There is no difference in hepatic fibrosis rates of patients infected with hepatitis C virus and those co-infected with HIV

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Some studies have suggested that human immunodeficiency virus (HIV) infection modifies the natural history of hepatitis C virus (HCV) infection, accelerating the progression of fibrosis and the development of cirrhosis. Our objective was to evaluate the fibrosis progression rate (FPR) in HCV/HIV-co-infected patients, and to identify factors that may influence it. HCV-mono-infected and HCV/HIV-co-infected patients with a known date of HCV infection (transfusion or injection drug use) and a liver biopsy were included. The FPR was defined as the ratio between the fibrosis stage (Metavir score) and the estimated length of infection in years and the result was reported as fibrosis units per year. The factors studied were gender, age at infection, consumption of alcohol, aminotransferase levels, histological activity grade, HCV genotype and viral load, CD4 cell count, HIV viral load, and the use of antiretroviral therapy. Sixty-five HCV-infected (group 1) and 53 HCV/HIV-co-infected (group 2) patients were evaluated over a period of 19 months. The mean FPR of groups 1 and 2 was 0.086 ± 0.074 and 0.109 ± 0.098 fibrosis units per year, respectively (P = 0.276). There was a correlation between length of HCV infection and stage of fibrosis in both groups. The age at infection, the aspartate aminotransferase level (r = 0.36) and the inflammatory activity grade were correlated with the FPR (P < 0.001). No difference in FPR was found between HCV-mono-infected and HCV/HIV-co-infected patients.

Key words: Hepatitis C; Human immunodeficiency virus; Hepatic fibrosis; Cirrhosis

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Introduction

Infection with hepatitis C virus (HCV) has an estimated prevalence of 3% in the world, meaning that there are around 170 million people chronically infected with HCV, thus representing a serious public health problem (1,2). Studies on the progression of hepatic fibrosis in HCV-infected patients and on the factors responsible for the wide variability shown in its natural history have been emphasized in the literature (3-6). Some investigators have concluded that fibrosis progression is time-dependent and that its estimate is an important piece of information in evaluating the vulnerability of an individual patient and the impact of treatment on the natural history of the disease (3-5).

Some factors identified in individual studies are associated with a greater progression of liver fibrosis: length of infection, age at the time of infection, male gender, abuse of alcohol (3,7), co-infection with hepatitis B virus (8) and with human immunodeficiency virus (HIV) (9), and the presence of elevated alanine aminotransferase (ALT) (10,11).

The natural history of liver fibrosis progression in patients with HCV/HIV co-infection has been studied during the last decade. The high prevalence of HCV/HIV co-infection is ascribed to the shared transmission route, the most important being the use of intravenous drugs, which continues to be the greatest risk for acute HCV infection (12-14). In the United States and Europe, the prevalence of HCV/HIV co-infection is approximately 30% (12). In Brazil, the prevalence depends on the geographic area, with values ranging from 8.9 to 54% (15-18).

Since the prognosis of HIV infection has improved...
significantly with antiretroviral (ARV) therapies, chronic liver diseases, initially mere coadjuvants in the spectrum of HIV infection, have become prominent and seem bound to be the leading cause of morbidity and mortality in the HCV/HIV-co-infected population (19-22).

Several studies conducted before the introduction of highly active antiretroviral therapy (HAART) have suggested that HIV infection changes the natural history of HCV infection, leading to more rapid progression of liver fibrosis (9,23-28). The progression, which typically takes 30 years or more in patients infected with HCV, could take less than half this time in co-infected patients (12).

The present study was designed to evaluate the fibrosis progression rate (FPR) and the factors influencing this progression in patients infected with HCV and in patients co-infected with HCV and HIV.

Patients and Methods

Patients with HCV infection (group 1) and with HCV/HIV co-infection (group 2) referred to the Gastroenterology Unit of Hospital Nossa Senhora da Conceição, a large public General Hospital in the South of Brazil, for a percutaneous liver biopsy during the period from January 2003 to August 2004 were evaluated. Considering the 45% prevalence of high grade fibrosis by Metavir (29) score (F3/F4) in co-infected patients and of 17% for those mono-infected with HCV (8), the estimated size of the sample was 49 patients in each group, for a 95% confidence interval and 80% statistical power.

