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Original article

# Sustained virological response to pegylated interferon plus ribavirin leads to normalization of liver stiffness in hepatitis C virus-infected patients

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ABSTRACT

*Introduction:* Pegylated interferon plus ribavirin (Peg-IFN/RBV) therapy leads to improvements in liver stiffness measurements (LSM) in hepatitis C virus (HCV)-infected patients. However, the rate of LSM return to normal values in response to Peg-IFN/RBV is unclear. Thus, our aim was to assess the probability and factors associated with LSM normalization in HCV-infected patients receiving Peg-IFN/RBV.
*Methods:* This prospective observational longitudinal study included 160 HCV-infected patients, 111 (69%) with human immunodeficiency virus and receiving Peg-IFN/RBV, with baseline LSM ≥7 kPa. The outcome variable was LSM normalization, i.e. a stable decrease in LSM below 7 kPa after starting Peg-IFN/RBV.
*Results:* After starting Peg-IFN/RBV, 56 [35%, 95% confidence interval (95% CI): 28–42%] patients showed LSM normalization. The probability of LSM normalization was 21% (95% CI: 13.2–32.4%) at 12 months, and 51.3% (95% CI: 39.9–63.9%) at 24 months after Peg-INF/RBV initiation for individuals with sustained virological response (SVR), and 8.3% (95% CI: 4–16.6%) at 12 months and 11.3% (95% CI: 6–20.7%) at 24 months for those without SVR ($p < 0.001$). For individuals with LSM ≥7 kPa 24 weeks after the pre-planned end of treatment, LSM normalizations were only observed among those with SVR. Achievement of SVR [Hazard ratio (HR, 95% CI): 6.84 (3.39–13.81)] and lack of baseline cirrhosis [HR (95% CI): 4.17 (1.69–10)] were independently associated with LSM normalization after starting Peg-IFN/RBV.
*Conclusions:* LSM normalizations during Peg-IFN/RBV treatment are more likely, and occur earlier among patients with SVR. In addition, LSM normalizations continue 24 weeks after the scheduled end of therapy, but only among individuals who reach SVR.



## Respuesta viral sostenida con interferón pegilado y ribavirina conduce a la normalización de la rigidez hepática en pacientes infectados por el virus de la hepatitis C

*Palabras clave:*
Hepatitis C
Elastometría transitoria hepática
Respuesta viral sostenida
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RESUMEN

*Introducción:* El retorno de la rigidez hepática (RH) a valores normales en respuesta al tratamiento de la infección por hepatitis C (VHC) con Peg-IFN/RBV no está claro. Por ello, evaluamos la probabilidad y los factores asociados con la normalización de la RH en pacientes tratados con Peg-IFN/RBV.
*Métodos:* Se incluyeron 160 pacientes infectados por VHC en este estudio longitudinal prospectivo, 111 (69%) de ellos por el virus de la inmunodeficiencia humana, con RH basal ≥ 7 kPa y que recibieron Peg-IFN/RBV. La variable principal fue la disminución estable de la RH < 7 kPa.
*Resultados:* Después de iniciar Peg-IFN/RBV, 56 (35%; intervalo de confianza del 95% [IC 95%]: 28–42%) pacientes normalizaron la RH. La probabilidad de la normalización de la RH fue del 21% (IC 95%: 13,2–32,4%) 12 meses y del 51,3% (IC 95%: 39,9–63,9%) 24 meses después de iniciar Peg-IFN/RBV en los pacientes con respuesta viral sostenida (RVS), y del 8,3% (IC 95%: 4–16,6%) 12 meses y del 11,3% (IC 95%: 6–20,7%) 24 meses en los sin RVS (p < 0,001). La normalización de la RH en los pacientes con ≥7 kPa 24 semanas después de finalizar el tratamiento se observó solo en aquellos con RVS. La RVS (hazard

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ratio [HR]: 6,84; IC 95%: 3,39–13,81) y la ausencia de cirrosis [HR (95%IC): 4.17 (1.69–10)] se asociaron independientemente con la normalización de la RH después de iniciar Peg-IFN/RBV.
*Conclusiones:* La normalización de la RH durante la terapia con Peg-IFN/RBV es más probable y ocurre más temprano en los pacientes con RVS; además, continúa 24 semanas después del fin de tratamiento, pero solo en aquellos con RVS.



