CONCISE COMMUNICATION

Lack of Association of Hepatitis C Virus Load and Genotype with Risk of End-Stage Liver Disease in Patients with Human Immunodeficiency Virus Coinfection

James J. Goedert,1 Angelos Hatzakis,4 Kenneth E. Sherman,2 and M. Elaine Eyster,3 for the Multicenter Hemophilia Cohort Study

In hepatitis C virus (HCV) infection, virus load and the risk for HCV-related end-stage liver disease (ESLD) are increased among persons with human immunodeficiency virus (HIV) coinfection. To clarify these relationships, 42 hemophilic patients who developed ESLD and random samples from 164 hemophilic patients with HCV infection alone and 146 with HCV-HIV coinfection were tested for HCV load and genotype. HCV genotype was unrelated to HIV and age. In contrast, HCV load was higher with older age (Pload = .0001) and with HIV coinfection (6.2 vs. 5.9 log10 genome equivalents/mL, P = .0001). During 16 years of follow-up of dually infected patients, ESLD risk was unrelated to HCV load over all (Pload = .64) or separately to HCV genotype 1 and genotypes 2 or 3 (Pload > .70). Irrespective of virus load, incidence of ESLD was marginally increased 2-fold (95% confidence interval, 0.8–5.6) with HCV genotype 1. Understanding the discordance between HCV load and ESLD, despite HIV’s link to each of these, may help clarify the pathogenesis of HCV-related disease.

Methods

Participants and specimens. The distributions of HCV load and genotypes were examined in stratified random samples of HCV-HIV coinfected and HCV-positive, HIV-negative participants of the Multicenter Hemophilia Cohort Study (MHCS): 20 participants each were from 8 birth cohorts (1 cohort consisting of those born before 1950, six 5-year cohorts of those born between 1950 and 1979, and 1 cohort of those born after 1979) [6]. Only MHCS centers in the United States were included. ESLD was defined as persistent ascites, bleeding esophageal varices, hepatic encephalopathy, or death, excluding nonhepatic causes [1]. The random samples did not differ from the MHCS US population in severe hepatitis A prevalence (P = .80), cumulative 16-year survival rate (P = .44), or ESLD incidence rate (P = .51; data not shown). Of the participants in the random samples, 310 (97%) had sufficient repositored specimens for determination of HCV load and genotype. In addition to the 12 ESLD case patients in the coinfected random sample, the 30 other coinfected US ESLD case patients with a prediagnostic specimen were included for case-cohort analysis of ESLD risk [7]. Differences over time in HCV load by HIV and ESLD status were evaluated by testing a second specimen of the prediagnostic specimen for determination of HCV load and genotype. In addition to the 12 ESLD case patients in the coinfected random sample, the 30 other coinfected US ESLD case patients with a prediagnostic specimen were included for case-cohort analysis of ESLD risk [7]. Differences over time in HCV load by HIV and ESLD status were evaluated by testing a second specimen of the same type (serum or plasma) that was obtained ~5 years earlier.

Virus load and genotype testing. HCV load was determined with branched DNA (bDNA) technology (Quantiplex HCV RNA 2.0 assay, Chiron) with a lower limit of sensitivity of 200,000 (5.3 log10) genome equivalents (GEq/mL). HCV load was transformed to log10 for all analyses, and, for continuous analyses, it was input as 100,000 (5.0 log10) GEq/mL for bDNA-negative specimens. For
genotyping, HCV RNA was reverse transcribed and amplified by the polymerase chain reaction (PCR), using nested biotinylated primers to the highly conserved 5′ noncoding region developed in our laboratory (A.H.) and/or using a commercially available HCV RNA assay (Amplicor HCV, Roche Diagnostic Systems). Genotyping of the PCR products was done on the labeled amplifieds, using a line-probe assay (LiPA, version 1.0; Immunogenetics) [8]. Specimens with no PCR product for the LiPA were considered to be PCR negative. Specimens with atypical band patterns were referred to as genotype unspecified. The rest were grouped into major genotypes/subtypes (HCV-1a, -1b, -2, -3, or -4).

