 14 are currently taking at least one medication for cardiovascular disease (versus none among the TOL group), 13 are on insulin and/or oral antidiabetic drugs because of new onset diabetes mellitus (versus none among the TOL group), and 16 require other drugs (versus only 1 in the TOL group). Of the non-TOL individuals, eight patients (36%) were switched during the follow-up to an antimetabolite (i.e., mycophenolate mofetil) because of CNI side effects (three for diabetes, two for hypertension, three for renal impairment).

Discussion

The onset of IFS is considered a positive event after solid organ transplantation because chronic toxicity derived from lifelong IS is one of the main problems of post-transplant morbidity and mortality [16].

Table 2. Duration of weaning, rejection episode during follow-up, and fibrosis progression rate of individual patients.

n	Follow-up from weaning start (months)	Follow-up from LT	Severity of acute rejection at weaning	Other rejection episode during follow-up	Current status	Yearly fibrosis progression rate
TOL					Alive	
1	111	191	–	–	Alive	0
2	139	205	–	–	Alive	0
3	109	184	–	–	Alive	−0.2
4	112	186	–	–	Alive	−0.1
5	109	181	–	–	Alive	−0.2
6	151	159	–	–	Alive	0.1
7	120	172	–	–	Alive- kidney Tx	0.4
8	74	175	–	–	Dead	–
Non-TOL						
1	114	157	Moderate	–	Alive	0.1
2	120	176	Mild	–	Alive	0.1
3	118	148	Mild	–	Alive	0.1
4	112	129	Mild	–	Alive	0
5	117	118	Mild	–	Alive	0.4
6	19	101	Mild	–	Dead	–
7	118	180	Mild	–	Alive	0.2
8	25	49	Mild	–	Alive	−0.1
9	107	168	Mild	–	Alive	0.2
10	15	50	Moderate	–	Alive	−0.3
11	45	124	Mild	–	Dead	–
12	128	132	Mild	–	Alive	0.1
13	79	172	Mild	–	Alive	0
14	107	161	Mild	–	Alive	0.4
15	121	148	Mild	–	Alive	0.1
16	113	120	Mild	–	Alive	0.1
17	128	164	Moderate	–	Alive	−0.2
18	113	185	Mild	–	Alive	0.2
19	80	104	Mild	–	Alive	0.2
20	115	177	Mild	–	Alive re-OLT	–
21	124	180	Mild	–	Alive	0
22	77	120	Mild	–	Dead	–
23	114	210	Mild	–	Alive	−0.2
24	105	165	Moderate	–	Alive	0.2
25	107	176	Mild	–	Alive	0
26	14	103	Mild	–	Dead	–

Table 3. Viral load did not differ between the groups. The number of patients presenting HCV-RNA load >500 × 10^3 IU/L is taken into account.

	TOL (%)	Non-TOL (%)	*P*
Baseline	1/8 (12.5)	13/26 (50)	n.s
3 years	1/8 (12.5)	6/26 (23)	n.s
6 years	1/6 (16)	11/22 (50)	n.s
10 years	3/6 (50)	9/22 (40)	n.s

The numerous immunosuppressants currently available are given to patients based on the principle that 'one-dose-fits-all'. This may cause an over-immunosuppression in some patients who are thus exposed to increased IS-related toxicity. Essentially, drug trough levels do not necessarily correspond to IS net state; this occurs because we have no means to thoroughly measure the net state of IS other than through trough levels. In fact, this merely relates to the pharmacokinetics of a specific molecule rather than its impact on the immune system. Another important reason for pursuing IFS is that it entails a reduction in daily medications that has a tremendous impact on a patient's quality of life [17].

Approximately, six decades of clinical experience in solid organ transplantation have demonstrated that IFS is extremely difficult to achieve and is also organ dependent [16]. Recipients of a liver graft are more capable of developing IFS because of the immune-privileged status of the liver [8,14], which is exemplified by the following observations that were recently highlighted by Lerut *et al.* [18]: (i) the

Table 4. Synoptic view of the main clinical findings at 10-year follow-up.

