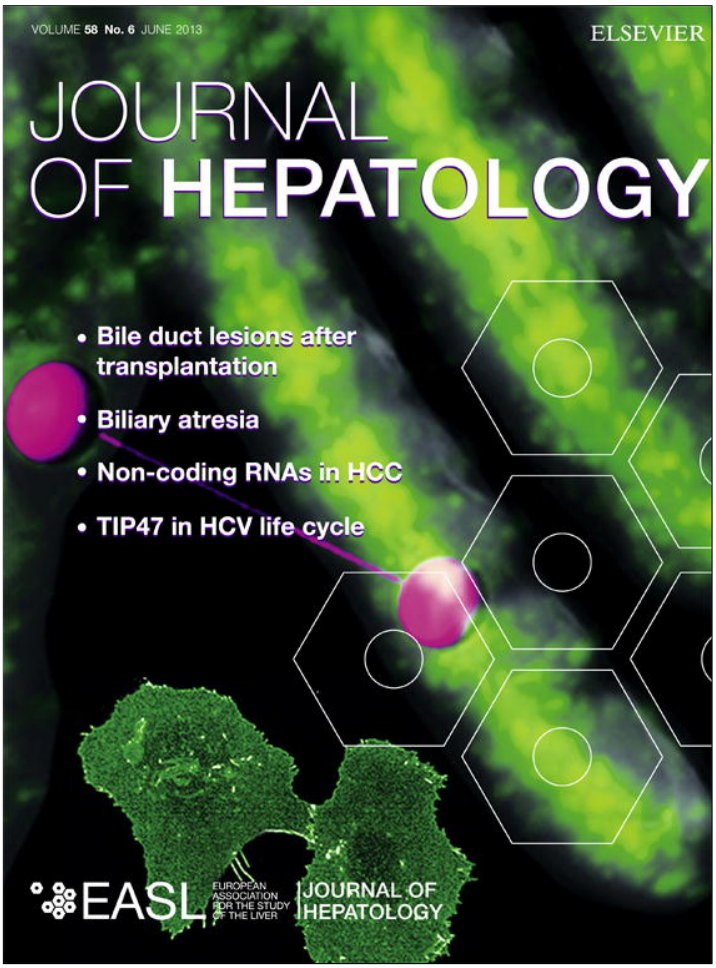

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Clinical effects of viral relapse after interferon plus ribavirin in patients co-infected with human immunodeficiency virus and hepatitis C virus

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Background & Aims: Sustained viral response (SVR) after therapy with interferon-ribavirin (IF-RB) reduces liver-related (LR) complications and mortality in HIV/HCV-co-infected patients. Here, we assess the impact of end-of-treatment response with subsequent relapse (REL) on LR events (LR death, liver decompensation, hepatocellular carcinoma, or liver transplantation), and liver stiffness (LS) by transient elastography.

Methods: We analyzed the GESIDA 3603 Cohort (HIV/HCV-co-infected patients treated with IF-RB in 19 centers in Spain). Response to IF-RB was categorized as SVR, REL, and no response (NR). The study started when IF-RB was stopped and ended at death or the last follow-up visit. Multivariate regression analyses were adjusted for age, sex, HIV category of transmission, CDC clinical category, nadir CD4+ cell count, HCV genotype, HCV-RNA viral load, and liver fibrosis.

Results: Of 1599 patients included, response was categorized as NR in 765, REL in 250 and SVR in 584. Median follow-up was more than 4 years in each group. Taking the group of patients with NR as reference, we found that the adjusted hazard ratios (95% confidence interval) of liver-related events (liver-related death, liver decompensation, hepatocellular carcinoma, liver transplantation) for patients with REL and for patients with SVR were 0.17 (0.05; 0.50) and 0.03 (0; 0.20), respectively. We also found that SVR was followed by less liver stiffness than both REL and NR. However, REL was associated with less liver stiffness than NR.

Conclusions: Best outcomes were achieved with an SVR. However, REL was associated with less LR mortality, decompensation, and liver stiffness than NR.

Keywords: HIV Infections/complications/drug therapy; Hepatitis C chronic/complications/drug therapy; Interferons/administration and dosage/therapeutic use; Follow-up studies; Treatment outcome.

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Abbreviations: HIV, human immunodeficiency virus; HCV, hepatitis C virus; cART, combination antiretroviral therapy; GESIDA, Grupo de Estudio del SIDA de la Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica (AIDS Study Group of the Spanish Society of Infectious Diseases and Clinical Microbiology); SEIMC, Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica (Spanish Society of Infectious Diseases and Clinical Microbiology); APRI, aspartate aminotransferase to platelet ratio index; ALT, alanine aminotransferase; IDU, injection drug use; CDC, Centers for Disease Control and Prevention; APRICOT, AIDS Pegasys Ribavirin International Co-infection Trial; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; IQR, interquartile range; HAI, histological activity index; sICAM-1, soluble intercellular adhesion molecule 1; sVCAM-1, soluble vascular cell adhesion molecule 1.

