

# Biochemical and virological response of patients with Hepatitis C virus-caused cirrhosis to treatment with interferon and ribavirin

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## ABSTRACT

Combination antiviral therapies have previously been used with success in patients suffering from hepatic cirrhosis caused by the hepatitis C virus (HCV). We present here the results of a multi-center clinical trial sponsored by the Institute of Gastroenterology, which included a total of 36 patients diagnosed with hepatic cirrhosis due to HCV, at class A stage in the Child scoring scheme. The patients were treated with interferon alfa 2b plus ribavirin during 48 weeks, estimating the efficacy of this combination through qualitative determinations of serum HCV RNA and the biochemical behavior of the hepatic enzyme alanine aminotransferase (ALAT). A sustained virological response was obtained in 25% of the patients, and 50% of the patients controlled their ALAT levels in a sustained manner. The results show that this treatment has a positive effect on cirrhotic patients. There are only a few studies in Cuba describing the results of this therapeutic combination in cirrhotic patients.

**Keywords:** HCV, hepatic cirrhosis, treatment, interferon, ribavirin

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## RESUMEN

**Respuesta bioquímica y virológica de pacientes cirróticos por virus de la hepatitis C tratados con interferón y ribavirina.** La terapia combinada antiviral se ha usado con éxito en pacientes con cirrosis hepática causada por el virus de la hepatitis C (VHC). Este artículo presenta los resultados de ensayos clínicos multicéntricos dirigidos por el Instituto de Gastroenterología de Cuba, para los cuales se seleccionó un grupo de 36 pacientes con cirrosis hepática por el VHC en estadio de Child A, tratados con interferón alfa 2b más ribavirina durante 48 semanas. Se evaluó la eficacia de la combinación terapéutica, mediante la determinación cualitativa del ARN del VHC y el comportamiento bioquímico de la enzima hepática alaninoaminotransferasa (ALAT). En el 25% de los pacientes hubo una respuesta viral sostenida, y en más del 50% de ellos, hubo una estabilidad de los parámetros normales de la ALAT. Los resultados mostraron el efecto positivo de este tratamiento en los pacientes cirróticos. Pocos estudios en Cuba describen los resultados de esta combinación terapéutica en pacientes cirróticos.

**Palabras clave:** VHC, cirrosis hepática, tratamiento, interferón, ribavirina

## Introduction

Hepatitis C remains a significant health problem on a global scale. For example, estimates for 2009 alone set the number of persons infected with its causative agent, the Hepatitis C Virus (HCV), at 170 million cases [1]. In 80% of these patients hepatitis evolves to chronicity, and 20 to 30% eventually suffer cirrhosis after 10 to 20 years of disease evolution. In the latter case, the disease evolves further to hepatocellular carcinoma in 5 to 10% of the patients. Cirrhosis and hepatocellular carcinoma constitute, together, the main causes of liver transplant [1-3].

Hepatic cirrhosis (HC) is the appearance of fibrotic scarring in the liver, to the point that the normal architecture of the liver is disrupted and replaced by nodules distributed diffusely. HCV infection constitutes one of the most common causes of cirrhosis [4].

The therapy of cirrhotic patients during early stages of the disease is aimed mainly at stopping disease progression. Such an outcome requires acting on the causative agent and controlling fibrogenesis, using antifibrotic agents that interfere with collagen synthesis and facilitate its degradation. It has been shown

that interferon alfa (IFN  $\alpha$ ) can stop the progression of fibrosis in HCV-infected patients, in addition to exhibiting antiviral and anti-necroinflammatory activities [5, 6]. Currently interferon-based treatments use mainly a combination of PEGylated IFN and ribavirin. The latter, in addition to its antiviral activity, has immunomodulatory effects that synergize with the antiviral properties of IFN [7, 8].

There are only a few studies reporting the use of IFN  $\alpha$  to treat cirrhotic patients in Cuba [9, 10]; none of which combines it with ribavirin.

HCV-caused HC is not only hard to treat, but constitutes one of the main causes of death in Cuba. The present study, therefore, examines the viral response and alanine aminotransferase levels of patients with HCV-caused HC treated with IFN and ribavirin [11, 12].

## Materials and methods

### Study design

The present was a descriptive study that included 36 patients affected with HCV-caused HC, recruited for a

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multi-center clinical trial sponsored by the Gastroenterology Institute that took place in Havana, from January 2002 to June 2005. Post-treatment patient follow-up lasted from February to December, 2005. Fifteen of the patients belonged to the National Gastroenterology Institute, seven to the *Dr. Luis Díaz Soto* Military Medicine Institute, four to the *Hermanos Ameijeiras* Clinical-Surgical Hospital, two to the *Carlos J. Finlay* Military Hospital, two to the *Calixto García* Clinical-Surgical Hospital, one to the Medical-Surgical Research Center, and one to the *Miguel Enríquez Cabrera* Hospital (all from Havana), three to the *Mario Muñoz Monroy* Military Hospital (Matanzas), and one from the *Gustavo Alderenguía Lima* Provincial Clinical-Surgical Teaching Hospital (Cienfuegos).