Statistical analysis. The coinfected cohort was followed, on average, for 16 years from individual input HIV seroconversion dates [6] to the date of the onset of ESLD, death, or last follow-up (typically in late 1998). Use of alternate starting dates, such as individual birth dates or the median HIV seroconversion date for the MHCS as a whole, had no substantive effect on the results (data not presented). Proportional hazards modeling (Epicure, HiroSoft International) for a case-cohort study [7] was used to determine the relative hazard and 95% confidence interval of ESLD by HCV genotype and virus load, adjusted for age. The relationship of HCV load to HIV status, genotype, hemophilia type and severity, and age was examined by analysis of variance. A Student’s t test was used to compare change in HCV load by HIV or ESLD status. The distribution of genotypes by birth cohort and HIV status was tested by χ² and Fisher’s exact tests.

Results

Of the 310 randomly selected US hemophilic participants, 173 (56%) had HCV genotype 1, including 92 (30%) with subtype 1a and 81 (26%) with subtype 1b. Thirty-two participants had genotype 2, 30 had genotype 3, and 3 had genotype 4. Seven participants (1% of HIV-negative and 3% of HIV-positive participants) had multiple genotypes detected, 17 (5%) had genotypes that could not be specified, and 48 (15%) were HIV negative, as determined by PCR. Genotypes did not differ by birth cohort or HIV status (table 1), but significantly more HIV-negative than HIV-positive participants had PCR-negative results (23% vs. 8%, P = .0001).

HCV load was higher among HIV-positive, compared with HIV-negative, participants (6.2 vs. 5.9 log₁₀ GEq/mL, P = .0001), and, over a mean of 4.9 years, it increased even more (0.42 vs. 0.16 log₁₀ GEq/mL, P = .0002). HCV load also was higher with older age, for both the HIV-negative participants (0.017 log₁₀ GEq/mL per year, P = .0001) and the HIV-positive participants (0.018 log₁₀ GEq/mL per year, P = .0001) (figure 1).

Irrespective of HIV status, mean HCV load was 6.3 log₁₀ GEq/mL with HCV types 1a, 1b, and 2, slightly lower (6.1 log₁₀ GEq/mL) with genotype 3, and nonsignificantly higher (6.6 log₁₀ GEq/mL) among the 7 participants with multiple genotypes. Likewise, mean virus loads were 6.1 log₁₀ GEq/mL in participants with mild, moderate, or severe hemophilia A or with factor VIII inhibitors. HCV load was lower (5.8 log₁₀ GEq/mL) among participants with hemophilia B, but this difference was not significant with adjustment for HIV status and age (data not shown).

Risk of ESLD with HCV-HIV coinfection appeared to be 2-fold higher with genotype 1 than with genotype 2 or 3 (P = .09; table 1). ESLD risk was unrelated to HCV load overall.