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Introduction

Human immunodeficiency virus (HIV) infection modifies the natural history of hepatitis C virus (HCV) infection, promoting more rapid progression to fibrosis and development of cirrhosis and end-stage liver disease [1]. Despite a decline in morbidity and mortality from opportunistic infections since the introduction of combination antiretroviral therapy (cART), liver disease secondary to HCV has emerged as a major cause of morbidity and mortality in HIV-infected persons [2].

The primary objective of hepatitis C treatment is the achievement of sustained viral response that is equivalent to the eradication of HCV infection [3]. Well-established benefits of sustained viral response include improvements in liver histology [4–6] and reversion of cirrhosis in some patients [6–8]; improved survival and reductions of liver decompensation in patients with advanced fibrosis or cirrhosis [9–12]; and reduction in the incidence of HCC in patients with HCV-related liver cirrhosis [13]. In HIV-infected patients with chronic hepatitis C, we previously showed that sustained viral response after therapy with interferon plus ribavirin reduces liver-related complications and mortality [14], as well as HIV progression and mortality not related to liver disease [15].

A significant proportion of patients with hepatitis C receiving interferon plus ribavirin achieve suppression of HCV viremia while on treatment, but experience a viral relapse after the anti-HCV therapy is interrupted. In HCV-monoinfected patients treated with pegylated interferon plus ribavirin, viral relapse can occur between 20% to 31.5% of those infected with HCV genotype 1 [16] and between 3% to 13% of those infected with HCV genotypes 2 or 3 treated for 24 weeks [17]. Similar rates of viral relapse after pegylated interferon plus ribavirin have been observed among HIV/HCV co-infected patients [18–20].

Little is known about the long-term clinical consequences of achieving an end-of-treatment response with subsequent viral relapse after treatment with interferon plus ribavirin. Our aim was to assess the impact of viral relapse on mortality and liver-related events in HIV/HCV-co-infected patients.

Materials and methods

Design and patient selection

The patients in this study were selected from the cohort of the 'Grupo de Estudio del SIDA' (AIDS Study Group, GESIDA) of the 'Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica' (Spanish Society of Infectious Diseases and Clinical Microbiology, SEIMC). This cohort was composed of patients naïve to anti-HCV therapy treated with interferon and ribavirin and was established in 2003 to follow HIV/HCV-co-infected patients who started therapy with these drugs between January 2000 and January 2008, at 19 institutions in Spain. The primary objective of this cohort study was to determine the effect of treatment response after therapy with interferon and ribavirin on the long-term clinical outcomes, including liver-related complications and liver-related mortality, of co-infected patients. The local ethics committees approved the analysis of anonymous routine clinical data without written informed consent with a view to scientific publication.

Anti-HCV therapy in Spain is provided by hospital pharmacies and is covered by the National Health System. The decision to administer interferon and ribavirin to co-infected patients was taken by infectious diseases physicians at each institution, according to national and international guidelines. The eligibility criteria for interferon and ribavirin therapy included absence of prior hepatic decompensation, CD4+ cell count >200 cells/μl, stable antiretroviral therapy or no need for antiretroviral therapy, absence of active opportunistic infections, and no active drug addiction. Patients were counseled against the use of alcohol. Anti-HCV therapy was stopped in all patients with detectable HCV-RNA at week 24 of treatment. Since 2002, anti-HCV was also stopped in patients with detectable HCV-RNA at week 12 of treatment and a reduction of <2 log IU/ml in HCV-RNA.

Investigations

All the information was entered directly into a common database at each institution by trained personnel using an online application that satisfied local requirements of data confidentiality. This database included all demographic, clinical, virological (HIV and HCV), and laboratory data. All the centers included in the cohort were monitored to verify that all the information in the database was consistent with the patient's medical history.

For each patient, we extracted the following data from the central database: age, sex, height and weight at the initiation of therapy, HIV transmission category, prior AIDS-defining conditions, baseline and nadir CD4+ cell counts, and baseline HIV viral load. We also recorded information about cART; including type, date of initiation, and whether or not it was maintained or changed during therapy. Information related to HCV infection included genotype, HCV-RNA levels, and estimated year of HCV infection (assumed to start the first year needles were unsafely shared in the case of injection drug users). Duration of HCV infection was considered to be unknown for patients infected through sexual contact. Patients were asked about their current alcohol intake. We considered the consumption of more than 50 g of alcohol per day for at least 12 months as a high intake.

Local pathologists, who all had extensive experience in scoring samples from patients with viral hepatitis, scored liver biopsy samples following the criteria established by the METAVIR Cooperative Study Group [21] as follows: F0, no fibrosis; F1, portal fibrosis; F2, periportal fibrosis or rare portal–portal septa; F3, fibrous septa with architectural distortion and no obvious cirrhosis (bridging fibrosis); and F4, definite cirrhosis. Diagnosis of liver fibrosis was also estimated using the aspartate aminotransferase to platelet ratio index (APRI) test, a non-invasive index developed in HCV-monoinfected patients [22] that has been validated in co-infected patients [23].

Assessment of response to interferon plus ribavirin

Response to interferon plus ribavirin therapy was classified in three categories: sustained viral response, defined as an undetectable serum HCV-RNA level 24 weeks after discontinuation of therapy; viral relapse defined as an undetectable serum HCV-RNA level at the end of programmed therapy (48 weeks), with subsequent relapse; and no response, when patients did not fulfill sustained viral response or viral relapse criteria. Safety was assessed by laboratory tests and evaluation of clinical adverse events during therapy.

