


SHORT COMMUNICATION

Direct-acting antivirals ability to clear intestinal HCV-RNA in liver transplant patients

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Abstract

The hepatitis C virus mainly infects the liver but is also able to infect and replicate in other body compartments by creating an extra-hepatic reservoir that may influence the persistence of the infection after transplantation. It is unknown whether antiviral drugs affect the viral extra-hepatic sites. We evaluated the ability of pegylated/interferon + ribavirin and sofosbuvir + ribavirin to clear the virus from the gastrointestinal mucosa of liver-transplanted patients with HCV recurrence after transplantation.

A total of 51 liver-transplanted patients, 30 treated with pegylated/interferon + ribavirin (ERA1) and 21 treated with sofosbuvir + ribavirin (ERA2), were enrolled, and blood serum and gastrointestinal tissues analyzed for the presence of HCV-RNA.

In the ERA1 group, the 46.6% of patients had a sustained viral response to antiviral treatment, and gastrointestinal biopsies were positive for HCV in 73.3% of cases, 54.5% of responders, and 45.5% of non-responders. In the ERA2 group, the 66.6% had a sustained viral response, and gastrointestinal HCV-RNA was present in the 14.3% of patients, all relapsers. Sofosbuvir + ribavirin cleared the intestinal HCV in 85.7% of patients with recurrent HCV infection, while pegylated/interferon + ribavirin cleared it in 26.6% of treated patients, demonstrating the better effectiveness of new direct antiviral agents in clearing HCV intestinal reservoir.

KEYWORDS

hepatitis C virus, interferon, liver transplantation, sofosbuvir

1 | INTRODUCTION

The hepatitis C virus (HCV) infects approximately 170 million people worldwide. Chronic HCV infection remains the leading indication for

liver transplantation (LTx)¹⁻⁶ which is the only curative treatment when significant hepatic decompensation occurs.⁷⁻⁹ However, if the patient is HCV-RNA positive at the time of transplantation, the graft is generally re-infected by HCV virions present in the blood or in extra-hepatic

Abbreviations: DAAs, direct antiviral agents; ER, extra-hepatic reservoir; GI, gastrointestinal; GIM, gastrointestinal mucosa; HCV, hepatitis C virus; LT, liver-transplanted; LTx, liver transplantation; NR, non-responders; pegIFN-alpha, pegylated interferon-alpha; R, responders; RBV, ribavirin; SOF, sofosbuvir; SVR, sustained virologic response.

Pietrosi and Russelli contributed equally to this work.

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reservoir (ERs).^{9,10} Although the liver is, in fact, the primary site of HCV replication, several reports have demonstrated that HCV is able to infect and replicate also in other body tissues.¹¹⁻¹⁶ The gastrointestinal tract has been considered a possible ER of HCV since viral RNA or proteins have been detected in intestinal cells and in stool samples.^{13,16-18} Indeed, we recently demonstrated that gastrointestinal mucosa (GIM) can represent an ER for HCV: viral minus-strand RNA was found in the cells of GIM and HCV quasiespecies detected in GIM resulted to be compartmentalized, in comparison with HCV present in the blood and similar to the quasiespecies found in the re-infected liver after transplantation, suggesting that HCV produced in GIM could contribute to graft re-infection¹⁶.

Until recently the standard of care for chronic HCV was the treatment with pegylated/interferon + ribavirin (PEG-IFN-alpha/RBV).¹⁹⁻²³ The introduction in the clinical practice of new direct antiviral agents (DAAs) has improved the clinical condition of patients with chronic hepatitis allowing to achieve sustained virologic response (SVR) in more than 95% of cases.^{24,25} It is unknown whether the ERs of HCV are affected by antiviral treatment or whether the disappearance of the viremia induces HCV clearance from the other body compartments. In this study, we addressed this issue taking advantage of the comparative evaluation of the effect of PEG-IFN-alpha/RBV and sofosbuvir + ribavirin (SOF/RBV) treatment on HCV-RNA clearance in GIM through the analysis of GIM biopsies taken from HCV-positive patients treated because of HCV infection recurrence after LTx.

2 | MATERIALS AND METHODS

2.1 | Patients and study design

We analyzed gastrointestinal (GI) biopsies of two groups of HCV-positive LT patients, ERA1 and ERA2, affected by HCV-related cirrhosis and HCV recurrence after liver transplantation, who were transplanted at ISMETT between August 1994 and November 2014. ERA1 group was composed by 30 patients treated with PEG-IFN-alpha (80 mcgs) and ribavirin (1000-1200 mg) daily and with an available GI biopsy (stomach = 15, duodenum = 2, large intestine = 13) taken during endoscopic examination after antiviral treatment. ERA2 group was composed by 21 patients treated daily and for six months with sofosbuvir (400 mg) and ribavirin (1000-1200 mg). The baseline characteristics of patients (age, gender, HCV genotype, immunosuppressive treatment, HCV viremia at enrollment, clinical status, and time of antiviral treatment) are showed in Table 1. The diagnosis of cirrhosis was clinical or histologically proven. The study was approved by ISMETT Institutional Research Review Board (IRRB5214) and by the Ethics Committee of Cervello Hospital, in Palermo, Italy. Signed informed consents were obtained before performing the endoscopic procedure and extracting blood samples.

