Short communication

Low current and nadir CD4+ T-cell counts are associated with higher hepatitis C virus RNA levels in the Swiss HIV Cohort Study

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Background: The aim of this study was to evaluate the effect of CD4+ T-cell counts and other characteristics of HIV-infected individuals on hepatitis C virus (HCV) RNA levels.

Methods: All HIV–HCV-coinfected Swiss HIV Cohort Study participants with available HCV RNA levels and concurrent CD4+ T-cell counts before starting HCV therapy were included. Potential predictors of HCV RNA levels were assessed by multivariate censored linear regression models that adjust for censored values.

Results: The study included 1,031 individuals. Low current and nadir CD4+ T-cell counts were significantly associated with higher HCV RNA levels (P=0.004 and 0.001, respectively). In individuals with current CD4+ T-cell counts <200/μl, median HCV RNA levels (6.22 log10 IU/ml) were +0.14 and +0.24 log10 IU/ml higher than those with CD4+ T-cell counts of 200–500/μl and >500/μl. Based on nadir CD4+ T-cell counts, median HCV RNA levels (6.12 log10 IU/ml) in individuals with <200/μl CD4+ T-cells were +0.06 and +0.44 log10 IU/ml higher than those with nadir T-cell counts of 200–500/μl and >500/μl. Median HCV RNA levels were also significantly associated with HCV genotype: lower values were associated with genotype 4 and higher values with genotype 2, as compared with genotype 1. Additional significant predictors of lower HCV RNA levels were female gender and HIV transmission through male homosexual contacts. In multivariate analyses, only CD4+ T-cell counts and HCV genotype remained significant predictors of HCV RNA levels.

Conclusions: Higher HCV RNA levels were associated with CD4+ T-cell depletion. This finding is in line with the crucial role of CD4+ T-cells in the control of HCV infection.

Introduction

Chronic hepatitis C virus (HCV) infection is a leading cause of death in HIV-infected individuals [1,2]. Co-infection with HIV is associated with faster progression to liver cirrhosis, a higher incidence of liver failure and higher levels of HCV viraemia [3–6]. The loss of HCV-specific T-cells in HIV infection contributes to the detrimental effects of HIV on HCV infection [7,8]. The effect of a reduced CD4+ T-cell count and other characteristics of HIV-infected individuals on HCV RNA levels is uncertain. Some reports suggest an inverse relationship between CD4+ T-cell count and HCV RNA levels [9–12]; however, other studies found no significant correlation between these parameters [13–15]. Older age [16], higher HIV RNA levels [9,13], higher alanine aminotransferase (ALT) levels [10], infection with HCV genotype 1 and HCV genotype 1 subtypes [16,17], higher body mass index and higher fibrosis scores [16] have been associated with higher HCV RNA levels. Although there is no clear evidence that HCV RNA levels influence the natural course of liver disease (reviewed in [18,19]), there is good evidence that HCV RNA levels are predictors of treatment response to pegylated interferon/ribavirin [20–22].

The aim of this study was to characterize the effect of CD4+ T-cell count and demographic and clinical
characteristics on HCV RNA levels in the Swiss HIV Cohort Study (SHCS).

Methods

The SHCS (www.shcs.ch) includes the majority of HIV–HCV-coinfected individuals in Switzerland. HCV serological testing is performed at study entry and subsequently every 2 years with a third-generation ELISA and confirmed by immunoblot. HCV RNA levels have been measured routinely since 2002 in all HCV-seropositive individuals.

HCV RNA was measured in 1,702 (58%) of 2,950 HCV-seropositive SHCS participants. Death or loss to follow-up before introduction of routine HCV RNA measurements was the most common reason (67% of cases) for not measuring HCV RNA levels despite HCV seropositivity. From the individuals with available HCV RNA results, 1,396 (82%) were found to have positive quantitative HCV RNA levels. We included only individuals with HCV RNA levels determined by the Roche COBAS Amplicor system (Roche Diagnostics, Rotkreuz, Switzerland) before starting HCV therapy with concurrent (within 1 month) measurement of CD4+ T-cell counts. All study participants gave written informed consent.

HCV RNA levels were measured in the laboratories participating in the SHCS by the COBAS Amplicor HCV Monitor assay and expressed as IU/ml. The first HCV RNA measurement was chosen for analysis, all measurements were performed before starting HCV therapy. Twenty-four percent of the HCV RNA measurements were above the upper limit of detection and were termed ‘censored’ for the statistical analyses. As the exclusion of censored values would have biased the results towards lower HCV RNA levels, we included all available values. Due to changing estimations of the linear range of the COBAS Amplicor system during the study period, the upper limit of detection varied: of those censored, 51% were censored at 0.5 IU/ml, 18% at 0.7 IU/ml, 23% at 0.85 and 8% at 1×10^6 IU/ml. Serial dilutions to determine HCV RNA levels above the upper limit of detection were performed at the discretion of the treating physicians. In these cases, the uncensored values were used for analysis. Censored linear regression models that adjust for censored values were used to estimate the effects of the different predictors on HCV RNA levels. Owing to the inclusion of censored values, medians were determined by Kaplan–Meier analysis. The statistical package STATA 9.2 was used for analyses.

