HIV-HCV RNA loads and liver failure in coinfected patients with coagulopathy

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ABSTRACT

Background and Objective. The aim of this study was to measure contemporaneously HCV-RNA load, HIV-RNA load and CD4+ lymphocytes count in HCV/HIV coinfected patients with coagulopathy and to examine the relationship between these parameters and the liver failure.

Design and Methods. A cross-sectional study was performed on 54 patients with severe coagulopathy: 39 HCV/HIV coinfected and 15 HCV+/HIV– comparable for age and HCV exposure time. HCV-RNA and HIV-RNA load, CD4+ lymphocytes count, biochemical and ultrasonographic parameters were evaluated at the time of entry to the study.

Results. Mean HCV-RNA load was significantly higher in coinfected patients (643,872±717,687 copies/mL) than in HCV+/HIV– (mean 161,573±276,896 copies/mL) (p = 0.01). The 39 HCV/HIV coinfected patients had a mean HIV-RNA load of 205,913±456,311 copies/mL (range 4,000-2,500,000) and a mean CD4+ lymphocyte count of 206.5±171/µL (range 5-693). Five of the 39 (12.8%) coinfected patients had liver failure. In these five patients the mean HCV-RNA load (770,200±996,426 copies/mL) was high but not significantly different from that in the 34 HCV+/HIV+ patients (623,496±682,239 copies/mL) without liver failure (p = 1.0). Cofinfected patients with liver failure had a significantly higher HCV-RNA load (mean 764,599±978,542 copies/mL) and lower CD4+ lymphocyte count (mean 52.6±55.6/µL) than those observed in coinfectected patients without liver failure (p = 0.007 and p = 0.03, respectively). A significant inverse correlation was found between CD4+ lymphocyte count and HIV-RNA load (r = –0.37, p = 0.01).

Interpretations and Conclusions. HCV-RNA load is significantly higher in HIV+ than in HIV– patients with coagulopathy. Liver failure was found only in the 39 HCV/HIV coinfected patients with severe immunodepression, expressed either by low CD4+ lymphocyte count or by high HIV-RNA load.

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Key words: HCV-RNA load, HIV-RNA load, liver failure, coinfection, coagulopathic patients

Infection with human immunodeficiency virus (HIV) seems to accelerate the natural course of HCV infection, increasing the risk of liver failure;1-4 HIV/HCV coinfection is frequent in intravenous drug users and transfused hemophiliacs.5 Early and self administration of clotting factor concentrates, a practice which began in 1970, has resulted in patients with coagulopathy having a longer and better quality of life, due to the rapid resolution of bleeding episodes. Before the use of virucidal methods in the processing of factor concentrates, over 50% of hemophilic patients revealed previous infections due to hepatitis B virus (HBV) or hepatitis C virus (HCV); this rate was higher than in normal population.6 HCV infection is frequently observed in patients with chronic liver disease coagulopathies.7,8 The onset of clinical cirrhosis is estimated to occur 12-18 years from the start of the use of clotting factor concentrates.9 It is now accepted that the HCV-RNA load is higher in patients coinfected with HIV than in HIV negative individuals, while the relationship between HCV-RNA load and CD4+ lymphocyte count is still controversial.10 HIV-RNA load and CD4+ lymphocyte count is still controversial.10-12 Two studies on HCV/HIV coinfected hemophiliacs reported high HCV-RNA load and low CD4+ lymphocyte count11 in patients with liver failure.

A single measurement of serum HIV-RNA has been reported as being of important prognostic value, and being strongly associated with rapid progression to AIDS and increased risk of mortality, regardless of treatment.11 Because variability in the measurement of HIV-RNA levels may significantly affect their interpretation in clinical practice, repeated measures of HIV-RNA levels are now routinely made both in clinical trials and clinical practice.12 To our knowledge, no study until now has reported the concomitant measurement of HCV-HIV RNA loads and CD4+ lymphocyte count in coinfectected hemophiliacs.