The inclusion criteria for consecutively attended patients were positive anti-HCV antibodies (in the mono-infected group), with verification of HCV infection by the polymerase chain reaction (PCR); association of anti-HCV with HIV infection (in the co-infected group) determined by the immunofluorescence test and Western blot; estimated date of viral infection (blood and/or blood product transfusion, or intravenous drug users), with infection considered to occur at first transfusion and at the beginning of intravenous drug use; age between 18 and 70 years, and no previous antiviral treatment for HCV.

Exclusion criteria were patients with risk factors for developing HCV infection other than intravenous drug use or transfusion; with decompensated cirrhosis or hepatocellular carcinoma; pregnant women, and associated hepatitis B infection (HBsAg positive).

The following data were evaluated: gender, age at the time of infection, serum levels of ALT and aspartate aminotransferase (AST), fibrosis and inflammatory activity grades, HCV genotype, viral load of HCV, infection transmission mode, drinking behavior, CD4 cell count, HIV viral load, and antiretroviral treatment.

HCV-RNA was detected by PCR using the Amplicor HCV Test, version 2.0 (Roche Diagnostics, Nutley, NJ, USA; detection limit: 50 IU/mL).

HCV-RNA was quantitated using the Amplicor HCV Monitor™ Test, version 2.0 (Roche Diagnostics; detection limit: 600 IU/mL).

Patients with positive HCV-RNA were tested for genotype by the technique of restriction fragment length polymorphism-PCR.

The CD4 cell count was performed by flow cytometry according to manufacturer instructions (Becton Dickinson, Heidelberg, Germany).

HIV viral load was determined by the b-DNA technique (Bayer Corporation, Tarrytown, NY, USA; detection limit: 50 copies/mL).

All patients were submitted to a percutaneous liver biopsy with a Menghini needle. The liver biopsy fragments were embedded in paraffin and stained with hematoxylin-eosin and picrosirius-red and then examined blindly by a pathologist. The biopsy was considered to be satisfactory when the fragments presented at least five complete portal tracts.

For each biopsy, the grades of fibrosis and inflammatory activity were determined using the Metavir classification (29).

The annual FPR was determined by the ratio of fibrosis stage (by the Metavir score) to the estimated duration of infection in years. The results are reported as fibrosis units per year. For example, for a patient with stage F2 fibrosis and length of infection estimated at 15 years, the FPR would be 2/15 = 0.133 fibrosis units per year.

This research was approved by the Ethics Committee of the Nossa Senhora da Conceição Hospital and posed minimum risk for the participants. All patients gave written informed consent to participate in the study.

For statistical analysis, the MS Excel 2000 software was used to store the data and the results were analyzed using the Statistical Package for the Social Sciences (SPSS 8.0). The quantitative variables are presented as means ± SD. ANOVA was used to compare means, followed by the multiple comparisons Tukey test. The qualitative variables are presented as frequency and percentage. To confirm the association between these variables the Pearson chi-square test was used, with the complementary resource of adjusted residues analysis to identify the location of associations. The level of significance was set at 5%.

Results

A total of 118 patients, 65 of whom were mono-infected
with HCV (group 1) and 53 co-infected with HCV/HIV (group 2), were evaluated. Table 1 shows the clinical and laboratory data of the population studied.

Table 2 shows the results of the histological evaluation. There was no statistically significant difference between groups regarding grade of fibrosis and inflammatory activity. Likewise, when more intense fibrosis grades (F3/F4) were evaluated as a whole, there was no difference across groups (36.5 vs 36.5%). Similarly, moderate or severe inflammatory activity (A2/A3) evaluated as a whole was present in 33.9% of patients in the mono-infected group and in 30.8% of the HCV/HIV co-infected group, with no statistically significant difference.

Figure 1 shows the relation between length of infection and grade of fibrosis.

The mean FPR for patients with HCV mono-infection and for patients with HCV/HIV co-infection was 0.086 and 0.109 ± 0.098 fibrosis units per year, respectively (P = 0.276). This rate did not show a normal distribution, with a median of 0.090 in both groups.

Among the patients in group 2, 40 were using ARV therapy. None of the patients was on monotherapy. The mean length of ARV treatment was 48.6 months before the liver biopsy. HIV viral load was determined in group 2, being undetectable in 60% of the cases. The mean CD4 cell count was 420 ± 172/mm³ (54-930/mm³; Table 1).