Variable	TOL	Non-TOL	*P* value
Number of patients	6	22	–
Median follow-up (months)	122 ± 18.3	104 ± 29.7	NS
ALT (n.v. 5–31 IU/l)	66 ± 30.4	67.4 ± 51.7	NS
AST (n.v. 5–31 IU/l)	42.5 ± 14	70.2 ± 70.4	NS
Alkaline phosphatase (n.v. 40–120 IU/l)	133.3 ± 82.9	186 ± 130.1	NS
GGT (n.v. 5–36 IU/l)	95.1 ± 58.7	116 ± 84.3	NS
Total bilirubin (n.v. 0.2–1.1 mg/dl)	1.22 ± 0.3	0.9 ± 0.3	NS
Serum creatinine (n.v. 0.4–1.1 mg/dl)	1.3 ± 0.3	1.1 ± 0.3	NS
Cholesterol (n.v. 110–200 mg/dl)	172 ± 48.9	176.6 ± 47.1	NS
Triglycerides (n.v. 40–160 mg/dl)	140.8 ± 65	159 ± 72.2	NS
Glycemia (n.v. 50–110 mg/dl)	102.8 ± 19.9	130.8 ± 39.4	0.05
Recurrent infectious disease	0	16	0.002
Cardiovascular disease	0	14	0.01
Diabetes	0	13	0.01
Other drugs	1	16	0.03
Staging at 10 years	2 ± 1.5	3.1 ± 1.8	NS
Grading at 10 years	3 ± 1.6	2.5 ± 1.2	NS
Fibrosis progression rate	−0.06 ± 0.12	0.1 ± 0.2	0.04
Chronic rejection	None	None	–

n.v. = normal value.

relative lack of an effect of either a positive cross-match or blood-type incompatibility; (ii) the irrelevance of human leukocyte antigen (HLA) matching; (iii) the irrelevant incidence of hyperacute rejection and spontaneous recovery after severe rejection; [19] (iv) the fact that acute rejection does not impact adversely on the overall outcome; (v) the ability of liver allografts to protect other extra-hepatic allografts from rejection in the case of multi-organ transplantations.

We can assume that about 200 LT patients are or have been off IS for at least 1 year, despite the fact that the precise number of LT recipients off IS cannot be precisely calculated because of the same patients having been the object of different investigations reported in different studies [7,8]. A quarter of our patients could remain off IS, while no intractable rejection occurred in any of the patients in whom it was tried to wean off IS. Importantly, patients who develop acute rejection during the different weaning protocols are not exposed to further risks of graft loss when maintenance IS is resumed; however, the risk for rejection still remains after successful weaning because of the balance between nonself antigens present on the liver graft and the immune system breaking down at any time owing to a myriad of variables [7,8,16,20]. As a corollary, the close follow-up of patients off IS is mandatory for the early diagnosis of rejection and subsequent prompt treatment. Furthermore, investigators who are following such patients should inform the transplant community of the outcomes to allow critical scrutiny and understanding of the real impact that IFS may have on organ transplantation that is achieved with the current means.

We herein report the 3rd longest follow-up of a successful weaning series, with the longest being Pittsburgh's (180 months) and London's (120 months) [8]. Moreover, the 34 patients enrolled in our study represent the 4th largest series after Miami (104 patients), Pittsburgh (95 patients), and Kyoto (63 patients) [8]. However, our series is unique in including only HCV LT patients for the reasons that have been illustrated in depth previously [5–8]. Briefly, we hypothesized that the reconstitution of a physiologic immune competence would mitigate the progression of HCV disease recurrence. In fact, it is well known that the natural history of HCV re-infection post-LT is characterized by a more aggressive evolution and a faster progression toward cirrhosis, with one of the main risk factors being the degree of impairment of the immune response, i.e., the total amount of IS administered to the patient [5,6,8]. Importantly, initial findings [5] showed that after a mean follow-up of 45.5 ± 5.8 months, IS-free patients showed stabilization/improvement of histologic fibrosis, lower necro-inflammation, and improved liver function tests compared with weaning-intolerants. The Immune Tolerance Network designed a prospective, randomized, multicenter trial to confirm our hypothesis in a large North American population.