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of participantsa</th>
<th>HIV-negative (n = 164)</th>
<th>HIV-positive (n = 146)</th>
<th>No. of HIV-positive case patients with ESLD</th>
<th>Adjusted relative hazard (95% CI)b</th>
<th>Pc</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCV subtype or genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1a</td>
<td>38</td>
<td>54</td>
<td>21</td>
<td>1.0 (referent)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1b</td>
<td>46</td>
<td>35</td>
<td>11</td>
<td>0.9 (0.4–2.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>13</td>
<td>19</td>
<td>3</td>
<td>0.4 (0.1–1.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>17</td>
<td>13</td>
<td>3</td>
<td>0.5 (0.1–2.1)</td>
<td>.63</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>Not evaluable</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multiple</td>
<td>2</td>
<td>5</td>
<td>0</td>
<td>Not evaluable</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative or unspecified</td>
<td>47</td>
<td>18</td>
<td>4</td>
<td>0.7 (0.2–2.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCV genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>84</td>
<td>89</td>
<td>32</td>
<td>2.0 (0.8–5.6)</td>
<td></td>
<td>.09</td>
</tr>
<tr>
<td>2 or 3</td>
<td>30</td>
<td>32</td>
<td>6</td>
<td>1.0 (referent)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCV load, log₁₀ GEq/mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5.3d</td>
<td>63</td>
<td>27</td>
<td>7</td>
<td>1.0 (referent)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.3–5.99</td>
<td>26</td>
<td>23</td>
<td>10</td>
<td>0.9 (0.3–3.0)</td>
<td>.64 (trend)</td>
<td></td>
</tr>
<tr>
<td>6.0–6.99</td>
<td>56</td>
<td>68</td>
<td>13</td>
<td>0.6 (0.2–1.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.0</td>
<td>19</td>
<td>28</td>
<td>12</td>
<td>0.9 (0.3–2.8)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NOTE. GEq, genome equivalents.
a Stratified random sample of US hemophilic population.
b Comparison of ESLD case patients with HIV-positive participants without ESLD, adjusted for age, using 3 strata: 0–16, 17–32, and 33–86 years.
c Log-rank tests for genotype; 1df trend test for HCV load.
d Limit of sensitivity of the assay.
Figure 1. Hepatitis C virus (HCV) load among participants of the Multicenter Hemophilia Cohort Study, by age (at time of sampling) and human immunodeficiency virus (HIV) infection status. Virus load was higher in HIV-positive than in HIV-negative subjects and increased with age in both groups. HCV RNA was measured by branched DNA (bDNA) technology; specimens with no detectable viremia, by polymerase chain reaction, were excluded. For reference, \(6.0 \log_{10}\) is \(10^6\) viral genome equivalents (GEq/mL). The lower limit of detection is \(5.3 \log_{10}\) (200,000 GEq/mL); specimens with levels not detected by bDNA were set to \(5.0 \log_{10}\) (100,000 GEq/mL).

\(P_{\text{trend}} = .64\); table 1) and in subgroups with HCV genotype 1 or genotypes 2 or 3 \(P_{\text{trend}} = .70\) and .81, respectively). Over a mean interval of 4.9 years between paired samples, HCV load increased 0.48 \(\log_{10}\) GEq/mL in coinfected participants who developed ESLD, compared with 0.41 \(\log_{10}\) GEq/mL in coinfected participants who did not develop ESLD \(P = .61\).

**Discussion**

ESLD in an HCV-HIV coinfected patient with hemophilia is a major clinical event that often leads rapidly to death [1, 4]. HCV-related liver fibrosis and, presumably, ESLD are associated with duration of infection, older age, male sex, high alcohol consumption, HIV infection, and low CD4+ lymphocyte count [2, 9]. We found recently that ESLD risk was increased with an atypical HCV antibody pattern (high anti-c100 and low anti-c22) [5]. We studied 310 patients who are highly representative of the US hemophilia population, to clarify the interaction of HIV with HCV genotypes, virus load, and ESLD risk.

Overall, with or without HIV, we found no relationship between HCV load and genotype, as noted usually but not always [3, 10, 11]. The associations noted between HCV load and HIV, including a significantly higher prevalence of HCV viremia and a nonsignificantly higher prevalence of multiple genotypes, deserve further study. HIV-related immune dysfunction presumably impairs clearance of HCV, but the components involved are unknown. Detection of multiple genotypes, as well as switches in genotypes over time [3, 8], in this repeatedly exposed population were not limited to those with HIV and may be a function of HCV load, which was 0.3 \(\log_{10}\) GEq/mL higher than in those with a single HCV genotype. The distribution of genotypes that we found was similar to that found in a previous study [12]. In addition, our representative sampling enabled us to show that HCV genotypes were independent of birth cohort, suggesting that there were no large genotype shifts in the plasma donor population before the mid-1980s.