2.1.1 | Tissue collection and HCV-RNA detection

Paraffin-embedded GI biopsies from 30 HCV-RNA-positive patients of the ERA1 population were obtained from ISMETT's Pathology

TABLE 1 Clinical characteristics of LT patients (ERA1 and ERA2)

	LT patients ERA1 (%)	LT patients ERA2 (%)	P value*
Numbers	30	21	–
Mean age [range]	63 [44-78]	62 [38-71]	.827
Gender, M:F	25:5	18:3	1.000
Genotype			
1a	1 (3.3)	2 (9.6)	.049
1b	24 (80)	16 (76.1)	
2	1 (3.3)	1 (4.7)	
3	4 (13.3)	2 (9.6)	
FK	19	17	.261
MMF + FK	7	3	
EVE	/	1	
Cyclosporin	3	/	
FK + EVE	1	/	
Time frame of LT	1995-2010	1996-2014	–
Mean serum HCV-RNA at enrollment [range]	14.5 x10 ³ [10.9-17.3] x10 ³	13.3 x10 ³ [6.8-15.4] x10 ³	.014
Antiviral treatment length	12 months (between 1995-2010)	6 months (between 2014-2015)	–
Time passed from the date of transplantation and the time of biopsy collection (mean years)	6.02	6.37	–

Abbreviations: EVE, everolimus; FK, tacrolimus; LT, liver-transplanted; MMF, mycophenolate mofetil.

*P value <.05 was considered statistically significant.

Service. GI biopsies were collected for diagnostic purposes during endoscopic examinations. Twenty-one fresh duodenal biopsies of the ERA2 population were collected during upper GI endoscopy and delivered to the Research Department of Ismett. HCV-RNA was isolated from fresh, submerged in "RNAlater[®]," and paraffin-embedded GI biopsies. The RNA purification was carried out using RNeasy Protect mini kit (Qiagen) from fresh samples and RNeasy FFPE kit (Qiagen) from five sections of 5 µm thickness paraffin-embedded GI samples. Purified RNA was retro-transcribed and amplified by RT-PCR with the Artus HCV RG RT-PCR kit (Qiagen) according to the manufacturer's instructions.

2.1.2 | Plasma HCV-RNA evaluation and HCV genotyping

HCV-RNA was purified and amplified from plasma samples with COBAS AmpliPrep/COBAS TaqMan HCV quantitative test, v2.0 (Roche Diagnostics) (range ≥1.50E + 01 IU/mL–≤1.00E + 08 IU/mL) using COBAS AmpliPrep/COBAS TaqMan system (Roche

Diagnostics). HCV genotype was determined by INNO-LipA HCV II kit (Innogenetics).

2.1.3 | Statistical analysis

Quantitative variables are expressed as mean and range, and qualitative variables as absolute and relative frequencies. Comparisons between groups of quantitative and qualitative variables were made with two-sample t tests with Satterthwaite approximation to the degrees of freedom, and Fisher's exact test, as appropriate. All tests were two-sided, with a *P* value of <.05 indicative of statistical significance. Data handling and analyses were done with Stata version 13.1 software (Stata).

3 | RESULTS

3.1 | HCV-RNA detection in GI biopsies

In ERA1 group, composed of 30 patients treated with PEG-IFN-alpha/RBV, at the end of therapy only 14 patients (46.6%) maintained an SVR. Twenty-two patients out of 30 (73.3%) showed a positive HCV-RNA on GI biopsy; of these 22, 12 were responders (54.5%) and 10 non-responders (45.5%). HCV was not detected in GIM in only 26.6% (8/30) of patients, of which 25% (2/8) of responders and 75% (6/8) of non-responders (Table 2). For 10 patients, we could analyze a GI biopsy available before starting PEG-IFN-alpha/RBV. HCV-RNA was found before treatment in 7 of these 10 biopsies, and after antiviral treatment, 5 biopsies continued to maintain HCV-RNA. Only in 2/7 cases (28.6%), therefore, HCV-RNA was cleared in GIM by the antiviral treatment.

In ERA2 group, composed by 21 patients treated with SOF/RBV, the 52.3% of LT patients (11/21) were been already treated with PEG-IFN-alpha/RBV between 2002 and 2010 with no response (Table 2). At the end of the therapy with SOF/RBV, all patients resulted

negative for serum HCV-RNA, but 24 weeks after treatment, 7 of them (33.3%) had HCV recurrence. Then, in the ERA2 group the 66.6% (14/21) of patients showed SVR. An intestinal biopsy was collected, from all patients, during a GI endoscopy for screening of esophageal varices after completing the antiviral course. A total of 18/21 (85.7%) GI biopsies were negative for HCV-RNA, 14 of which were responders (78%) and 4 non-responders (22%). None of the responders showed a positive intestinal biopsy for HCV-RNA. A total of 3/21 patients (14.3%) showed a positive HCV-RNA on GI biopsy, and all these three patients were non-responders. For 13 LT patients, an intestinal biopsy was analyzed for the presence of HCV-RNA before starting SOF/RVB. HCV-RNA resulted positive in 5/13 (38%) cases, and 4 of 5 (80%) biopsies became negative after antiviral treatment, all responders.