Follow-up

Completion of treatment was followed by active monitoring every 6 months to analyze clinical and laboratory parameters, including survival, presence of liver decompensation, antiretroviral therapy, CD4+ cell count, HIV viral load, HCV-RNA, and assessment of liver fibrosis. The length of the study was calculated from the date interferon plus ribavirin was stopped to death or the last follow-up visit. The administrative censoring date was July 31, 2010.

Clinical end points

We assessed the following clinical end points: (a) liver-related complications, including ascites, hepatic encephalopathy, variceal bleeding, hepatocellular carcinoma, and liver transplantation. Ascites was confirmed by paracentesis and/or ultrasound. Hepatic encephalopathy was established on clinical grounds after the reasonable exclusion of HIV-associated encephalopathy based on clinical and laboratory parameters (i.e., CD4+ cell counts, HIV viral load, and neuroimaging techniques). The source of gastroesophageal bleeding was confirmed by endoscopy, whenever possible. For patients who had more than 1 event, only the first was included in the analyses of the association between sustained viral response and "any event"; and (b) mortality (death reports, autopsy reports (if available), and standard forms were requested). All the information related to death (death reports, autopsy reports (if available), and standard forms) was reviewed by JB and JGG. Both authors were blind to the category of treatment response and classified deaths in accordance with the opinion of the attending clinician as follows: (i) liver-related death, when the train of events that ended in death was caused by liver decompensation or hepatocellular carcinoma; (ii)

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Table 1. Characteristics of 1599 HIV/HCV+ patients stratified according to response to interferon plus ribavirin.

Characteristic	No response (n = 765)	Viral relapse (n = 250)	Sustained viral response (n = 584)	Total (n = 1599)
Male sex - n (%)	583 (76.3)	180 (72.3)	431 (73.9)	1194 (74.8)
Age - yr, median (IQR) (Baseline)	40.2 (37; 43.5)	40.1 (36.6; 42.9)	40.3 (36.6; 43.3)	40.2 (36.7; 43.3)
Weight - kg, median (IQR)	68 (60; 75)	69 (60; 77)	68 (60; 75)	68 (60; 75)
Prior injection drug use - n (%)	612 (80.7)	199 (79.9)	476 (82.2)	1287 (81.1)
CDC disease category - n (%)*				
A	353 (47.4)	129 (52.4)	326 (57.1)†	808 (51.7)
B	207 (27.8)	58 (23.6)	134 (23.5)	399 (25.5)
C	185 (24.8)	59 (24.0)	111 (19.4)	355 (22.7)
CD4+ cells nadir-n/mm^3, median (IQR)	200 (104; 310)	215 (120; 330)	218 (118; 334)	209 (110; 326)
CD4+ cells baseline-n/mm^3, median (IQR)	510 (365; 708)	546.5 (419; 741)	529 (402; 731)	527 (391; 723)
HIV-RNA <50 copies/ml baseline - n (%)	493 (65.8)	179 (73.4)	419 (73.9)†	1091 (69.9)
Duration of HCV infection, median (IQR)	18 (13; 22)	19 (14; 22)	18 (14.5; 22)	18 (13; 22)
HCV genotype - n (%)**				
1 or 4	606 (81.8)	141 (57.8)†	235 (41.3)†‡	982 (63.2)
2 or 3	135 (18.2)	103 (42.2)†	334 (58.7)†‡	572 (36.8)
HCV-RNA ≥500,000 IU/ml - n (%)#	512 (78.8)	146 (67.9)	321 (60.9)†‡	979 (70.3)
METAVIR fibrosis score - n (%)¶				
F ≤2 - n (%)	318 (54.8)	106 (59.9)	281 (71.0)†‡	705 (61.1)
F ≥3 - n (%)	262 (45.2)	71 (40.1)	115 (29.0)†‡	448 (38.9)
Advanced fibrosis (F ≥3 or APRI >2) - n (%) ¶¶				
Yes	304 (43.3)	83 (37.6)	146 (28.3)†‡	533 (37.0)
No	398 (56.7)	138 (62.4)	370 (71.7)†‡	906 (63.0)
HBsAg - n (%)				
Positive	34 (4.5)	4 (1.6)	17 (2.9)	55 (3.5)
Negative	694 (92.3)	235 (95.5)	550 (94.8)	1479 (93.7)
Unknown	24 (3.2)	7 (2.8)	13 (2.2)	44 (2.8)
Current alcohol intake >50 mg/d - n (%)	46 (6.9)	14 (6.7)	16 (3.1)†	76 (5.4)
Current methadone use - n (%)	102 (15.1)	38 (16.4)	50 (9.7)†‡	190 (13.3)
Months in HCV treatment, median (IQR)	6.6 (4.4; 10.6)	11.1 (7.2; 12.0)†	11.1 (9.0; 12.0)†	9.9 (5.9; 11.7)

CDC, Centers for Disease Control and Prevention; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; IQR, interquartile range.
*A, asymptomatic, acute HIV, or persistent generalized lymphadenopathy; B, symptomatic, conditions not C, C, AIDS-indicator conditions.
**HCV genotype was determined in 1554 patients.
#HCV-RNA was determined in 1392 patients.
¶Assessment of baseline fibrosis by liver biopsy was performed in 1153 patients.
¶¶Assessment of baseline fibrosis by liver biopsy or APRI score was performed in 1439 patients.
†p <0.05 with the group 'No response'.
‡p <0.05 with the group 'Viral relapse'.