As in the study by Thomas et al. [13], HCV load in our study was higher with older age. In addition, we and others found that HCV load was much higher and increased more steeply over time with HIV coinfection [3, 13, 14]. Despite this association, age-adjusted ESLD risk was unrelated to HCV load in the coinfected participants overall and in the coinfected subgroups with HCV genotype 1 or with genotypes 2 or 3. As in
other studies [3, 10, 11], our power to investigate subgroups
was limited except with genotype 1. Irrespective of virus load,
the risk of ESLD was nonsignificantly increased 2-fold for coin-
fected participants with HCV genotype 1. In contrast, in a
recent multivariate analysis of 125 HCV-HIV coinfected and
185 HCV singly infected hemophilic patients, genotype 1 was
associated with a 7.8-fold (95% confidence interval, 2.0–30.8)
increased risk of liver death [4]. Additional studies will be
needed to reconcile these findings. We found no difference in
ESLD risk between HCV subtypes 1a and 1b.

In summary, HIV infection is associated with increased HCV
load, with an increased rate of liver fibrosis [9], and with ESLD
[1, 3, 4]. Despite these links, we found that HCV load was not
associated with ESLD, even in subgroups defined by HCV ge-
notype. This paradox is quite unlike the straightforward as-
sociation between HIV load and AIDS, perhaps reflecting the
relationship between liver fibrosis and intrahepatic T lympho-
cyte cytotoxicity, which appears to be unrelated to HCV load
[15]. These and other such studies are likely to lead to improved
understanding of the pathogenesis of HCV-related diseases and
their treatment and prevention.

Acknowledgements

We thank Brad Goebel, Mike Walters, and Andrea Stossel (Scientific
Applications International Corporation), for performing the assays;
Susan Wilson (Research Triangle Institute) and Frances Yellin (Com-
puter Sciences Corporation), for computer programming; and Virginia
Lamprecht and Barbara Kroner (Research Triangle Institute), for study
management. We are especially grateful to the study participants, he-
mophilia center staff members, and collaborators of the Multicenter
Hemophilia Cohort Study for their tireless contributions.

References

1. Eyster ME, Diamondstone LS, Lien JM, Ehmman WC, Quan S, Goedert JJ.
   Natural history of hepatitis C virus infection in multitransfused hemo-
   philiacs: effect of coinfection with human immunodeficiency virus. J Ac-
3. Eyster ME. Effect of HIV infection on hepatitis C. Viral Hepatitis Rev 1998;4:
   189–206.
   of HCV in a cohort of haemophilic patients infected between 1961 and
5. Goedert JJ, Hatzakis A, Maloney EM, Eyster ME. Increased liver decompensation
6. Kroner BL, Rosenberg PS, Aledort LM, Alvord WG, Goedert JJ. HIV-1 in-
   fection incidence among persons with hemophilia in the United States and
7. Wacholder S, Gail M, Pee D. Selecting an efficient design for assessing ex-
   posure-disease relationships in an assembled cohort. Biometrics 1991;47:
   63–76.
   and changes in hepatitis C virus genotypes among multitransfused persons
   with hemophilia. The Multicenter Hemophilia Cohort Study. J Infect Dis
   human immunodeficiency virus and hepatitis C virus coinfected patients.
10. Ahmed MM, Mutimer DJ, Martin B, Elias E, Wilde JT. Hepatitis C viral
    load, genotype and histological severity in patients with bleeding disorders.
11. Perez-Gracia T, Galan F, Fernandez-Gutierrez C, Giron JA, Rodriguez-
    Iglesias M. Relationship of hepatitis C viremia to HIV state and to in-
    virus genotyping systems to sera from chronic hepatitis C patients in the
14. Eyster ME, Fried MW, Di Bisceglie AM, Goedert JJ. Increasing hepatitis C
    virus RNA levels in hemophilia: relationship to human immunodefici-
    ency virus infection and liver disease. The Multicenter Hemophilia Co-
15. Nelson DR, Marousis CG, Davis GL, et al. The role of hepatitis C virus
    specific cytotoxic T lymphocytes in chronic hepatitis C. J Immunol 1997;
    158:1473–81.