After caring for a patient with coagulopathy, who was coinfected by HCV and HIV and who rapidly developed liver failure, we designed a cross-sectional study to measure HCV-RNA load, HIV-RNA load and CD4+ lymphocyte count in coinfectected patients with severe coagulopathy and examined the relationship between these parameters and the liver failure.
Design and Methods

In January 1996 we performed a cross-sectional study of 163 patients with severe coagulopathy attending the hematology Department of “La Sapienza” University in Rome. Severe coagulopathy was defined as by plasma levels of factor VIII:C, IX:C and VII:C below 2%. The patients who fulfilled the following criteria were evaluated: a) severe coagulopathy in patients who had received clotting factors concentrates which had not undergone virus-inactivation; b) HCV and HIV coinfection; c) absence of HBsAg; d) absence of interferon treatment.

Of the 163 initial patients, 39 patients (Group 1) fulfilled the entry criteria; there were 36 males and 3 females, 33 of them had hemophilia A, 3 hemophilia B and 3 factor VII:C deficiency. Of the remaining 124 females, 33 of them had hemophilia A, 3 hemophilia B and HIV-RNA viral loads were measured in a virology laboratory (Istituto Superiore di Sanità, Rome, Italy), member of the World Viral Quality Control (VQC). The HCV-RNA load was measured using the Amplicor HCV Monitor test (Roche Diagnostic System Inc.). Extraction, amplification and detection were performed using standard techniques.

Virologic methods

All frozen specimens were retrieved and immediately tested. HBsAg, HBeAg, HBeAb, HBCab and HBsAb tests were performed using standard techniques.

HCV positivity was detected by the ELISA test (Ortho Diagnostics). The HCV-RNA and HIV-RNA viral loads were measured in a virology laboratory (Istituto Superiore di Sanità, Rome, Italy), member of the World Viral Quality Control (VQC). The HCV-RNA load was measured using the Amplicor HCV Monitor test (Roche Diagnostic System Inc.). Extraction, amplification and detection were performed according to the manufacturer’s instructions, starting from 100 µL of serum. This test has a sensitivity of 1,000 copies HCV-RNA/mL.

HIV positivity was detected by the ELISA test and confirmed by a Western blot method. The HIV-RNA viral load measurement was performed by the NASBA QT system (Organon Teknika). This test has a sensitivity of 4,000 HIV-RNA/mL copies.

The stability of serum HCV-HIV RNA loads was tested twice/day on three consecutive days for randomly chosen samples.

Imaging methods

Hepatic longitudinal diameter, longitudinal splenic diameter, portal vein diameter, and presence of ascites and collateral vessels were evaluated by ultrasound using an electrically focused 3.75 MHz sectorial probe (Toshiba SSH-140A). Duplex Doppler hemodynamic evaluation was performed in order to evaluate the average speed of portal flow and the spectrum characteristic of the flow in the suprahepatic and infraportal veins. The accuracy of these evaluations was validated in 54 patients by means of Doppler color flow imaging (Toshiba SSH-140A). The diagnosis of hepatic cirrhosis was based on the presence of at least one of the following parameters: ascites, splenomegaly, and portal hypertension as defined by the IASL.

Viral therapy and prophylaxis

Thirty-four out of the 39 coinfected patients received antiretroviral monotherapy (zidovudine or didanosine or zalcitabine). No antiretroviral therapy was administered to the remaining five coinfected patients who had a CD4+ lymphocyte count above 400/µL. Out of twenty patients with a CD4+ lymphocyte count below 200/µL, 18 received Pneumocystis carinii prophylaxis with trimethoprim (800 mg) and sulphamethoxazole (160 mg) twice daily three times every week. The other two patients, who could not tolerate trimethoprim-sulphamethoxazole were treated with aerosol administered pentamidine (300 mg once a month).

From December 1996 to March 1997 all patients with a CD4+ lymphocyte count below 200 µL received protease inhibitors as HIV viral therapy.

None of the coinfected patients received therapy or prophylaxis for mycobacterial or cytomegaloviral infections.

Biochemical evaluation

All measurement were made using standard methods. Serum ferritin (range 10-190 ng/mL) levels were measured by Microparticle Enzyme Immunoassay (MEIA) (Abbott Diagnostic).