AIDS-related death, when death was directly related to an AIDS-defining condition; and (iii) non-liver-related non-AIDS-related deaths. For patients who had more than 1 event, only the first was included in the analyses of the association between category of response and "any event".

Liver stiffness following interferon plus ribavirin

Liver stiffness by transient elastography was measured in some patients, depending on hospital availability, following interferon plus ribavirin treatment. Transient elastography was performed using a FibroScan® device (EchoSens, Paris, France). A median value, expressed in kilopascals (kPa), of 10 successful acquisitions was considered the representative measurement of liver stiffness. We considered 10 acquisitions with a success rate ⩾60% and an IQR <30% of the median value as representative measurements [24]. The transient elastography cut-off values for each stage of fibrosis (METAVIR) were as follows: minimal fibrosis (F0–F1) ⩽7 kPa; moderate fibrosis (F2) ⩾7.1 kPa and <9.5 kPa; advanced fibrosis (F ⩾3) ⩾9.5 kPa; cirrhosis (F4) ⩾14.5 kPa [24].

Statistics

Differences between groups were analyzed using the Chi-square test, t test, or Mann–Whitney test, as appropriate. Analysis of normality was performed with the Kolmogorov–Smirnov test. Logistic regression models were used to explore baseline factors predicting sustained viral response. We calculated the frequency, incidence rate, and survival function (Kaplan–Meier) for the different end points. Multivariate analysis was performed using Cox regression analysis. The statistical analysis was performed using SPSS 18.0 (SPSS Inc., Chicago, Illinois, USA).

As several patients underwent retreatment with interferon plus ribavirin, we performed three types of analysis: (a) in the first one, that was the primary analysis, the follow-up of retreated patients was censored the same day of initiation of the second course with interferon plus ribavirin, (b) in the second analysis, those who achieved sustained viral response after retreatment were included in the sustained viral response group, (c) in the third analysis, patients that were retreated were excluded from the analysis.

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Table 2. Frequency and rate of events during follow-up in 1599 HIV/HCV+ patients stratified according to response to interferon plus ribavirin.

Event	Frequency of events - N° (%)			Rate/100 person-yr (95% CI)		
	NR (n = 765)	REL (n = 250)	SVR (n = 584)	NR (n = 765)	REL (n = 250)	SVR (n = 584)
Lost to follow-up - n (%)	169 (22.1)	35 (14)*	63 (10.8)*	4.65 (3.95-5.36)	3.16 (2.11-4.21)*	2.22 (1.67-2.77)*
Overall mortality - n (%)	69 (9)	7 (2.8)*	8 (1.4)*	1.9 (1.45-2.35)	0.63 (0.16-1.1)*	0.28 (0.09-0.48)*
Liver-related - n (%)	47 (6.1)	1 (0.4)*	3 (0.5)*	1.29 (0.9-1.66)	0.09 (0-0.27)*	0.11 (0-0.23)*
AIDS-related - n (%)	3 (0.4)	2 (0.8)	0 (0)	0.08 (0-0.18)	0.18 (0-0.43)	0 (0-0)*
Non-liver-related non-AIDS-related - n (%)	19 (2.5)	4 (1.6)	5 (0.9)	0.52 (0.29-0.76)	0.36 (0.01-0.71)	0.18 (0.02-0.33)*
CDC category C disease - n (%)	26 (3.4)	11 (4.4)	7 (1.2)*†	0.73 (0.45-1)	1.01 (0.41-1.61)	0.25 (0.06-0.43)*†
Liver decompensation - n (%)	93 (12.2)	9 (3.6)*	5 (0.9)*†	2.7 (2.15-3.25)	0.82 (0.29-1.36)*	0.18 (0.02-0.33)*†
Hepatocarcinoma - n (%)	21 (2.8)	2 (0.8)	2 (0.3)*	0.58 (0.33-0.83)	0.18 (0-0.43)	0.07 (0-0.17)*
Liver transplantation - n (%)	18 (2.4)	2 (0.8)	3 (0.5)*	0.5 (0.27-0.73)	0.18 (0-0.44)*	0.11 (0-0.23)*

SVR, sustained virologic response; REL, end-of-treatment response with subsequent relapse; NR, no response; CDC, Centers for Disease Control and Prevention.
Median follow-up times in months (interquartile range) for NR, REL, and SVR were 57.9 (36.7; 77.6), 52.3 (34.2; 71.4), and 56.4 (37.8; 77.4) respectively.
*p <0.05 with the group 'NR'.
†p <0.05 with the group 'ETR'.