Results

Table 1 shows HCV RNA levels according to demographic characteristics. In unadjusted analyses, female sex and HIV transmission through male homosexual contacts were significantly associated with lower HCV RNA levels. Age and ALT levels, which were correlated with HCV RNA levels in previous studies [9–11], were not significant predictors of viral loads. Body weight and HCV subtypes 1a/1b, which have been shown to correlate with HCV RNA levels [9–11], were not significant predictors in our study.

HCV RNA levels according to immunological and virological characteristics are shown in Table 2. Lower CD4+ T-cell counts within 1 month (‘current’) of the measurement of HCV RNA and lower nadir CD4+ T-cell counts were associated with higher HCV RNA levels in unadjusted censored linear regression. As compared with HCV genotype 1, HCV genotype 4 was associated with lower HCV RNA levels, whereas HCV genotype 2 was associated with higher HCV RNA levels.

All patient characteristics shown in Tables 1 and 2 were adjusted for variables with a significant association in unadjusted analyses (sex, transmission mode of HIV, HCV genotype and CD4+ T-cell count). As current and nadir CD4+ T-cell counts were strongly correlated (P<0.0001, R²=0.37), the results shown in Table 1 were adjusted for current CD4+ T-cell counts only. In adjusted analyses, only CD4+ T-cell count and HCV genotype were significant predictors of HCV RNA levels. The same factors were significantly associated with HCV RNA levels when adjusting with nadir CD4+ T-cell counts.

The inverse correlation between HCV RNA levels and current and nadir CD4+ T-cell counts remained when excluding censored HCV RNA levels (unadjusted P-values 0.001 and <0.001, adjusted P-values 0.03 and <0.001 for current and nadir CD4+ T-cell counts, respectively). Similarly, the association between HCV genotypes and HCV RNA levels remained when excluding censored values (unadjusted P-values 0.03 and 0.007, adjusted P-values 0.03 and 0.02 for the association with HCV genotypes 2 and 4, respectively). The proportion of censored measurements did not correlate with CD4+ T-cell counts, HCV genotypes, sex or year of measurement of HCV RNA levels (P-value >0.3 for all comparisons).

We stratified individuals according to being never (<1% of days), partially (1–90%) or always (>90% of days) treated with antiretroviral drugs in the year before measuring HCV RNA levels. We found no significant correlation between these categories and HCV RNA levels (Table 2). A similar trend for an inverse correlation between HCV RNA levels and current CD4+ T-cell counts was present in all three groups stratified by antiretroviral therapy (ART) duration (β-coefficients -0.01, -0.03 and -0.01; P-values 0.1, 0.02 and 0.09 for individuals never, partially or always treated).
Individuals with <200 CD4+ T-cells/µl and detectable (>400 copies/ml) HIV RNA had higher HCV RNA levels than individuals with <200 CD4+ T-cells/µl and undetectable HIV RNA (6.53 versus 6.20 log10 IU/ml; \( P = 0.02 \)). Higher HCV RNA levels in individuals with detectable versus undetectable HIV RNA were also present for those with 200–500 and >500 CD4+ T-cells/µl, however, these differences were not statistically significant.

All patient characteristics shown in Tables 1 and 2 were included in a multiple censored linear regression analysis (Table 3). Predictors with a \( P \)-value >0.05 were stepwise removed from the models. Due to the strong correlation of nadir and current CD4+ T-cell counts, two separate analyses were performed adjusting for either current or nadir CD4+ T-cell count. Lower current (model 1) and nadir (model 2) CD4+ T-cell counts and HCV genotypes were the only significant predictors of HCV RNA levels.

**Discussion**

HCV RNA levels were inversely correlated with current and nadir CD4+ T-cell counts and differed markedly between HCV genotypes. The associations between HCV RNA levels and CD4+ T-cell counts were similar in untreated and treated individuals and after excluding censored HCV RNA measurements. As current and nadir CD4+ T-cell counts were highly correlated, we could not assess the relative importance of current versus nadir CD4+ T-cell counts.