Liver failure was defined as the contemporary evidence of at least three of the following parameters: albumin < 3.5 g/dL, prolonged prothrombin time, jaundice and ascites. The prothrombin time, expressed as a ratio versus the value from pooled normal plasma, was considered prolonged when the values were above the upper limit of the normal range (normal range as ratio = 0.84-1.18). Jaundice was defined as a bilirubin concentration higher than twice the upper limit of the normal range. Ascites was detected by ultrasonographic evaluation.

Liver biopsy in patients with severe coagulopathy may cause serious hemorrhagic complications in about 20% of patients. In our Department, liver biopsy was not, therefore, performed in such patients, and chronic hepatitis C was evaluated by indirect parameters.

Definition of liver failure

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atic vein. Esophageal varicosity was diagnosed by esophagogastroduodenoscopy (EGDS).

Statistical analysis
Statistical analysis was performed using BMDP statistical software (Berkeley, Ca., USA). Mean values and standard deviations of all evaluated variables were calculated for both groups. The analysis of variance was performed between mean values of parameters in coinfected patients (Group 1) and in the control group (Group 2).

In the 39 coinfected patients, the non-parametric Mann-Whitney U test was used to measure the differences between the five patients with liver failure and the remaining without liver failure.

Pearson’s r coefficient was calculated for correlation analysis.

Results
Analyses of variance of biochemical, ultrasonographic and virological parameters in coinfected and not coinfected patients are reported in Table 1. The significant differences between HCV/HIV coinfected and HCV+/HIV– patients were: HCV-RNA load (p = 0.01), longitudinal splenic diameter (p = 0.02) and portal vein diameter (p = 0.04). Alanine aminotransferase, albumin, ferritin and longitudinal hepatic diameter were not different between the two groups of patients (Table 1). The HCV-RNA load was not correlated with ALT levels (r = 0.21, p = 0.1).

Of the 54 patients selected according to the study criteria all had serologic evidence of past HBV infection (HBSAb/HBcAb positivity).

In coinfected patients a significant inverse correlation was found between CD4+ lymphocyte count and HIV-RNA load (r = -0.37, p = 0.01), while CD4+ lymphocyte count did not correlate with HCV-RNA load (r = -0.23, p = 0.08).

The 39 HCV/HIV coinfected patients had a mean HIV-RNA load of 205,913±456,311 copies/mL (range 4,000-2,500,000) and a mean CD4+ lymphocytes count of 206.5±171/µL (range 5-693).

Of the 39 coinfected patients five (12.8%) had liver failure at the time of entering the study. At that time, liver failure was not present either in the 15 patients of Group 2 selected for age and HCV exposure time, or in the remaining 90 HCV+/HIV– patients. Age, HCV exposure time and HIV seroconversion data were not significantly different in the five coinfected patients with liver failure from those in the remaining 34 HCV+/HIV+ patients (Table 2).

In the 39 coinfected patients the Mann-Whitney U test showed a significantly higher HIV-RNA load (p = 0.007) and lower CD4+ lymphocyte count (p = 0.03) in the five patients with liver failure than in the remaining 34 (Table 2). The HCV-RNA load was similarly high in coinfected patients with liver failure (770,200±996,426 copies/mL) and without (623,496±682,239 copies/mL) (p = 1.0)(Table 2).

Hepatic and splenic longitudinal diameters were significantly increased in patients with liver failure (p = 0.003 and 0.0008, respectively). The average speed of portal flow was significantly lower in the five patients with liver failure (p = 0.01)(Table 2).

Table 1. Analysis of variance between HCV/ HIV coinfected patients and those only HCV positive (control group).