The 10-year results of our study confirm that IFS is beneficial to HCV LT recipients in both the pathology and clinical perspectives. With regard to pathology, the TOL patients definitely showed better results than the non-TOL patients over time with the staging score developing as a proper predictor of outcome. In fact, when the baseline biopsies are compared with the 10-year biopsies, the TOL patients showed no differences in terms of staging, whereas in the non-TOL patients, the staging increased significantly to the point that 40% of them were showing frank cirrhosis at the last follow-up biopsy. Although there was no statistical difference between the two groups in term of staging at 10 years of follow-up, the striking difference between the two groups was reflected in the fibrosis progression rate (Fig. 3a), which takes into account the disease progression and not only the last "fibrosis picture". Overall, in this specific setting, the TOL patients may be considered as slow fibrosers, while the non-TOL patients may be considered as fast fibrosers [21,22]. Why the comparison between 10 years and baseline liver biopsies demonstrated an improvement in a grading score is not clear at present? However, grading score is not a reliable measure of disease

progression in particular in transplanted patients [23]. In fact, both groups in our study showed a fluctuation of the necro-inflammatory index that ultimately did not correlate with the overall clinical picture (Fig. 3b). Interestingly, our findings are consistent with what is normally observed in nontransplant patients [19], which is that necro-nflammatory activity, as expressed by grading, in chronic hepatitis C typically waxes and wanes over time; whereas, fibrosis tends to progress more linearly and at a velocity that is determined by numerous variables, the most important of which, in our case, seems to be the degree of impairment of the immune system. It is also worth reporting that in immunocompetent non-LT HCV patients, it is common practice that grading, unless particularly severe, is not as important as the stage of fibrosis regarding the decision on whether or not to pursue treatment. Lastly, from a clinical perspective, the TOL patients presented better overall results and were subjected to less drug intake, as only one of them was receiving metforal and atenolol, versus the 16 non-TOL patients (see above).

The strength of our study derives from the availability of yearly protocol liver biopsies in all patients enrolled. As the approximate 300 biopsies performed in more than 34 patients over a 10-year period were studied by the same pathologist, this allowed us to closely monitor the histologic evolution of recurrent HCV-related disease while minimizing potential biases attributable to sampling errors or interpretation by different pathologists. While patients off IS deserve the most strict surveillance, we believe that in this scenario, early protocol biopsies should be mandatory regardless of well-known biopsy-related mortality and morbidity, as well as costs and even patient compliance/approval.

In conclusion, most patients in the subgroup of HCV LT patients with successful weaning off IS do well 10 years after complete IS withdrawal. Importantly, the achievement of stable and durable IFS is beneficial because it slows down the progression of disease recurrence while diminishing side effects from IS. We believe that HCV LT recipients who have been transplanted for at least 1 year before should be offered the option of being weaned off IS to slow down disease recurrence if no sustained viral response can be achieved.

Even if graft HCV-fibrosis is established, we believe that it is never too late to wean the recipient off IS, not only because of the possibility of avoiding cirrhosis recurrence but also to prevent long-term IS side effects and because of the cost reduction implied. Obviously, the earlier IFS is attempted after LT, the slower disease progression could be; in this regard, in the near future, biological markers [24,25] could be useful in identifying those recipients in which IS withdrawal can be attempted immediately after LT.

Authorship

TMM: analyzed data, wrote the paper. RA: collected and analyzed data. LB: analyzed data. LT: wrote the paper. PC: collected data. GP: pathologist who analyzed the liver specimens. MA and GO: reviewed the paper. GT: designed the study, wrote, and reviewed the paper.

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