### Patient characteristics

The study included patients with HCV-caused cirrhosis having detectable anti-HCV antibody and viral RNA in serum, and exhibiting the liver damage patterns typical of cirrhosis, as determined by laparoscopy and liver biopsy.

Other inclusion criteria were a minimum age of 18 years, class A cirrhosis in the Child-Pugh scoring scheme, and the availability of signed informed consent. Exclusion criteria were: having been subjected to previous antiviral treatments, previous use of hormonal contraception in the case of fertile women, pregnancy, breast-feeding, presence of hemoglobinopathies, hemoglobin levels below 12 and 12 g/dL for women and men respectively, and a clinical history of hypersensitivity to IFN or ribavirin.

Cirrhotic patients were classified as A, B, or C according to the severity of hepatic dysfunction, following the Child-Pugh scoring scheme. This scheme uses five scoring variables: bilirubin, serum albumin, presence of ascites, degree of liver encephalopathy, and prothrombin time. Liver dysfunction is estimated with these variables [13, 14].

Child A cirrhotic patients receive the maximum score (5 points); these patients exhibit no clinical signs and do not have symptoms of ascites or hepatic encephalopathy. In addition, their complementary tests yield normal values: bilirubin stays below 2 mg/dL, serum albumin stays above 3.5 g/dL, and prothrombin time stays below 4 seconds.

The patients were also classified as treatment virgin (with no previous history of antiviral treatment) or recidivist (having been treated with antiviral vaccines at some point during the year preceding their inclusion).

### Treatment

The contents of one vial containing  $3 \times 10^6$  U of recombinant IFN  $\alpha$ -2b (Heberón alfa R, Heber Biotec, produced at the Center for Genetic Engineering and Biotechnology, Havana) were administered subcutaneously every other day, three times per week. Ribavirin (1- $\beta$ -D-ribofuranosyl-1H-1, 2,4-triazole-3-carboxamide; produced at the Center for Drug Research and Development (CIDEM) and presented in 200 mg capsules) was administered at a daily dose of 1000 mg for patients weighing less than 75 kg and 1200 mg for patients weighing 75 kg or more. This treatment was administered for a period of 48 weeks. Clinical follow-up after treatment concluded lasted for 24 weeks.

### Variables

#### Determination of hepatic enzymes

Levels of alanine aminotransferase (ALAT) were determined in the available chemical autoanalyzers of clinical laboratories at the participating institutions, using commercially available diagnostic kits. Values two-fold higher than the norm (up to 49 U/L) were considered abnormal. ALAT levels were measured before starting the treatment and at weeks 24, 48 and 72.

At week 24, the patients were classified as:

- a) Early responders: Patients whose ALAT values have returned to normal levels.
- b) Early partial responders: Patients whose ALAT values decreased to at least 50% of the initial value, without reaching normal levels.
- c) Non responders: Patients whose ALAT levels did not decrease or increased.

At week 48 (end of the treatment), the patients were classified as:

- a) Responders: Patients whose ALAT values have returned to normal levels.
- b) Partial responders: Patients whose ALAT values decreased, but did not reach normal levels.
- c) Non responders: Patients whose ALAT levels did not decrease or increased.

At week 72 (end of follow-up) the patients were classified as:

- a) Sustained response: Patients whose ALAT values remained normal.
- b) Relapse: Responders whose ALAT values increased.
- c) Non responders: Patients whose ALAT values remained above the norm, without reductions compared to their initial value.

#### Virological response

Virological response was evaluated with a qualitative HCV-specific Polymerase Chain Reaction (PCR) using UMELOSA HCV kits from the Immunoassay Center (Havana, Cuba). This technique can detect down to 50 copies/mL.

At week 24, the patients were classified, according to their viral RNA levels, as:

- a) Early responders: Patients with undetectable levels of HCV RNA.
- b) Non-responders: Patients where HCV RNA remained above the detection limit.

At week 48 (end of treatment) the patients were classified, according to their viral RNA levels, as:

- a) Responders: Patients with undetectable levels of HCV RNA.
- b) Non responders: Patients where HCV RNA remained above the detection limit.

At week 72 (end of follow-up), responders were classified as having a:

- a) Sustained virological response (SVR): Responders whose HCV RNA levels remained below the detection limit.
- b) Relapse: Responders who became positive again for HCV RNA.