## Introduction

Chronic hepatitis C virus (HCV) infection is a frequent cause of cirrhosis and hepatocellular carcinoma. Improvement in not only liver fibrosis, even reversal of cirrhosis, but also a lower risk of decompensated liver disease and liver-related death are observed among HCV-infected individuals who attain sustained virological response (SVR) after antiviral therapy.[1–9] Short-term liver fibrosis changes have been mainly assessed with paired biopsies obtained before antiviral treatment and shortly afterwards.[1–3] A single clinical trial has provided data on liver biopsy changes over longer periods of follow-up in patients with advanced liver fibrosis who did not reach SVR.[10] Repeated liver biopsies after antiviral therapy are difficult to obtain outside clinical trials, particularly in patients achieving HCV clearance. This fact accounts for the paucity of data on long-term liver damage changes in patients treated against HCV infection.

On the contrary, liver fibrosis evaluated through liver stiffness measurement (LSM) by transient elastometry is easily repeated.[11] In addition, LSM fulfills other characteristics that make the technique very adequate for follow-up determinations as high reproducibility,[11–14] accurate diagnosis of cirrhosis[11] and good performance to detect and exclude significant fibrosis.[11] Moreover, LSM correlates with the hepatic venous pressure gradient[15] and, as a consequence, can predict esophageal varices[16] and liver-related events.[17–20] Because of this, changes in LSM are important from a clinical point of view, in addition to the variations in histology that they reflect.

Sequential LSM have been applied to follow patients during anti-HCV therapy.[21–24] LSM decrease significantly among patients with chronic HCV infection who achieve SVR with antiviral treatment.[21,23,24] The rates of improvement in fibrosis stage between paired liver biopsies in clinical trials, with second biopsy performed 24 weeks after the end of treatment, are similar to those observed with follow-up LSM.[1–3] However, the time between paired assessments of liver fibrosis has also been short in studies on LSM[21–24] after treatment with pegylated interferon (Peg-IFN) plus ribavirin (RBV). Due to the lack of long-term studies both with biopsies and LSM, there are very limited data on the rates of histology or LSM normalization among patients treated with interferon-based antiviral therapy, since fibrosis regression is a very slow process.[25]

The objective of this study was to assess the probability of return of LSM to normal values in HCV-infected patients who received Peg-IFN plus RBV and the factors associated with LSM normalization.

## Patients and methods

### *Study population and follow-up*

This was a prospective observational longitudinal study. All patients with chronic hepatitis C, with or without human immunodeficiency virus (HIV)-coinfection, followed at the Infectious Diseases Units of the participating hospitals, who fulfilled the following criteria were included in this analysis: (1) treatment with Peg-IFN plus RBV started after January 2006 and pre-planned date of evaluation of SVR before June 2010; (2) baseline LSM equal or greater than 7 kPa; and (3) consented to be followed with serial LSM. Patients were excluded if a LSM were not regarded as valid. LSM during the follow-up coincident with acute ALT elevations ≥200 IU/mL were regarded as not valid, but LSM after the ALT flare was resolved were considered as valid.

Patients were evaluated during the anti-HCV therapy as previously described.[28] SVR was defined as an undetectable serum HCV-RNA 24 weeks after the completion of treatment. All subjects underwent LSM at baseline, 4 weeks, 12 weeks, 24 weeks, and every 24 weeks afterwards, regardless of the treatment response.

### *Treatment regimen*

All individuals received Peg-IFN alfa-2a at a dose of 180 μg per week or Peg-IFN alfa-2b at a dose of 1.5 μg/kg per week plus weight-based RBV, at a dose of 800–1200 mg per day. Programmed treatment length was 48–72 weeks in all individuals infected by HCV genotype 1 or 4. Thus, in patients without complete early virological response, defined as an HCV-RNA above the detection level at week 12, treatment was prolonged to 72 weeks if it was well tolerated and the patient agreed to do it. Individuals with HCV genotype 1 or 4 and rapid virological response were treated for 48 weeks. HCV genotype 3 carriers were treated for 24 weeks, if they were uninfected with HIV. HIV-coinfected patients with genotype 2 or 3 were treated for 48 weeks, except when they showed undetectable HCV-RNA at week 4 of therapy, because in this case therapy was shortened to 24 weeks. HCV therapy was stopped if a reduction of at least 2 logs in HCV-RNA levels was not reached at week 12 or HCV-RNA was still detectable at week 24.