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>HCV/HIV</th>
<th>HCV/HIV</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>34.7±12.4</td>
<td>32.1±13</td>
<td>0.51</td>
</tr>
<tr>
<td>HCV exposure time</td>
<td>22.8±4.0</td>
<td>21.3±3.0</td>
<td>0.17</td>
</tr>
<tr>
<td>HCV-RNA load</td>
<td>643,872±17,687</td>
<td>161,573±276,896</td>
<td>0.01</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>4.3±0.5</td>
<td>4.1±0.8</td>
<td>0.19</td>
</tr>
<tr>
<td>Ferritin (ng/mL)</td>
<td>336±508</td>
<td>104±94</td>
<td>0.08</td>
</tr>
<tr>
<td>Alanine aminotransferase (ALT) &lt; 40 IU/L</td>
<td>70±37</td>
<td>73±39</td>
<td>0.77</td>
</tr>
<tr>
<td>Hepatic longitudinal diameter (mm)</td>
<td>142±12</td>
<td>140±9</td>
<td>0.59</td>
</tr>
<tr>
<td>Splenic longitudinal diameter (mm)</td>
<td>134±22</td>
<td>119±16</td>
<td>0.02</td>
</tr>
<tr>
<td>Portal vein diameter (mm)</td>
<td>12.5±1.3</td>
<td>11.5±1.6</td>
<td>0.04</td>
</tr>
<tr>
<td>Average speed of portal flow (cm/sec)</td>
<td>17.5±3</td>
<td>18.4±1.7</td>
<td>0.20</td>
</tr>
</tbody>
</table>

Mean values±standard deviations are reported.

Table 2. Mann-Whitney U test of HIV-RNA load, HCV-RNA load, CD4+ lymphocyte count and ultrasonographic parameters between coinfected patients with liver failure and without.

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>Liver failure</th>
<th>No liver failure</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>37.6±15.5</td>
<td>34.3±12</td>
<td>0.8</td>
</tr>
<tr>
<td>HIV seroconversion date (year)</td>
<td>1,983.6±1.12</td>
<td>1,983±0.57</td>
<td>0.20</td>
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<tr>
<td>HCV exposure time (years)</td>
<td>24.8±1.9</td>
<td>22.5±4.2</td>
<td>0.25</td>
</tr>
<tr>
<td>HCV-RNA load (copies/mL)</td>
<td>764,599±978,542</td>
<td>131,562±246,049</td>
<td>0.007</td>
</tr>
<tr>
<td>HIV-RNA load (copies/mL)</td>
<td>770,200±996,426</td>
<td>623,496±682,239</td>
<td>1.0</td>
</tr>
<tr>
<td>CD4+ lymphocytes count (n/µL)</td>
<td>52.6±55.6</td>
<td>229.1±170.3</td>
<td>0.03</td>
</tr>
<tr>
<td>Hepatic longitudinal diameter (mm)</td>
<td>156±22</td>
<td>140±12</td>
<td>0.003</td>
</tr>
<tr>
<td>Splenic longitudinal diameter (mm)</td>
<td>170±18</td>
<td>129±17</td>
<td>0.0008</td>
</tr>
<tr>
<td>Portal vein diameter (mm)</td>
<td>13.3±0.4</td>
<td>12.4±1.6</td>
<td>0.24</td>
</tr>
<tr>
<td>Average speed of portal flow (cm/sec)</td>
<td>13.8±2</td>
<td>18.1±2</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Mean values±standard deviations are reported.
Out of the 54 patients studied, six coinfected patients died; five of liver failure within the 9th month of follow-up and one in the 27th month due to non-Hodgkin’s lymphoma.

Discussion
The main finding of this cross-sectional study is that HIV infection influences HCV infection in patients with HCV/HIV coinfection and coagulopathy.

HCV-RNA load was significantly higher in coinfected patients than in HCV+/HIV patients, in agreement with data reported by others.1,2,3,10-12,18,19 Teffer et al.2 reported an increase of HCV-RNA among 29 HIV+ hemophiliacs compared with that in 29 HIV controls, however, among the HIV positive patients there was no correlation between HCV-RNA load and CD4+ cell count. The lack of correlation between HCV-RNA load and CD4+ cell count was in agreement with data reported by Sherman et al.10 on 13 HIV seropositive and 30 HIV seronegative subjects. On the other hand, Eyster et al.12 reported a higher HCV-RNA load in 37 HIV+ hemophiliacs than in 17 HIV+ hemophiliacs, with a correlation between HCV-RNA load and CD4+ cell count. In agreement with Teffer2 and Sherman,10 we did not find any correlation between HCV-RNA load and CD4+ lymphocyte count in 39 HCV/HIV coinfected patients with coagulopathy.