#### Statistical analysis

A descriptive analysis of the data was performed, calculating absolute and relative frequencies for adverse

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clinical and hematological events in treatment-virgin or recidivist HC patients. Chi-squared tests were run to examine the presence of statistically significant ( $p < 0.05$ ) differences between these groups for each of the biochemical and hematological assays performed. The SPSS version 14.0 statistical software package was used throughout.

### Ethical issues

Signed informed consent was requested and obtained from each patient by the principal investigator before their inclusion in the study, as a proof of voluntary participation. Each volunteer received a copy of this document. The study was performed under compliance with the principles of the Helsinki Declaration.

### Results

There were no statistically significant differences regarding the epidemiological characteristics of the two patient groups studied (data not shown). Whites predominated, and there was a slight predominance of females among treatment-virgin and recidivist patients ( $p > 0.05$ ). Average age among treatment-virgin patients was  $52.9 \pm 7.8$  years, and  $50.1 \pm 9.6$  years among recidivists ( $p > 0.05$ ).

ALAT values remained at normal levels, especially in the treatment-virgin group (Table 1). For 72.7% of the individuals of this group, ALAT values stayed at normal levels for 24 weeks. This percentage, however, decreased 24 weeks after treatment conclusion (week 72). The recidivist group exhibited the lowest percentages of biochemical response for each and every period, although it should be stressed that differences with the treatment-virgin group were never statistically significant ( $p > 0.05$ ). A general analysis of these evaluations revealed a biochemical response rate of 65% during treatment (weeks 24 and 48) that was not, however, maintained 24 weeks after treatment conclusion (week 72).

Table 2 contains the virological evaluation data. No statistically significant differences were detected at any of the analyzed time points. A comparison of the evaluations at different time points reveals a higher virological response in treatment-virgin patients. This difference was sustained from treatment conclusion (week 48) to the end of the follow-up period (week 72). The two patients who did not exhibit a SVR belonged to the non-responder group before treatment. Nine patients (25%) exhibited an SVR for all evaluated time points.

### Discussion

The only commercially available drugs against HCV exhibiting higher-than-marginal efficacy are IFN (standard or PEGylated) and ribavirin. They, however, have a number of side effects and are poorly tolerated [15, 16], representing therefore a viable alternative only for those patients able to tolerate the treatment, although hepatic dysfunction in the latter group is usually smaller.

IFN produces neutropenia, thrombocytopenia and anemia through medullary suppression. Ribavirin produces anemia, also through medullary suppression and, in addition, hemolysis. The induction and/or worsening of pre-existing cytopenias in cirrhotic

Table 1. Biochemical evaluation of the levels of alanine aminotransferase<sup>a</sup>

Week	Evaluations Patient classification	Patient group according to previous treatment Virgin N = 22	Recidivist N = 14	Total N = 36
24	Early responder	16 (72.7%)	9 (64.3%)	25 (69.4%)
	Early partial responder	2 (9.1%)	4 (28.6%)	6 (16.7%)
	Non-responder	4 (18.2%)	1 (7.1%)	5 (13.9%)
48 <sup>b</sup>	Responder	16 (72.7%)	8 (57.1%)	24 (66.7%)
	Partial responder	2 (9.1%)	4 (28.6%)	6 (16.7%)
	Non-responder	4 (18.2%)	2 (14.3%)	6 (16.7%)
72 <sup>c</sup>	Sustained response	13 (59.1%)	6 (42.9%)	19 (52.8%)
	Relapse	6 (27.3%)	7 (50.0%)	13 (36.1%)
	Non-responder	3 (13.6%)	1 (7.1%)	4 (11.1%)

<sup>a</sup> Data taken from Case Report Files. No statistically significant differences ( $p > 0.05$ ) were found between virgin and recidivist patients for any of the evaluated time points, as determined with a Chi-squared test.

<sup>b</sup> Week 48, treatment conclusion.

<sup>c</sup> Week 72, end of follow-up.

patients can have serious consequences, such as infections and hemorrhages; for this reason, these drugs are usually administered only to compensated patients [17, 18]. Child's scoring scheme is often used to evaluate the degree of liver dysfunction, in order to select patients for further antiviral treatment [19, 20].

A number of studies published during the last decade have demonstrated that the smallest rates of response to antiviral treatment are found among patients infected with genotype 1 of HCV. Treatment is most successful in genotype 2-infected patients, followed by those infected with genotype 3 and 4, in that order [21].

Although the present study did not determine viral genotype, previous studies have demonstrated that genotype 1 predominates among Cuban HCV-infected patients [22].

There are only a few studies addressing specifically the efficacy of antiviral treatment in cirrhotic patients where the viral genotype has been determined. In the study by Hadziyannis *et al.* [23], the SVR rate in patients with bridged fibrosis or HC (most of which were infected with genotype 1) was 41% after treatment with PEGylated IFN  $\alpha$ -2a (180  $\mu$ g/week) and ribavirin (1000-1200 mg/day) for 48 weeks. The present study cannot be directly compared to that of Hadziyannis *et al.* [23], as no genotyping was performed in the present case, and only standard IFN was used.