### *Liver stiffness measurement*

Hepatic transient elastography was carried out according to a standardized technique[11] by one experienced operator at each center. Each operator had performed more than 1000 examinations. At least ten acquisitions of LSM were obtained from each patient. LSM were considered as valid if the interquartile range (IQR) was <30% of the median value of LSM and the success rate was ≥60%.[11] LSM were regarded as normal if values were <7 kPa. This value was selected because 95% healthy adults show LSM ≤6.8 kPa[26] and ≤6.7 kPa[27] in two studies. Normalization of LSM was defined as achievement of values <7 kPa in at least two consecutive determinations. Declines in LSM below 7 kPa with subsequent elevation above this value were not considered as normalizations. Subjects with LSM ≥9 kPa were classified as having liver fibrosis extended beyond the portal tracts, i.e. significant fibrosis.[29] Patients with LSM ≥14.6 kPa were classified as individuals with cirrhosis.[30]

### *Laboratory methods*

HCV genotype was determined by line-probe assay (HCV Genotype 2.0 Assay-LIPA, Versant HCV, Siemens Tarrytown, NY, USA). Plasma HCV-RNA load measurements were performed using a quantitative PCR assay according to the available technique (Cobas Amplicor HCV Monitor; Roche Diagnostic Systems Inc., Branchburg, NJ, USA: detection limit of 600 IU/mL; Cobas AmpliPrep-Cobas TaqMan; Roche Diagnostic Systems Inc., Meylan, France: detection

limit of 50 IU/mL; Cobas TaqMan; Roche Diagnostic Systems Inc., Pleasanton, CA, USA: detection limit of 10 IU/mL).

### *Statistical analysis*

The decrease of LSM to values below 7 kPa, i.e. the return of LSM to normal values, from the date of starting therapy with Peg-IFN plus RBV was the primary end-point. For this end-point, the baseline date was the day of starting Peg-IFN plus RBV. The normalization of LSM after the SVR assessment date among those patents with LSM ≥7 kPa at that date was the secondary end-point. The baseline date for the secondary end-point was the day of SVR assessment. The time to the event was the length of time since baseline until the first date reaching LSM <7 kPa in a subject with confirmed LSM normalization in a subsequent determination. For patients who did not reach the LSM below 7 kPa during the follow-up, the analysis was censored at the date of their last LSM. The associations between the time to the normalization of LSM and the following factors were examined: age, gender, route of acquisition of HCV infection, alcohol consumption, HCV genotype, baseline HCV viral load, HIV coinfection, ALT serum levels at baseline, type of Peg-IFN, daily dose of RBV, achievement of SVR, and baseline LSM. The time to the normalization of LSM was evaluated using Kaplan–Meier curves. Survival curves were compared applying the log-rank test. Continuous variables were dichotomized by their median value for this analysis, except for LSM. Factors with a level of association $p \leq 0.2$ in univariate analyses were included in Cox's regression models. Proportional hazard assumptions were checked by use of Schoenfeld's residuals. Adjusted associations with $p \leq 0.05$ were considered as significant. Data were analyzed using SPSS 15.0 (SPSS Inc., Chicago, IL, USA) and Stata SE 9.0 (Statacorp, College Station, TX, USA).

### *Ethical aspects*

This study has been designed and performed according to the Helsinki declaration and was approved by the Ethics Committee of Hospital Universitario de Valme.

## Results

### *Characteristics of the study population*

One hundred and seventy-nine patients were selected because they fulfilled the inclusion criteria. Fourteen (7.8%) individuals were lost due to follow-up and one (0.6%) patient died. Four (2.2%) patients were excluded due to a non-valid LSM, two of them by each operator. Finally, 160 (89%) patients were analyzed. In this group, there were 111 (69%) HIV-infected patients, 109 (98%) of whom were on antiretroviral therapy at baseline. The baseline characteristics of the study patients are shown in Table 1. The median (interquartile range, IQR) length of Peg-IFN plus RBV treatment was 8 (5–12) months.

### *Treatment outcomes*

Globally, 73 (46%) patients reached SVR, 27 (55%) were HCV-monoinfected and 45 were (41%) HIV/HCV-coinfected individuals. Among patients without SVR, 16 (10%) relapsed, 49 (30%) showed non-response and 4 (2.5%) a viral breakthrough. In addition, 8 (5%) subjects discontinued treatment due to adverse events and 10 (6.3%) voluntarily dropped-out. SVR was achieved by 40 (36%) individuals with genotype 1 or 4 and 32 (67%) with genotype 2 or 3.