Of the 39 HCV/HIV coinfected patients, five (12.8%) had liver failure; by contrast none of the 105 HCV+/HIV had liver failure, which is consistent with data reported by Eyster,1 Teffer2 and Makris.4

The five patients with liver failure had a significantly high HIV-RNA load, low CD4+ lymphocyte count and advanced liver disease as suggested by ultrasonographic evaluation. Ultrasonography is considered an accurate method for predicting the diagnosis of decompensated liver cirrhosis.20 Recently, ultrasonographic liver surface nodularity and average speed of portal flow have been shown to be independently associated with the diagnosis of compensated liver cirrhosis.21 The average speed of portal flow was significantly reduced in the five patients with liver failure reported in this study.

Potential confounders to the clinical association of worsening liver disease in our group of HCV/HIV coinfected patients could be concomitant or prior administration of hepatotoxic drugs, presence of other infections diseases that may affect the liver (e.g. Mycobacterium avium, Cytomegalovirus) and the genotype of the HCV strain.

That hepatotoxic drugs had a role in determining liver failure in our five patients who received zidovudine or didanosine, sulphasemethoxazole and trimethoprim cannot be excluded because the patients who received anti retroviral and prophylactic therapy did not undergo histologic liver evaluation.

None of our coinfected patients with severe coagulopathy developed Mycobacterium avium complex disease or Cytomegalovirus infection.

It has been suggested that the genomic heterogeneity observed in HCV infection could have epidemiological, clinical and therapeutic implications.22 Recently, it has been reported that liver damage in HIV+ patients with chronic hepatitis C seems to be directly influenced by HCV genotype 1b.23 Other authors, however, have found that HIV infection is responsible for an increase in HCV viremia, irrespective of HCV genotype.24 Moreover, despite the fact that different hepatitis C virus genotypes have been hypothesized to have potentially different relative infectivity and pathogenicity in hemophiliacs, no evidence supporting these findings was found by Jarvis et al.25

Another important point to consider is the duration of the HCV infection. Several studies26,27 have shown that HCV infection may induce a deleterious effect in case of concomitant HIV infection. Nevertheless, many years are needed to observe significant morbidity and mortality related to HCV infection. Mean estimated duration of HCV infection (> 20 years) was similar in the two groups (Table 1) of patients evaluated in this study and emphasizes the influence of HIV disease on HCV infection.

Chronic hepatitis C is considered an emergent problem in the management of HCV/HIV coinfected patients with coagulopathy both because of the risk of death4 and because of the low therapeutic index of interferon treatment.28,29 Recently, several studies30-32 showed that in HCV/HIV coinfected patients, protease inhibitor antiretroviral therapy reduces HIV replication and increases CD4+ lymphocyte count, even if it does not seem to reduce HCV-RNA load.

The absence of other cases of liver failure over 30 months of follow-up in the remaining coinfected patients with coagulopathy may support the hypothesis that therapy with protease inhibitors improves immune function in HCV/HIV coinfected patients with coagulopathy.

In conclusion, HCV-RNA load was higher in HIV+ than in HIV+ patients with coagulopathy. Liver failure was found in HCV/HIV coinfected coagulopathic patients with severe immunodepression, expressed either by low CD4+ lymphocyte count or by high HIV-RNA load. A longitudinal study is essential in order to establish whether HIV disease influences the outcome of HCV infection.

Contributions and Acknowledgments
FD, AC and GG were the principal investigators, designed the study and wrote the paper. MGM was involved in patients management. SV was involved in the evaluation of HIV-RNA load. UDC and MET performed statistical analysis. GP contributed by the evaluation of HCV-RNA load. VS was involved in the radiological and ultrasonographic evaluation. PM reviewed the manuscript.

We would like to thank Dr. Flavia Chiariotti and Dr. Maria Puopolo (Laboratory of Organ and System Pathophysiology, Istituto Superiore di Sanità, Rome, Italy) for the evaluation of estimated date of HIV seroconversion in HIV+ patients evaluated in this study.
Disclosures
Conflict of interest: none.
Redundant publications: <50%. Preliminary data from this study were presented at the XVIth Congress of the International Society on Thrombosis and Haemostasis, held in Florence, Italy, June 6-12, 1997, abstract n. 1849.

Manuscript processing
Manuscript received November 20, 1998; accepted March 10, 1999.

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