Results

Patient characteristics

The data of 1599 patients who started treatment between January 2000 and January 2008 were included in the database. Their baseline characteristics are shown in Table 1. In brief, 74.8% were male, the median age was 40.2 years, 22.7% had prior AIDS-defining conditions, the median baseline CD4 cell count was 527 cells/mm^3, 69.9% had an undetectable HIV viral load, 63.2% were infected by genotypes 1 or 4, 70.3% had an HCV-RNA ⩾500,000 IU/ml. Baseline liver biopsy was performed in 1153 patients and 448 (38.9%) had bridging fibrosis or cirrhosis. Assessment of baseline fibrosis by liver biopsy or APRI score was performed in 1439 patients and 553 (37.0%) were considered to have advanced fibrosis. A total of 790 (49%) patients were treated with PegIFN-α2a plus ribavirin, 602 (38%) were treated with PegIFN-α2b plus ribavirin, and 207 (13%) were treated with standard thrice-weekly IFN-α plus ribavirin.

During treatment of hepatitis C, 1262 (79%) patients were on cART. The most common combinations were 2 NRTI plus 1 NNRTI in 573, 2 NRTI plus one PI in 386, 3 NRTI in 89, and other combinations in 214 cases.

Treatment response

Treatment response was categorized as sustained viral response in 584 (36%) patients; sustained viral response was observed in 235 (24%) cases for those infected by genotypes 1 or 4, and 334 (58%) cases for those infected by genotypes 2 or 3 (Table 1).

Treatment response was categorized as viral relapse in 250 (16%) patients; viral relapse was observed in 141 (14%) cases for those infected by genotypes 1 or 4, and 103 (18%) cases for those infected by genotypes 2 or 3 (Table 1).

Treatment response was categorized as no response in 765 (48%) patients; no response was observed in 606 (62%) cases for those infected by genotypes 1 or 4, and 135 (24%) cases for those infected by genotypes 2 or 3 (Table 1).

Of the 1599 patients, 174 (11%) received a second course of interferon plus ribavirin and 43 (25%) achieved sustained viral response after the second course of anti-HCV treatment.

We developed a multiple logistic regression analysis model to identify pretreatment factors that were predictive of sustained viral response. The model included baseline factors that were associated with sustained viral response by univariate regression analysis: prior AIDS-defining conditions, nadir CD4+ cell count, HCV genotype, and HCV-RNA level. It also included the type of interferon used (pegylated *vs.* non-pegylated), the presence or not of advanced fibrosis (F ⩾3 or APRI >2), and alcohol intake higher than 50 g per day. The model did not include variables not achieving a significance threshold by univariate analysis. The final model identified 5 variables that were independently associated with increased odds of sustained viral response: interferon pegylated (OR, 1.64; 95% CI, 1.08–2.51; p = 0.022, HCV genotype 2 or 3 (OR, 4.41; 95% CI, 3.34–5.82; p <0.001), HCV-RNA level <500,000 IU/ml (OR, 1.75; 95% CI, 1.31–2.33; p <0.001), non-advanced fibrosis (OR, 1.84; 95% CI, (1.38–2.46); p <0.001) and absence of current alcohol intake higher than 50 g per day (OR, 2.17; 95% CI, (1.07–4.42); p = 0.033).

Clinical outcomes

The median follow-up in months (interquartile range), since the date interferon plus ribavirin was stopped for non-responders, relapsers, and responders, was 57.9 (36.7; 77.6), 52.3 (34.2; 71.4), and 56.4 (37.8; 77.4). The frequencies and rates of events during follow-up stratified by response to interferon plus ribavirin are shown in Table 2; the main findings can be summarized as follows:

Mortality: Overall mortality rates and liver-related mortality rates were significantly less in patients with sustained viral response and those with viral relapse in comparison with patients with no response. AIDS-related and non-liver-related, non-AIDS-related mortality rates were significantly less in patients with sustained viral response in comparison with patients with no response.

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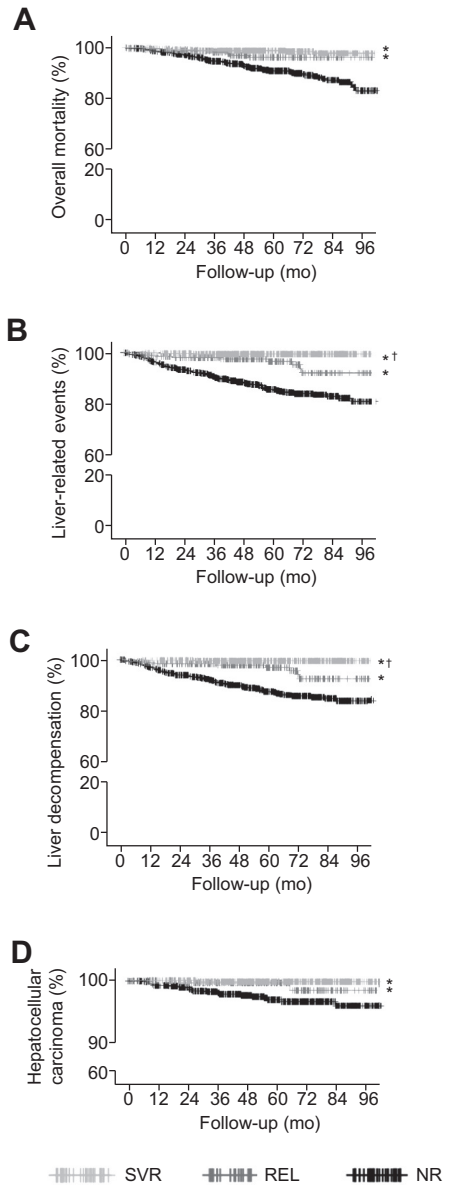