**Table 1**
Characteristics of 160 HCV-infected patients with liver stiffness equal to or greater than 7 kPa at starting pegylated-interferon plus ribavirin.

| Variable | Value |
|---|---|
| *Age (years)*[a] | 43 (40–75) |
| *Male gender, n (%)* | 133 (83) |
| *Intravenous drug users, n (%)* | 132 (83) |
| *Alcohol intake ≥50 g/day, n (%)* | 26 (16) |
| *HBsAg positive, n (%)* | 4 (2.5) |
| *HCV genotype, n (%)* | |
| 1 | 95 (59) |
| 2 | 2 (1.3) |
| 3 | 47 (29) |
| 4 | 16 (10) |
| *$Log_{10}$ plasma HCV RNA (IU/mL)*[a] | 6.18 (5.56–6.71) |
| *ALT (IU/mL)*[a] | 58 (36–84) |
| *AST (IU/mL)*[a] | 56 (37–85) |
| *LSM*[b] *(kPa)* | 10.4 (8.5–17.3) |
| *Significant fibrosis*[c]*, n (%)* | 109 (68) |
| *Cirrhosis*[d]*, n (%)* | 50 (31) |
| *Type of pegylated-interferon, n (%)* | |
| Alfa-2a | 129 (81) |
| Alfa-2b | 31 (19) |
| *Ribavirin dose, n (%)* | |
| 800 mg/day | 18 (11) |
| 1000 mg/day | 85 (53) |
| 1200 mg/day | 57 (36) |

[a] Median (IQR).
[b] LSM, liver stiffness measurement.
[c] Significant fibrosis, LMS ≥9 kPa.
[d] Cirrhosis, LSM ≥ 14.6 kPa.

### *Rate of liver stiffness measurement normalization*

After starting Peg-IFN plus RBV, 56 [35%, 95% confidence interval (95% CI): 28–42%] patients showed stable reductions in LSM below 7 kPa during a median (IQR) follow-up of 26 (18–36) months. The probability of LSM normalization after Peg-IFN plus RBV initiation was 14.1% (95% CI: 9.5–20.6%) at 12 months and 30.4% (95% CI: 23.5–38.9%) at 24 months.

LSM normalization was detected during the follow-up in 26 (25%) of 104 patients with LSM ≥9 kPa at starting Peg-IFN plus RBV. Among individuals with LSM ≥ 9 kPa at starting therapy but without LSM normalization at the date of SVR evaluation, 9 (16%) of 55 patients with follow-up after the SVR time point of assessment showed decreases below 7 kPa. Six (12%) of 50 subjects with LSM ≥ 14.6 kPa at starting therapy showed LSM normalizations during the follow-up. Among these six cirrhotic patients with LSM normalizations, LSM reductions below 7 kPa were observed in five after the SVR date of evaluation.

### *Liver stiffness normalization after sustained virological response*

At the date of SVR assessment, 72 (45%) individuals had LSM ≥ 7 kPa. Among them, decreases of LSM below 7 kPa were observed in 12 (17%, 95% CI: 8–25%) for a median (IQR) length of follow-up after the SVR time point of evaluation of 12 (12–24) months. The probability of LSM normalization after the SVR date of assessment was 18.4% (95% CI: 10–32.3%) at 12 months and 27.8% (95% CI: 16.5–44.4%) at 24 months.

### *Factors associated with normalization of liver stiffness measurement*

Normalization of LSM after starting Peg-IFN plus RBV was significantly associated with achievement of SVR (Fig. 1). The probability of LSM normalization after Peg-IFN plus RBV initiation was 21%

**Table 2**
Predictors of liver stiffness measurement normalization, i.e. reduction below 7 kPa, after starting pegylated-interferon plus ribavirin treatment in HCV-infected patients.

| Variable | Patients with LSM[a] normalization *n* (%) | *p* univariate | Hazard ratio (95% CI)[b] | *p* multivariate |
|---|---|---|---|---|
| *Gender* | | 0.808 | | |
| Male | 46 (35) | | | |
| Female | 10 (37) | | | |
| *Risk group* | | 0.166 | | |
| IDU | 42 (32) | | 0.81 (0.39–1.69) | 0.575 |
| Non-IDU | 14 (50) | | | |
| *HIV infection* | | 0.023 | | |
| Yes | 33 (30) | | 1.22 (0.62–2.42) | 0.568 |
| No | 23 (47) | | | |
| *Alcohol intake* | | 0.138 | | |
| <50 g/day | 49 (37) | | | |
| ≥50 g/day | 7 (27) | | 0.60 (0.27–1.33) | 0.208 |
| *Baseline ALT* | | 0.827 | | |
| <59 IU/ml | 28 (33) | | | |
| ≥59 IU/ml | 28 (37) | | | |
| *Baseline LSM*[a] | | <0.001 | | |
| <14.6 kPa | 50 (45) | | | |
| ≥14.6 kPa | 6 (12) | | 0.24 (0.10–0.59) | 0.002 |
| *Baseline $log_{10}$ HCV RNA* | | 0.111 | | |
| <6.2 IU/ml | 24 (30) | | | |
| ≥6.2 IU/ml | 32 (40) | | 1.45 (0.84–2.52) | 0.187 |
| *HCV genotype* | | 0.738 | | |
| 1 or 4 | 40 (36) | | | |
| 2 or 3 | 16 (33) | | | |
| *Type of Peg-IFN*[c] | | 0.853 | | |
| Alfa-2a | 46 (36) | | | |
| Alfa-2b | 10 (32) | | | |
| *SVR*[d] | | <0.001 | | |
| Yes | 46 (64) | | 6.84 (3.39–13.81) | <0.001 |
| No | 10 (11) | | | |

