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The Tor Vergata weaning off immunosuppression protocol in stable HCV liver transplant patients: The updated follow up at 78 months

Giuseppe Orlando [b,c], Tommaso Manzia [a], Leonardo Baiocchi [d], Alberto Sanchez-Fueyo [e], Mario Angelico [d], Giuseppe Tisone [a,*]

[a] Transplant and General Surgery, Tor Vergata University of Rome, S. Eugenio Hospital, Rome, Italy
[b] Wake Forest Institute for Regenerative Medicine, Winston Salem, NC, USA
[c] Department of Surgery, Nuffield University Hospital, University of Oxford, Oxford, UK
[d] Chair of Gastroenterology and Hepatology, Tor Vergata University of Rome, Rome, Italy
[e] Liver Transplant Unit, Hospital Clinic Barcelona, IDIBAPS, University of Barcelona, Barcelona, Spain

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ABSTRACT

Background: We report the update of the Tor Vergata immunosuppression (IS) weaning protocol in stable hepatitis C virus (HCV) liver transplant (LT) recipients.
Methods: The weaning off IS was attempted in 34 patients who had received a LT 63.5±20.1months earlier, for HCV-related end stage liver disease. Patients were observed over a period of 6.5years. During this time, yearly protocol liver biopsies were performed. Primary endpoints were determined as the feasibility of weaning off IS and its impact on the long term disease progression. Secondary endpoints were defined as the impact on patient morbidity and quality of life.
Results: Of the 8 originally tolerant patients, 7 remain alive and in good condition, while 1 died of severe HCV recurrence 10years post-LT and 6years after complete removal of IS. Four out of 26 intolerant individuals died of HCV recurrence (2×), lung carcinoma (1×) and acute myocardial infarction (1×), after a mean follow up period from LT of 115 (range 100–124). The 10-year survival from LT was comparable (89% vs. 87.5%). Liver graft pathology showed no significant differences between the two groups in terms of staging, fibrosis progression rate, and grading. Quantitative HCV RNA assay showed a significant non-logarithmic difference between the two groups ($p = 0.03$). The two groups were comparable in terms of liver function tests and lipid profile, whereas they differed with regards to glycaemia. While all tolerant individuals were euglicemic, 11 intolerant individuals developed new onset diabetes that required specific treatment ($p = 0.03$). Finally, significantly more intolerant patients are suffering from either cardiovascular (14/22 vs. 0/7, $p = 0.01$) or infectious diseases (13/22 vs. 0/7, $p = 0.01$).
Conclusions: After a 6.5-year follow up, the complete withdrawal of IS in HCV LT recipient remains safe and beneficial to patients, because it reduces the IS-related morbidity and increases the quality of life. The impact on HCV disease recurrence is less marked than after 3.5years.

1. Introduction

An earlier study published in 2006 by the current authors disclosed the results of a 4year prospective trial in which immunosuppressive drugs were withdrawn from stable hepatitis C virus (HCV) liver transplant (LT) patients [1]. It was shown that the progression of HCV disease recurrence is slowed down in response to the reconstitution of the immune surveillance in the host. In fact, patients who could permanently be weaned off immunosuppression (IS) showed a significant reduction in the fibrosis progression rate and a marked improvement of liver function tests (LFT). In addition, it was demonstrated that the occurrence of acute rejection was not harmful to the patient, as it did not have any impact on patient outcome and LFT normalized after prompt resumption of IS.

The present study describes the results over the course of 78months post IS withdrawal. Specifically, these findings demonstrate that the complete withdrawal of IS in HCV LT recipients remains safe and is also beneficial to patients because it reduces the IS-related morbidity and increases the quality of life. However, the impact on HCV disease recurrence is less marked than after 4years. Importantly, our data provide further evidence that HCV-related end stage liver

Abbreviations: LT, liver transplantation; HCV, hepatitis C virus; IS, immunosuppression; LFT, liver function tests; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma glutamyl transferase; AP, alkaline phosphatase.