The predominance of whites in the sample is odd. Skin color data were taken from ID documents; however, classification into race based on skin color is notoriously unreliable, and it is not uncommon to find mulattoes classified as white. Our data cannot, therefore, be compared with that of other peer-re-

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Table 2. Virological evaluation, according to the presence or absence of HCV RNA<sup>a</sup>

Week	Evaluations Patient classification (HCV RNA +/-)	Patient group according to previous treatment Virgin N = 22	Recidivist N = 14	Total N = 36
24	Early responder (-)	9 (40.9%)	3 (21.4%)	12 (33.3%)
	Non-responder (+)	13 (59.1%)	11 (78.6%)	24 (66.7%)
48 <sup>b</sup>	Responder (-)	8 (36.4%)	3 (21.4%)	11 (30.6%)
	Non-responder (+)	14 (63.6%)	11 (78.6%)	25 (69.4%)
72 <sup>c</sup>	Sustained viral response (-)	8 (36.4%)	1 (7.1%)	9 (25.0%)
	Relapse (+)	14 (63.6%)	13 (92.9%)	27 (75.0%)

<sup>a</sup> Determined with an UMELOSA assay (Immunoassay Center, Havana, Cuba). Data taken from ambulatory patient records. No statistically significant differences were found ( $p > 0.05$ ) between virgin and recidivist patient groups for any of the analyzed time points (determined by Chi-squared test).

<sup>b</sup> Week 48, treatment conclusion.

<sup>c</sup> Week 72, end of follow-up.



viewed studies describing, at evidence level IV, that Afro-Americans predominate over Caucasians among patients afflicted with this disease, without evident differences of race among Latino patients [24, 25]. Statistically speaking, the results of our study cannot be compared to those of research based on studies of ethnicity rather than skin color.

Ages ranged from 50 to 58 years, which corresponds with data previously published by other authors [26].

Biochemical response rates were higher than virological response rates for all evaluated time points. This result indicates that normalization of ALAT levels and serum clearance of viral particles take place independently [27, 28]. A decrease in necroinflammatory activity, as evaluated biochemically through the levels of the cytolytic enzyme ALAT, without an accompanying decrease in viremia, has also been described by other authors in comparable studies [29-31]. In some patients who have been treated fundamentally with IFN, HCV RNA levels remain unaffected, while ALAT stays only slightly elevated [32].

The results of the virological evaluation resemble those described by other studies [33, 34]. For instance, the study published by Poynard, Marcellin and Lee [35] reported an SVR rate of 24% in 41 patients with compensated HC, quite close to the 25% rate we obtained with 36 patients.

The behavior of the studied variables in non-responders also corresponded with results published by other studies, where combined antiviral treatments with IFN  $\alpha$  and ribavirin in patients who had previously received IFN  $\alpha$  monotherapy produced SVR rates of 15 to 20% [23, 36].

Despite the appearance of adverse events in 77.8% of the patients, the treatment was well tolerated. Most of these events were of low intensity, and treatment dis-

continuation, temporal or definitive, was not necessary in any case. However, 40% of the patients had to decrease the ribavirin dose at some point of the treatment due to hemoglobin levels falling below 10 g/L [37].

We suggest that the treatment can be used in compensated cirrhotic patients, taking into account that multi-center studies performed in hospitals from Italy, Japan and Argentina have provided evidence suggesting that IFN decreases the risk of hepatocarcinoma by twofold. This effect is thought to be mediated by its antiproliferative activity and the suppression of viral replication, which eliminates in turn the carcinogenic effect of the accumulation of viral proteins in the hepatocytes. These proteins may lead to the proliferation and malignant transformation of this cell type [38].

One of the limitations of the present work was the use of standard IFN. Hadziyannis *et al.* [23] obtained an SVR of 41% after treating the patients with PEGylated IFN  $\alpha$ -2a (180  $\mu$ g/week) and ribavirin (1000-1200 mg/day) for 48 weeks. This response rate was similar to that obtained by Fried *et al.* [36], and also by Manns *et al.* [39], who obtained an SVR rate of 55% with 44 patients in advanced stages of the disease that received a daily dose of ribavirin equal to or higher than 10.6 mg/kg of body weight [39].

The treatment of HCV infection with IFN  $\alpha$  in combination with ribavirin produced levels of sustained virological and biochemical response equal to those obtained in patients with a diagnosis of HC [36].

We recommend the application of antiviral treatment in patients with cirrhosis caused by HCV, due to its significant impact in viral clearance and the delay it produces, according to the evidence, in disease progression and the appearance of complications, guaranteeing that the patient is better prepared for an eventual liver transplant [40].

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