[a] LSM, liver stiffness measurement.
[b] 95% CI, 95% confidence interval.
[c] Peg-IFN, pegylated-interferon.
[d] SVR, sustained virological response.

(95% CI: 13.2–32.4%) at 12 months and 51.3% (95% CI: 39.9–63.9%) at 24 months for individuals with SVR, and 8.3% (95% CI: 4–16.6%) at 12 months and 11.3% (95% CI: 6–20.7%) at 24 months for patients without SVR ($p < 0.001$). Normalization of LSM after starting Peg-IFN plus RBV was also associated with baseline cirrhosis (Table 2).

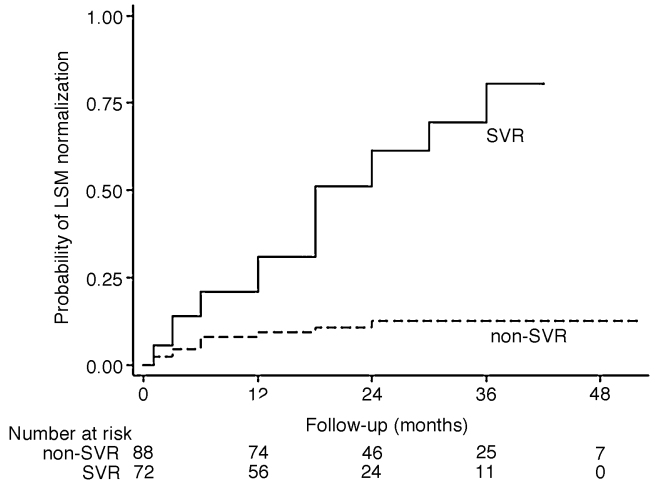


**Fig. 1.** Probability of liver stiffness measurement normalization, i.e. reduction below 7 kPa, after starting pegylated-interferon plus ribavirin treatment in HCV-infected patients. Patients with sustained virological response (SVR): solid line; patients without SVR: dashed line.

After multivariate analysis, achievement of SVR and lack of baseline cirrhosis were independently associated with normalization of LSM after starting Peg-IFN plus RBV (Table 2).

Among 72 patients with LSM ≥ 7 kPa at the SVR evaluation date and follow-up afterwards, none of those without SVR reached LSM normalization. For individuals with SVR, LSM reductions below 7 kPa were observed in 12 (50%) of them. The probability of LSM normalization was 48.7% (95% CI: 29–73%) 12 months after SVR in these individuals. Among 48 individuals without LSM normalization at the SVR assessment date, four (8.3%) decompensations of cirrhosis were observed during the follow-up. All of them showed baseline LSM ≥ 14.6 kPa and none had achieved SVR. No patient with LSM normalization developed liver events.

## Discussion

LSM normalizations during Peg-IFN plus RBV treatment are significantly more likely and occur earlier among patients with SVR. In addition, LSM normalizations continue beyond 24 weeks after the scheduled end of therapy only among individuals who reach SVR. Patients with baseline cirrhosis are more unlikely to return to normal LSM. Moreover, individuals with cirrhosis normalize LSM mainly after SVR is achieved. Thus, sequential LSM could be useful to follow patients after SVR is attained.