Fig. 1. Proportion of patients free from events. (A) Overall mortality, (B) liver-related events (liver-related mortality, liver decompensation, hepatocellular carcinoma, liver transplantation) according to treatment response, (C) liver decompensation, and (D) hepatocellular carcinoma. *p <0.05 with the NR group (Log-Rank test); †p <0.05 with the REL group (Log-Rank test). SVR, Sustained virologic response; REL, end-of-treatment response with subsequent relapse; NR, no response.

As mentioned before, baseline liver biopsy information was available for 1153 out of 1599 patients, and there were 36 liver-related deaths among those 1153 patients. Liver-related deaths were documented in 12 out of 704 patients with baseline liver biopsy with F0 to F2 (1.7%), and in 24 out of 448 patients with liver biopsy with F3-F4 (5.4%); $p = 0.001$. This means that liver-related deaths, although more frequently documented in patients with advanced liver biopsy, were not exclusively seen in this group.

Liver-related events: Liver decompensation (i.e., ascites, hepatic encephalopathy, and variceal bleeding) rates were significantly less in patients with sustained viral response than in patients with viral relapse and patients with no response. Likewise, liver decompensation rates were significantly less in patients with viral relapse in comparison to patients with no response. Hepatocellular carcinoma rates were significantly less in patients with sustained viral response than in those with no response. Liver-transplantation rates were significantly less in patients with sustained viral response and in those with viral relapse in comparison with patients with no response.

Kaplan–Meier estimates of the survival function showed that the hazard of liver-related events (liver-related death, liver decompensation, hepatocellular carcinoma, liver transplantation) was significantly lower in patients with sustained viral response than in patients with viral relapse and in patients with no response. Likewise, the hazard of liver-related mortality or liver-related events was significantly lower in patients with viral relapse in comparison with patients with no response (Fig. 1).

We performed a multivariate analysis of factors associated with liver-related events by Cox regression analysis, adjusted for age, sex, history of IDU, CDC clinical category, nadir CD4+ cell count, HIV RNA at baseline, HCV genotype, HCV-RNA viral load, and liver fibrosis. Taking the group of patients with no response as reference, we found that the adjusted hazard ratio (HR) (and 95% CI) of liver-related events, for patients with viral relapse and for patients with sustained viral response, were 0.17 (0.05; 0.50) and 0.03 (0; 0.20), respectively (Table 3).

The results of the 2 sensitivity analyses (the first one considering those who achieved sustained viral response after retreatment in the sustained viral response group, and the second one in which we excluded from the analysis patients that were retreated) did not change the observations of the primary analysis (data not shown).

Liver stiffness following interferon plus ribavirin

Of the 1599 HIV/HCV-co-infected patients included in the cohort, we studied 416 patients who had a baseline liver biopsy and a post-treatment transient elastography measurement and who had not been retreated. Transient elastography results, categorized by baseline liver biopsy stages (F0–F2 and F3–F4) and response, are shown in Fig. 2.

In the F0–F2 fibrosis category, there were no significant differences in the proportion of patients with baseline F0, F1, F2 between the different categories of response; likewise, in the F3–F4 fibrosis category, there were no significant differences in the proportion of patients with baseline F3 and F4 between the different categories of response (Fig. 2). Of note, there were also no significant differences in the median time to last post-treatment transient elastography measurement between the three categories of response in both baseline liver fibrosis stages (Fig. 2).

For assessing the effect of treatment response on liver stiffness, we compared: (1) last transient elastography values between the different categories of response in each baseline liver fibrosis stage, and (2) the proportion of different transient elastography cut-off values between the different categories of response in each baseline liver fibrosis stage. Both analyses showed that sustained viral response after interferon plus ribavirin was followed by less liver stiffness than both viral relapse and

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Table 3. Multivariate analysis of factors associated with liver-related events* by Cox regression analysis.

Variable	Adjusted HR	95% CI	*p* value
No response	1.00	-	-
Viral relapse	0.17	(0.05; 0.5)	0.003
Sustained viral response	0.03	(0; 0.2)	<0.001
Age	0.99	(0.94; 1.05)	0.817
Male sex	1.01	(0.57; 1.8)	0.968
Genotype 1-4 *vs.* 2-3	0.86	(0.44; 1.68)	0.665
HCV-RNA ≥500,000 IU/ml	1.27	(0.7; 2.29)	0.426
Undetectable HIV RNA viral load at baseline	0.75	(0.45; 1.23)	0.251
CDC category C *vs.* A/B	0.61	(0.33; 1.13)	0.112
Nadir CD4+ cells	1.00	(1; 1)	0.392
Prior injection drug use	0.96	(0.45; 2.07)	0.922
Advanced fibrosis (F ≥3)	4.18	(2.39; 7.31)	<0.001

CDC, Centers for Disease Control and Prevention; CI, confidence interval; HR, hazard ratio.
*Liver-related death, liver decompensation, hepatocellular carcinoma, and liver transplantation.

no response. However, viral relapse was associated with less liver stiffness than no response (Fig. 2).