* Corresponding author. Tor Vergata University of Rome, General and Transplant Surgery, Ospedale S. Eugenio Piazzale dellUmanesimo 10 00144 Rome, Italy. Tel.: +39 06 51002280; fax: +39 06 5922681.
E-mail address: tisone@med.uniroma2.it (G. Tisone).

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disease does not represent a contraindication to the withdrawal of IS [2,3], but instead it should be considered as one of the most valuable indications.

2. Materials and methods

Test subjects consisted of 34 individuals whose baseline characteristics have been extensively described earlier [1]. Throughout the 78months of the follow up evaluation, patients were assessed through routine blood tests including LFT, renal function tests, lipemic profile (cholesterol and triglycerides), glycaemia and immunosuppressant blood trough levels. Liver biopsies were taken annually to establish staging, grading, and fibrosis progression rate (defined as the difference between the staging score in the last and the baseline liver biopsy divided by the years of follow up [1]), and to exclude features of acute or chronic rejection. Additional biopsies were taken whenever there was suspicion of acute rejection. Grading and staging were assessed according to Ishak [4]. Acute rejection was defined according to standard criteria [5]. Chronic rejection was assessed according to Banff classification [6]. We received written approval from all patients involved for this study.

Patients who could be permanently weaned off IS are referred to as tolerant, whereas those who developed acute rejection and thus required the resumption of IS are referred to as intolerant.

3. Endpoints

The primary endpoint was the long term feasibility and safety of the weaning protocol. The secondary endpoint was to assess whether the achievement of a sustained IS-free state have any impact on the progression of HCV recurrence in the future. The tertiary endpoint was to verify the impact of the IS-free state on patient quality of life, as expressed by IS-related morbidity (namely, the onset of cardiovascular, tumoral, infectious and metabolic complications) and the prescribed number of medications per patient.

4. Statistical analysis

We used the survival analysis to assess mortality. Survival rates were calculated using the Kaplan–Meier method. For normal distribution continuous data analysis, we used the parametric test (Student *t*-test); categorical variables were evaluated according to the non-parametric test (Fisher exact test). A *p*-value of ≤0.05 was considered significant.

The program used for statistical analysis was SPSS® 13.0 (233 South Wacker Drive, Chicago, USA).

5. Results

5.1. Survival

No patient was lost to follow up. After a mean follow up period of 78 (range 57–109) months, the participation to the weaning protocol did not cause either patient or graft loss. Of the original 8 patients whom the IS could be successfully and steadily weaned off (tolerant group), 1 died due to severe cholestatic HCV recurrence 10years after transplantation, 6years following the complete withdrawal of cyclosporine. Out of the 26 individuals who did not tolerate the tapering of IS (intolerant group) (76.5%), 4 died after a mean of 115 (range 100–124) months following LT: 2 due to severe cholestatic HCV recurrence, 1 from lung carcinoma, and 1 due to acute myocardial infarction. Overall, the 10-year survival rate from the LT was 88%, with no significant difference between the tolerant and intolerant groups (Table 1).

5.2. Liver pathology

At least 7 consecutive yearly liver biopsies were available from each patient, in addition to the one performed at the time of enrollment in the study.

Overall, the significant differences observed 3 years following withdraw from IS were unable to be confirmed 3years later (i.e., 6years after the weaning). In fact, within the tolerant group, the comparison of 3-year versus 6-year biopsies showed an improvement of grading in 5 patients (71%) and a stabilization in 3 patients. In addition, an improvement (namely, a reduction) of at least 1 unit of the staging score was observed in 4 patients (57%), while the score did not vary in the remaining 3 patients. Similar features were detected in the intolerant patient population, where the grading worsened in 4 (18%) patients, improved in 9 (41%) and remained unchanged in 9 (41%); the staging deteriorated in 8 (30%) patients and remained unchanged in the remaining 14.