In the present study, about one third of the patients normalized LSM after starting Peg-IFN plus RBV. Independent predictors of LSM normalization were achievement of SVR and absence of

baseline cirrhosis. During the follow-up after the SVR evaluation date, LSM normalizations were only detected in subjects with SVR. Thus, no significant benefit could be observed among patients without SVR after completing therapy. This was particularly important for patients with baseline cirrhosis. A small proportion of them showed reductions below 7 kPa after starting Peg-IFN plus RBV. All patients with cirrhosis and normalization of LSM had attained SVR. This result is in agreement with previous data on paired liver biopsies in patients receiving anti-HCV therapy.[1] In the present study, most LSM normalizations among patients with cirrhosis were seen throughout the follow-up after reaching SVR.

The LSM threshold that we selected to define normal LSM values was derived from two large studies on healthy adults.[26,27] One of them examined individuals attending a systematic free medical check-up[26] and the other blood donors.[27] Similar median LSM values were reported in both studies.[26,27] We chose the value of 7 kPa because it was close to the 95th percentile value in both studies.[26,27] The use of a stringent category to define the outcome variable, as LSM below 7 kPa, is a strength of the present study. Small decreases in LSM 24 weeks after the scheduled end of treatment date have been reported.[21–23] The clinical meaning of small variations in LSM is not clear. On the contrary, reductions of LSM below values seen in the majority of healthy adults are more easily interpretable. Indeed, since lower values of LSM are associated with lower portal pressure,[15] reduced likelihood of esophageal varices[16] and, in turn, a smaller rate of clinical events,[17–19] it is conceivable that normal LSM could be associated with no further risk of liver-related complications. In the present study, we found that liver decompensations were only observed in the short-term among individuals with cirrhosis without LSM normalization. However, the relationship between LSM decreases to normal values and clinical outcomes needs to be investigated.

Higher levels of liver inflammation may increase LSM to some extent.[11,31] Given that antiviral treatment improves both liver fibrosis and inflammatory activity, improvements in liver inflammation during treatment might also decrease LSM. In the present study, baseline ALT levels showed no association with LSM reductions below 7 kPa. Due to this, baseline ALT levels and, hence, in initial liver inflammatory activity, was not a confounder for LSM normalization. This suggests that decreases in LSM actually reflect improvements in fibrosis, rather than reductions in liver inflammation.

This study has a few limitations. First, the proportion of patients who end up reaching normal LSM values is likely to be higher than that found herein, especially for patients with cirrhosis at baseline. Surely, a much longer follow-up is needed to see more cases of cirrhosis reversion. However, we provide relevant data on LSM normalization in patients receiving Peg-IFN plus RBV therapy. Thus, this study clearly shows that patients without SVR have few chances of LSM normalization after starting antiviral treatment. For patients with SVR and with LSM equal or greater than 7 kPa at 24 weeks after the end of therapy, the likelihood of LSM normalization after two years of follow-up is nearly 70%. Individuals with cirrhosis and SVR do show reversions of cirrhosis, but clearly a longer follow-up is needed for them. Second, the study population was not homogeneous, as it was composed of HCV-infected individuals with and without HIV coinfection. HIV infection was associated with a lower probability of LSM normalization. However, this association was confounded by the lower rates of SVR and higher proportion of subjects with cirrhosis among HIV-coinfected patients. After adjustment in the multivariate analysis, SVR and cirrhosis were the only independent predictors of LSM normalization and HIV-coinfection did not remain associated with LSM return to normality anymore.

In conclusion, LSM normalization is a continuous phenomenon during Peg-IFN plus RBV treatment and afterwards in patients who attain SVR. This fact led us to believe that eventually most, if not all, patients with SVR will achieve a normal LS, if sufficient time goes by, perhaps as an expression of a normalization in liver histology. Patients without SVR do not show LSM normalization after the SVR evaluation time point. If normal LSM were associated with lack of liver complications in patients with SVR, sequential LSM could be used to identify patients with and without continued risk of liver decompensations after anti-HCV therapy. In this way, repeated LSM could aid targeting screening and preventive measures at those patients who previously had LS indicative of cirrhosis.

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## Conflict of interest

Juan A. Pineda has carried out consultancy work for GlaxoSmithKline, Bristol-Myers Squibb, Abbott Pharmaceuticals, Gilead, Merck Sharp and Dome, Schering-Plough, Janssen Cilag and Boehringer Ingelheim. He has received research grants from GlaxoSmithKline, Roche, Bristol-Myers Squibb, Schering-Plough, Abbott Pharmaceuticals y Boehringer Ingelheim and financial compensation for conferences by GlaxoSmithKline, Roche, Abbott Pharmaceuticals, Bristol-Myers Squibb, Gilead, Merck Sharp and Dome, Janssen Cilag, Boehringer Ingelheim and Schering-Plough.

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