Alanine aminotransferase levels

We analyzed alanine aminotransferase (ALT) levels at baseline, at the end of interferon plus ribavirin therapy, and 24 weeks after the end of interferon plus ribavirin therapy (Table 4). No significant differences were found in the levels of ALT at baseline between the three different response categories; however, levels of ALT were significantly lower, with sustained viral response, in comparison with viral relapse and with no response, both at the end and 24 weeks after the end of interferon plus ribavirin therapy. Likewise, levels of ALT were significantly lower for viral relapse in comparison with no response at both follow-up time points.

When we analyzed the proportion of patients with normal ALT levels, defined as <41 international units/liter (U/L), we found that the proportion of patients with normal ALT levels at baseline was significantly higher among patients with viral relapse in comparison with patients with sustained viral response. However, sustained viral response was followed by a higher frequency of normal ALT levels in comparison with viral relapse and with no response, both at the end and 24 weeks after the end of interferon plus ribavirin therapy. Likewise, viral relapse was followed by a higher frequency of normal ALT levels in comparison with no response at both follow-up time points.

Discussion

In the present study, we evaluated the clinical course of a large cohort of HIV/HCV-co-infected patients who were followed up for a median period close to four years after therapy with interferon plus ribavirin, with the main objective of assessing the long-term consequences of viral relapse. The results of our study, confirm prior findings from our group, i.e., that sustained viral response reduces the incidence of liver-related deaths, liver decompensation, and liver transplantation in HIV/HCV-co-infected patients [14]. Now, with more patients in the cohort and more prolonged follow-up, we also found that sustained viral response was associated with a reduction in the incidence of hepatocellular carcinoma; something that we did not find before and that highlights the importance of continued surveillance of these patients, particularly those with advanced fibrosis and cirrhosis.

In our cohort, treatment response was categorized as viral relapse in 16% of patients (14% for genotypes 1 or 4 and 18% for genotypes 2 or 3). Best clinical outcomes were obtained with sustained viral response; however, viral relapse was clearly associated with less liver-related events than no response. The most likely mechanism behind this finding is that relapsers obtain some degree of histological benefit from interferon plus ribavirin, as patients with sustained viral response do. This is supported by our assessment of liver stiffness by transient elastography, whose results showed that during follow-up, sustained viral response was followed by less liver stiffness than both viral relapse and no response. However, viral relapse was associated with less liver stiffness than no response. The evolution of serum ALT concentrations after the end of interferon plus ribavirin therapy in our cohort also supports the concept that relapsers obtain some degree of histological benefit in comparison with non-responders. This is because in chronic hepatitis C, patients with normal ALT levels had significantly lower inflammation and fibrosis scores on liver biopsy examination than patients with elevated ALT levels [25].

The notion of some degree of fibrosis regression after therapy with interferon plus ribavirin, in HIV/HCV-co-infected patients with viral relapse, is further supported by a subanalysis with paired liver biopsies from the multinational AIDS Pegasys Ribavirin International Co-infection Trial (APRICOT), in which the investigators found that a substantial proportion of patients who did not achieve sustained viral response, experienced histological improvement [26]; likewise, in another study of repeated biopsies in HIV/HCV-co-infected patients, the achievement of end-of-treatment response following anti-HCV treatment, was independently associated with slower fibrosis progression [27].

We found that sustained viral response was also associated with a reduction in non-liver-related non-AIDS-related mortality, as we have reported elsewhere [15]. Patients with viral relapse, however, did not achieve this benefit. It must be taken into account that in HIV-infected individuals, active HCV infection

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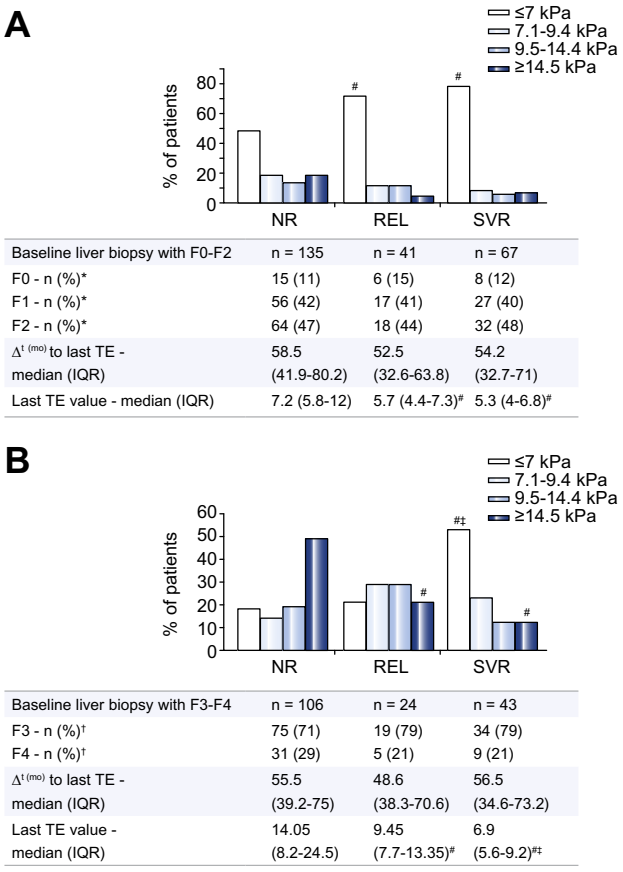