In contrast to previous data recorded after 4-year follow up [1], the fibrosis progression rates calculated for the tolerant and intolerant groups at 72months of follow up were comparable, despite tolerant patients showed a slight trend toward a slower progression rate (0.26±0.13 and 0.43±0.29 respectively, p = n.s.). However, focal ductopenia was occasionally observed in protocol liver biopsies, always being limited to less than 20% of portal spaces (usually less than 10%). Of the patients of the present series, there was no evidence of early or late chronic rejection, in consistence with our data at 4years [1].

Table 1
Synoptic view of the main clinical findings at 6.5-year follow up

	Tolerant	Intolerant	*p* value
Number of patients	7	22	
10-year survival rate	89%	87.5%	n.s.
Median follow up (months)	80±15.1	74.5±7.1	n.s.
ALT (n.v. 5–31 IU/L)	74	40 (10–85)	n.s
AST (n.v. 5–31 IU/L)	43	35 (8–45)	n.s
Alkaline phosphatase (n.v. 40–120 IU/L)	113 (58–249)	154 (51–287)	n.s.
GGT (n.v. 5–36 IU/L)	86 (23–156)	57 (20–138)	n.s.
Total bilirubin (n.v. 0.2–1.1 mg/dl)	1.1 (1.0–2.0)	0.9 (0.6–2.0)	n.s.
Serum creatinine (n.v. 0.4–1.1 mg/dl)	1.3 (0.8–6)	1.3 (0.8–7.2)	n.s.
Cholesterol (n.v. 110–200 mg/dl)	127 (107–219)	170 (107–270)	n.s.
Triglicerydes (n.v. 40–160 mg/dl)	137(55–221)	130 (53–393)	n.s.
Glycaemia (n.v. 50–110 mg/dl)	112 (85–197)	129 (86–333)	n.s.
Recurrent infection disease	0	13	0.01
Cardiovascular disease	0	14	0.01
Diabetes	0	11	0.03
Other drugs	1	16	0.03
Staging at 6 years	1.5±0.9	2.8±1.5	n.s.
Grading at 6 years	3.1±0.7	3.7±1.6	n.s.
Final yearly fibrosis progression rate	0.23±0.16	0.48±0.26	n.s.

Legend: n.v. normal values.

5.3. HCV virology

At 6years from study entry, tolerant patients presented significantly lower non-logarithmic mean HCV RNA titers compared to intolerant individuals [205,800 (range 0–993,000) IU/L vs. 3,129,588 (0–16,400,000) IU/L] (p = 0.03), in consistence with our previous report [1].

5.4. Blood biochemistry

Both tolerant and intolerant groups scored similarly in liver and renal function tests, as well as their lipid profile (Table 1). Although glycaemia tests were also comparable between the two groups, 11 of the 22 surviving intolerant patients (50%) are currently under hypoglicemizing drugs following the onset of de novo diabetes mellitus.

5.5. IS-related morbidity

Out of 22 intolerant patients who are still alive, 14 (63%) suffer from cardiovascular disturbances and are currently taking at least one medication active on the cardiovascular system. On the contrary, none of the tolerant individuals are being treated with any similar medication, nor have they suffered from any arterial hypertension (p = 0.01).

In addition, none of the tolerant patients suffer from recurrent infections, whereas 13 (59%) intolerant patients have developed urinary and/or pulmonary (bacterial and/or viral and/or fungal) infections requiring specific treatment during the study period (p = 0.01).

Sixteen out of 22 (72%) intolerant patients are also receiving other medications, specifically allopurinol, levothyroxine, antipsychotics, or bisphosphonates, whereas only 1 tolerant (14%) is taking allopurinol (p = 0.03).

6. Discussion

The study is peculiar for two reasons. First, we did not administer any drug, nor did we adopt any specific regimen, expected to be tolerogenic, but – more simply and pragmatically – we based our