Our findings are concordant with some previous reports showing a significant association of lower CD4+ T-cell counts with higher HCV viral loads [9–11]. Thomas and colleagues [9] found higher HCV RNA levels in individuals with lower CD4+ T-cell counts and two studies in haemophiliacs demonstrated an inverse relationship between CD4+ T-cell count and HCV RNA levels [10,12]. In a multivariate analysis, a CD4+ T-cell count <400/µl was the only factor significantly associated with higher HCV RNA levels [11]. Fishbein and colleagues [13] found similar median HCV titres to those in our study (6.03, 5.96 and 5.88 log10 IU/ml for <200, 200–500 and >500 CD4+ T-cells/µl, respectively); however, these differences were not statistically significant in a smaller population (\( n = 142 \)). Other studies found no association between CD4+ T-cell counts and HCV RNA levels [14,15].

Previous studies have also found higher HCV RNA levels in individuals infected with HCV genotype 1 compared with other genotypes [17]. To our knowledge, our study is the first report of an association between
HCV genotype 4 infection and lower HCV RNA levels measured by the Roche Amplicor system. Chevaliez and colleagues [23] found a substantial underestimation of HCV RNA levels in genotype 2 and genotype 4 samples using the COBAS Taqman platform. Although all HCV measurements have been performed by the Roche Amplicor assay, which should provide consistent results for all genotypes analysed, we cannot exclude that our results might have been influenced by genotype-specific amplification of PCR products.

HCV RNA levels increase shortly after initiation of ART, possibly followed by a gradual decrease in the long-term associated with better immunological control of HCV [24–27] (reviewed in [28]). Because the time-points of HCV measurements were independent of the commencement of ART, we could not assess the effect of ART initiation on HCV RNA levels.

The small absolute effects of the predictors point to further important determinants of HCV RNA levels not analysed here. For example, we could neither address the effect of innate immune responses nor the role of HCV-specific cellular immunity. Considering the complex virus–host interactions that determine HCV RNA levels, it is perhaps not surprising that the absolute effects of

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<th>Table 2. Immunological and virological characteristics and HCV RNA levels in 1,031 HIV–HCV-coinfected individuals</th>
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<td><strong>Patient characteristic</strong></td>
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<tr>
<td>Current CD4+ T-cell count CDC category</td>
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<tr>
<td>&lt;200/ml</td>
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<td>Nadir CD4+ T-cell count CDC category</td>
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<td>HCV genotype</td>
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<td>ART during past year</td>
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<td>Never treated</td>
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<td>Partially (1–90% of days)</td>
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<td>Continuously (&gt;90% of days)</td>
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*The total number of patients does not always add up to 1,031, as not all characteristics were available in all patients within 1 month of measurement of HCV RNA levels. †Censored linear regression as implemented in the statistical package STATA 9.2. To satisfy normality assumptions, CD4+ T-cell counts were square-root-transformed for statistical analysis. ‡Censored linear regression adjusted for sex, risk of HIV transmission, HCV genotype, and current CD4+ T-cell count. § Value given per CD4+ T-cell increase (square-root-transformed). CDC, Centres for Disease Control and prevention; HCV, hepatitis C virus.

<table>
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<th>Table 3. Significant predictors of HCV RNA levels in multivariate analysis</th>
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<td><strong>Variable</strong></td>
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</tr>
<tr>
<td>Intercept</td>
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<tr>
<td>CD4+ T-cell count</td>
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<tr>
<td>HCV genotype 2*</td>
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<td>HCV genotype 4*</td>
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*Compared with HCV genotype 1. CI, confidence interval.
single predictors are small and that different predictors were identified in diverse populations. Nevertheless, our findings are in line with functional studies that demonstrate a crucial role for CD4+ T-cell responses for the control of HCV infection [29,30].

In conclusion, HCV RNA levels were independently associated with CD4+ T-cell depletion as well as HCV genotype. Our study provides further evidence that HIV-associated immune deficiency contributes to the higher HCV RNA levels observed in HIV–HCV-coinfected individuals. The slower progression of liver disease observed in individuals on effective ART [1,31] supports consideration of earlier ART initiation in HIV–HCV-coinfected individuals. Longitudinal studies are warranted to definitively clarify whether ART-induced CD4+ T-cell gains lead to a decrease in HCV RNA levels and whether such a decrease is substantial enough to improve the outcome of HCV therapy, as lower HCV RNA levels predict a better response to pegylated interferon/ribavirin [20–22].

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Disclosure statement

The authors declare no conflicts of interest.

Additional file

An additional file ‘Supplementary material’ detailing members of the Swiss HIV Cohort Study can be accessed via the Volume 13 Issue 3 contents page for Antiviral Therapy, which can be found at www.intmedpress.com (by clicking on ‘Antiviral Therapy’ then ‘Journal PDFs’).

References