Age at the time of HCV infection, inflammatory activity degree, and serum AST level were the factors associated with higher mean FPR (P < 0.001). There was no correlation between gender (P = 0.50), drinking behavior (P = 0.98), mode of transmission (P = 0.91), genotype (P = 0.64), HCV viral load (P = 0.40), total CD4 cell count (P = 0.71), and ARV treatment (P = 0.61).

Table 1. Characteristics of the study population.

<table>
<thead>
<tr>
<th></th>
<th>HCV (N = 65)</th>
<th>HCV/HIV (N = 53)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (years)</td>
<td>45.86</td>
<td>40.29*</td>
</tr>
<tr>
<td>Female gender</td>
<td>55.3%</td>
<td>20.7%**</td>
</tr>
<tr>
<td>Transfusion</td>
<td>75.4%</td>
<td>20.7%**</td>
</tr>
<tr>
<td>Intravenous drug use</td>
<td>24.61%</td>
<td>79.24%**</td>
</tr>
<tr>
<td>Drinking &gt;50 g/day</td>
<td>23.5%</td>
<td>52.0%**</td>
</tr>
<tr>
<td>Mean length of infection (years)</td>
<td>20.64</td>
<td>18.73</td>
</tr>
<tr>
<td>Mean age at the time of infection (years)</td>
<td>25.18</td>
<td>22.95</td>
</tr>
<tr>
<td>Genotype 1</td>
<td>55.90%</td>
<td>51.10%</td>
</tr>
<tr>
<td>Genotype 2</td>
<td>1.70%</td>
<td>2.10%</td>
</tr>
<tr>
<td>Genotype 3</td>
<td>42.40%</td>
<td>46.80%</td>
</tr>
<tr>
<td>HCV-VL (IU/mL)</td>
<td>1,433,842 ± 3,252,323</td>
<td>1,068,236 ± 2,080,291</td>
</tr>
<tr>
<td>CD4 (cell/mm³)</td>
<td>ND</td>
<td>420 ± 172</td>
</tr>
<tr>
<td>HIV-VL (IU/mL)</td>
<td>ND</td>
<td>55,802 ± 136,152</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>101 ± 51</td>
<td>120 ± 84</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>61 ± 55</td>
<td>81 ± 31*</td>
</tr>
</tbody>
</table>

Data are reported as means ± SD, percent or years. HCV-VL = hepatitis C virus viral load; ND = not determined; HIV-VL = human immunodeficiency virus viral load; ALT = alanine aminotransferase; AST = aspartate aminotransferase. *P < 0.05 compared to HCV group (ANOVA). **P < 0.05 compared to HCV group (Pearson χ² test).

Table 2. Comparison of liver histopathology (Metavir score).

<table>
<thead>
<tr>
<th></th>
<th>HCV (N = 65)</th>
<th>HCV/HIV (N = 53)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrosis stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F0</td>
<td>18 (27.69%)</td>
<td>12 (22.64%)</td>
</tr>
<tr>
<td>F1</td>
<td>10 (15.38%)</td>
<td>12 (22.64%)</td>
</tr>
<tr>
<td>F2</td>
<td>14 (21.53%)</td>
<td>11 (20.75%)</td>
</tr>
<tr>
<td>F3</td>
<td>13 (20.00%)</td>
<td>13 (24.52%)</td>
</tr>
<tr>
<td>F4</td>
<td>10 (15.38%)</td>
<td>5 (9.43%)</td>
</tr>
<tr>
<td>Inflammatory activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A0</td>
<td>9 (13.84%)</td>
<td>5 (9.43%)</td>
</tr>
<tr>
<td>A1</td>
<td>36 (55.38%)</td>
<td>33 (62.26%)</td>
</tr>
<tr>
<td>A2</td>
<td>15 (23.07%)</td>
<td>11 (20.75%)</td>
</tr>
<tr>
<td>A3</td>
<td>5 (7.69%)</td>
<td>4 (7.54%)</td>
</tr>
</tbody>
</table>

HCV = hepatitis C virus; HIV = human immunodeficiency virus. There were no statistical differences between groups for fibrosis stage or activity degree (Pearson χ² test).

Figure 1. Length of HIV infection vs stage of fibrosis.
patients than in HCV-mono-infected patients, and HCV/
HIV co-infection was a significant risk factor for the develop-
ment of cirrhosis only in the pre-HAART era.

The results of the present study showed a similar FPR
for mono- and co-infected patients. Despite the results of
previous studies, the lack of difference regarding fibrosis
progression between groups is in agreement with a recent
report by Brau et al. (32), considering that most co-infected
patients included in the present study were young, were on
treatment with ARV and presented good immunity, as
reflected by CD4 cell count and low HIV viral load (unde-
tectable in 60% of the cases).