Treatment with SOF/RVB was able to clear the intestinal HCV-RNA in 85.7% (18/21) of treated patients, while the treatment with PEG-IFN-alpha/RBV was successful in only 26.6% (8/30) of treated patients (*P* < .001). In ERA1 group, only 2 of 14 responders had negative intestinal HCV-RNA after antiviral treatment, while in ERA2 all responders had clearance of the intestinal HCV (*P* < .001).

4 | DISCUSSION

The standard treatment for chronic HCV, previously based on PEG-IFN-alpha/RBV,¹⁹⁻²³ has been replaced in the last few years by the introduction of the DAAs, which have greatly increased the SVR rate and shortened duration of therapy.^{24,25} SVR after antiviral therapy is evaluated according to the clearance of HCV-RNA from serum, but it is unknown whether the HCV-ERs are equally cleared by the antiviral treatment. In this study, we analyzed a population of patients affected by HCV recurrence after LTx, treated both with PEG-IFN-alpha/RBV and with SOF/RBV. Particularly, we evaluated the effect of the different antiviral treatments on the clearance of HCV-RNA in the gastrointestinal tract because of the previous evidence that HCV produced in GIM could contribute to graft re-infection.¹⁶

TABLE 2 Response to treatment results and HCV-RNA in GI biopsies of ERA1 and ERA2 patients

	LT patients ERA1 = 30	LT patients ERA2 = 21	<i>P</i> value*
Treatment with Peg-IFN + Riba	30	11	-
Response to Peg-IFN + Riba (%)	14 (46.6)	0 (0)	-
Treatment with SOF + Riba	/	21	-
Response to SOF + Riba (%)		14 (66.6)	
Mean serum HCV-RNA at time of GI biopsy (IU/ml), [range]	14.5 x10 ³ [10.9-17.3] x10 ³	12.6 x10 ³ [10.9-17.3] x10 ³	.001
GI biopsies in which HCV-RNA was evaluated after treatment	30	21	<.001
GI negative biopsies for HCV-RNA (%)	8 (26.6)	18 (85.7)	<.001
GI negative biopsies for HCV-RNA in responders (%)	2/14 (14.3)	14/14 (100)	

Abbreviations: GI, gastrointestinal; NR, non-responders; R, responders.

**P* value <.05 was considered statistically significant.

SVR was observed in 66.6% of the patients after treatment with SOF/RBV and in 46.6% of those treated with PEG-IFN-alpha/RBV. Regarding the presence of HCV-RNA in GI biopsies, SOF/RBV treatment resulted to be able to clear HCV in 85.7% of cases, whereas PEG-IFN-alpha/RBV cleared GIM HCV-RNA only in 26.6% of the patients ($P < .001$). The evidence that 45.5% of patients treated with PEG-IFN-alpha/RBV showed persistence of HCV-RNA in the GI tract indicates the inability of the treatment to clear the intestinal reservoir of the virus that, together with other factors, could contribute to restore viremia and then induce non response to the therapy. Indeed, in case of SOF/RBV treatment all the patients who were GIM HCV-RNA positive did not respond to the treatment. On the opposite, 100% of the responders were characterized by a complete HCV-RNA clearance in GI tract. This might imply that elimination of both serum and GI reservoir of HCV is mandatory to achieve a sustained complete response to any anti-HCV antiviral therapy. Indeed, it must be pointed out that the small percentage of HCV-infected patients non-responding to DAA includes particularly patients with severe clinical status (liver cirrhosis, liver transplantation, renal impairment, etc), who might be characterized by different pharmacokinetics and pharmacodynamics of DAAs.²⁶ Moreover, the compartmentalized molecular evolution of HCV-RNA in GI reservoir¹⁶ could cause the emergence of viral variants resistant to DAAs not detectable in the blood. Thus, according to these considerations, in order to pursue HCV clearance the findings of our study draw attention to consider the effects of DAAs to non-hepatic reservoirs of the virus. Indeed, although the new DAA therapy is highly efficacious (>95% cure rate) still a number of patients are NR and the reasons of DAA failure remain partially unclear. Our study suggests that, in order to effectively clear HCV infection the antiviral therapy should achieve the clearance of HCV not only from the hepatic but also from non-hepatic reservoirs.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

AUTHORS' CONTRIBUTION

GP involved in conception and design of the work, biopsy sample collection, data analysis, data interpretation, and editing. GR involved in conception and design of the work, clinical data and sample collection, sample laboratory and data analysis, and drafting of the work. FB involved in sample laboratory analysis. GC involved in biopsy sample collection. FT involved in statistical data analysis. AG involved in analysis and interpretation of data, and editing and critically revising of the work for important intellectual content. RV-GV involved in critically revising of the work for important intellectual content. PGC involved in data analysis, editing, and critically revising of the work for important intellectual content. All authors reviewed and approved final version of the manuscript.

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