Baseline liver biopsy with F0-F2	n = 135	n = 41	n = 67
F0 - n (%)*	15 (11)	6 (15)	8 (12)
F1 - n (%)*	56 (42)	17 (41)	27 (40)
F2 - n (%)*	64 (47)	18 (44)	32 (48)
$\Delta^{t\,(mo)}$ to last TE - median (IQR)	58.5 (41.9-80.2)	52.5 (32.6-63.8)	54.2 (32.7-71)
Last TE value - median (IQR)	7.2 (5.8-12)	5.7 (4.4-7.3)#	5.3 (4-6.8)#

Baseline liver biopsy with F3-F4	n = 106	n = 24	n = 43
F3 - n (%)†	75 (71)	19 (79)	34 (79)
F4 - n (%)†	31 (29)	5 (21)	9 (21)
$\Delta^{t\,(mo)}$ to last TE - median (IQR)	55.5 (39.2-75)	48.6 (38.3-70.6)	56.5 (34.6-73.2)
Last TE value - median (IQR)	14.05 (8.2-24.5)	9.45 (7.7-13.35)#	6.9 (5.6-9.2)#‡

Fig. 2. Liver stiffness following interferon plus ribavirin. Last transient elastography results according to treatment responses in patients with (A) baseline liver fibrosis with F0–F2, and (B) baseline liver fibrosis with F3–F4. NR, no response; REL, end-of-treatment response with subsequent relapse; SVR, sustained viral response; IQR, interquartile range; Δ^t (months) to last TE, time (months) from the date interferon plus ribavirin was stopped to last transient elastography measurement. *$p = 0.98$ for differences between the 3 groups; †$p = 0.47$ for differences between the 3 groups; #$p < 0.05$ in comparison with NR; ‡$p < 0.05$ in comparison with REL.

has been found to impair CD4 lymphocyte recovery [28] and is associated with high grades of immune activation [29]; factors that have been causally related to the development of non-AIDS morbidity and mortality in HIV-infected individuals [30]. In addition, active HCV infection is associated with increased serum levels of the soluble adhesion molecules sICAM-1 and sVCAM-1 [31]; a factor that may contribute to increase the risk of death from cardiovascular events [32].

Our study has several limitations, the most important being that it is not entirely prospective. We believe, however, that its characteristics make it unlikely that the results differ considerably from those that would have been obtained in an entirely prospective study. This is because follow-up was done by the same physicians in the same reference hospitals throughout the course of the disease, with standard clinical and laboratory parameters every 6 months. Furthermore, all the information in the database was monitored to verify that it was consistent with the patient's medical history. In addition, we performed sensitivity analyses in order to overcome the potential bias of patients that underwent retreatment with interferon plus ribavirin, the results of which confirmed the findings of the main analysis. Our study is also limited by the lack of information about some characteristics of our population, such as educational level, drug adherence, life-style factors (exercise, diet, smoking) and social support; therefore, we cannot rule out the possibility that differences in these variables could have affected the outcome. Finally, the frequency and rate of lost to follow-up were higher for patients with no response, followed by patients with viral relapse and then by patients with sustained viral response. We believe, however, that the potential biases of these differences in follow-up would be in the line of underestimating our findings (best outcomes for sustained viral response, followed by viral relapse and then by no response), but not in the opposite way.

In summary, our results suggest that in HIV/HCV-co-infected patients treated with interferon plus ribavirin, best outcomes are achieved by those with sustained viral response. However, those with viral relapse have less liver-related events and less fibrosis progression than those with no response.

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Table 4. Alanine aminotransferase levels at different time points stratified according to response to interferon plus ribavirin.

Variable	No response (n = 765)	Viral relapse (n = 250)	Sustained viral response (n = 584)	Total (n = 1599)
ALT serum levels - U/L, median (IQR)				
Baseline (n = 764/250/584)	91 (61-140)	95 (62-142)	99 (66-156)	95 (62-147)
EOT (n = 731/245/573)	49 (32-80)	31 (21-47)†	27 (20-37)†‡	35 (24-58)
24 weeks after EOT (n = 673/237/580)	71 (49-108)	49 (27-85)†	24 (18-32)†‡	42 (24-79)
ALT serum levels <41 U/L - n (%)				
Baseline (n = 764/250/584)	71 (9.3)	30 (12)	39 (6.7)‡	140 (8.8)
EOT (n = 731/245/573)	275 (37.6)	165 (67.3)†	462 (80.6)†‡	902 (58.2)
24 weeks after EOT (n = 673/237/580)	118 (17.5)	99 (41.8)†	505 (87.1)†‡	722 (48.5)

ALT, alanine transaminase; U/L, international units/liter, EOT, end of interferon plus ribavirin therapy.
†$p < 0.05$ with the group 'No response'.
‡$p < 0.05$ with the group 'Viral relapse'.

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Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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