Moreover, in the present series, there was no associa-
tion between co-infection and fibrosis stage. When more
intense fibrosis stages (F3/F4) were analyzed, there was
no difference between the two groups of patients.

In the evaluation of the various factors implicated in the
progression of liver fibrosis, only age at the time of HCV
infection, inflammatory activity grade, and AST serum
level were significantly correlated with FPR.

Although the co-infected patients were younger, there
was no significant difference between groups regarding
mean age at the time of infection or mean length of infection.

The role of inflammatory activity as an independent
factor for fibrosis progression is a controversial matter (3).
Poynard et al. (4) failed to demonstrate any impact of inflam-
matory activity on the progression of fibrosis, considering the
first 20 years after infection. Only the absence of activity was
associated with slower progression. However, in more re-
cent studies (33,34), HCV-mono-infected and HCV/HIV-co-
infected patients who presented moderate or severe inflam-
matory activity had more rapid fibrosis progression than
those with minimum or absent activity. Likewise, as ob-
serve for fibrosis grade, in the present study, there was no
difference when histological activity was evaluated in the co-
infected group. It is thus impossible to conclude for a greater
hepatic involvement in this group.

It is recognized that an AST/ALT ratio above 1 sug-
gests a diagnosis of cirrhosis in patients with chronic HCV
infection (35), and some investigators (11,33) have ob-
served a relationship between aminotransferase levels
and fibrosis grade. In the present study, patients with more
advanced fibrosis presented high AST and ALT levels, but
only AST level proved to be correlated with FPR.

Regarding alcohol intake, although the co-infected
patients had a greater intake than mono-infected patients,
no difference in FPR was observed. However, the fact that
the co-infected group consisted of younger patients may
have contributed to this result.

Viral aspects have been studied in the context of
progress of liver disease caused by HCV, with conflicting
results in the literature (3,4,33,36-38). The influence of
genotype and of viral load remains in discussion. In the
present study, the different HCV genotypes or viral load did
not affect the histological findings or FPR in the co-infected
or mono-infected group.

Regarding the HCV/HIV-co-infected patients, some
aspects such as CD4 cell count and the use of ARV
therapy should be highlighted. A CD4 count equal to or
below 200/mm$^3$ is associated with a higher rate of liver
fibrosis progression (9,39). Mohsen et al. (33), evaluating
HCV/HIV-co-infected patients, failed to find an association
between fibrosis and ARV treatment. In the present study,
CD4 cells and the use of ARV therapy were not correlated
with FPR in co-infected patients. However, the CD4 cell
count was below 200/mm$^3$ in only one patient, reproducing
an immunity profile similar to that of immunocompetent
patients, and this can be an important reason for the
absence of a difference in FPR between groups.

The present study has limitations, the most relevant of
which is possibly related to the model used to calculate the
FPR in HCV-infected and in HCV/HIV-co-infected patients.
Ideally, in the calculation of fibrosis progression, consecu-
tive biopsies should be performed with a long interval be-
tween them in patients untreated for HCV, which would be
ethically unacceptable. The present model, described else-
where (3), permits the evaluation of the cumulative effect of
fibrosis progression according to the duration of infection.
Although previously validated, this model may be criticized
since it considers the patient to have no fibrosis at the time of
HCV infection. The accuracy of the calculation of the esti-
mated length of infection can also be criticized, as it is based
on patient history. In order to adequately analyze the dura-
tion of HCV infection, the population evaluated here in-
cluded only patients with a history of blood transfusion or
intravenous drug users, since in this population contamina-
tion generally occurs during the first transfusion or in the first
year of intravenous drug use in as many as 90% of cases
(38). Although not an ideal method, this estimate using a
single biopsy remains a useful tool in the study of the natural
history of chronic hepatitis C. Besides, the groups presented
some differences related to epidemiological aspects that
possibly influenced the results when comparative analyses
were performed.

In the present study, no difference in FPR was found
between HCV-mono-infected and HCV/HIV-co-infected
patients. HCV/HIV co-infection had no relevant negative
impact on the progress of these patients as compared to
HCV-mono-infected ones. However, more studies are nec-
essary to evaluate FPR in co-infected HCV/HIV patients in
order to better understand the natural history of HCV
infection in this population.
References


