Low-level liver enzyme elevations during HAART are not associated with liver fibrosis progression among HIV/HCV-coinfected patients

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Objectives: To assess the association between non-severe liver enzyme elevations (LEEs) during antiretroviral treatment and liver fibrosis in HIV/HCV-coinfected patients.

Methods: All co-infected patients from an Infectious Disease Unit who had received treatment with highly active antiretroviral therapy (HAART) for at least 12 months before undergoing a liver biopsy were included in the study.

Results: One-hundred and sixteen patients met the inclusion criteria of the study. Advanced liver fibrosis was observed in 32 (38%) of 84 patients who developed non-severe LEEs and in 11 (34%) of 32 subjects who developed severe (grade ≥ 3) LEEs, (P = 0.7). Seven (6%) of 116 patients showed grade 3 or 4 LEEs for at least 30% of the follow-up. Advanced liver fibrosis was observed in five (71%) of these patients and in 38 (35%) of the 109 subjects who did not develop long-term severe LEEs (P = 0.05). Eight (10%) of 84 patients showed grade 2 LEEs for at least 30% of the follow-up. Advanced liver fibrosis was observed in 28 (37%) of 76 subjects who did not develop long-term grade 2 LEEs and in three (38%) of eight patients who developed them (P = 0.9).

Conclusions: In HIV/HCV-coinfected patients, non-severe LEEs, whether persistent or not, are not associated with advanced liver fibrosis. On the other hand, long-term severe LEEs are associated with more severe liver fibrosis in this population.

Keywords: hepatotoxicity, HIV/HCV coinfection, antiretroviral agents, highly active antiretroviral therapy

Introduction

Human immunodeficiency virus (HIV) infection is associated with an accelerated progression of chronic hepatitis C.¹,² HIV-related immunosuppression is a factor that can modify the course of hepatitis C virus (HCV) infection.³ Thus, chronic hepatitis C progression could be attenuated as a consequence of the immune system recovery induced by highly active antiretroviral therapy (HAART). In fact, studies carried out in asymptomatic HIV/HCV-coinfected patients have shown that the mortality as a result of liver disease is increased in subjects with low CD4 cell counts.⁴,⁵ Similarly, HAART use leads to a reduction in the rate of deaths resulting from liver disease.⁴,⁵ This beneficial effect of HAART is also observed in end-stage liver disease.⁶

However, HAART-related liver toxicity could theoretically enhance liver disease progression. Hepatotoxicity grade 1 or 2, non-severe liver toxicity is a very frequent event during HAART use, particularly among HIV/HCV-coinfected patients.⁷ Usually, this is a self-limited and asymptomatic problem. Because of this, physicians seldom take notice of non-severe liver enzyme elevations (LEEs) during HAART. However, long-term low-level alanine aminotransferase (ALT) increases could translate to persistent liver inflammation, with fibrosis as the final outcome. Nonetheless, the effect of LEEs in liver fibrosis is far from clear. In this regard, we found that significant LEEs were associated with more severe liver fibrosis in HIV/HCV-coinfected patients.⁸ Mehta et al.⁹ have described that coinfected patients with significant elevations of liver enzymes for over one-third of their follow-up had an increased risk of advanced fibrosis. It is not known whether persistent non-severe (grade 2 or lower) LEEs are also associated with progression of liver fibrosis in HIV/HCV coinfection. Similarly, it remains unclear whether any pattern...
of liver enzyme increase could be related to advanced liver fibrosis.

We hypothesized that persistent non-severe LEEs could increase the risk of developing advanced liver fibrosis in HIV/HCV coinfection. Persistent low-level LEEs could reflect continued necroinflammatory activity that would eventually result in liver fibrosis progression. Thus, the aim of this study was to assess the association between non-severe LEEs, equal or lower than grade 2, and liver fibrosis in HIV/HCV-coinfected patients. Additionally, we evaluated the relationship between different patterns of liver enzyme increases and liver fibrosis in this population.

Patients and methods

Study design and patients

This is a retrospective cohort study. Patients were selected from a cohort of 1067 HIV-infected patients who are prospectively followed since 1987 at the Infectious Diseases Unit of the Hospital Universitario de Valme, in Seville, Southern Spain. Patients undergo a scheduled visit every 3 months that involves a complete physical examination and blood testing. Measurements of ALT level are included in routine blood determinations.

We included all HIV/HCV-coinfected patients who fulfilled the following inclusion criteria: (i) to have a liver biopsy to determine the severity of liver disease; (ii) to have been using HAART before the liver biopsy for at least 12 months; and (iii) to have completed all scheduled visits for at least 12 months. The baseline date for this study was the date of starting HAART.

Clinical data

The following variables related to epidemiological features and clinical and virological characteristics of HIV/HCV coinfection were collected: age, sex, risk factors for HIV and HCV infection, daily alcohol intake, nadir CD4+ cell count, baseline CD4+ cell count, baseline HIV viral load, diagnosis of AIDS, antiretroviral treatment, HCV viral load by the date of liver biopsy, HCV genotype and length of HCV infection. The duration of HCV infection was calculated from the date of HCV seroconversion or estimated from the first year of HCV infection. The duration of HCV infection was calculated following the Knodell histological activity index modified by Scheuer. A line-probe assay (INNOLIPA HCV; Innogenetics, Ghent, Belgium) was used to determine HCV genotype. CD4 cell count was measured by standard flow cytometry.

Liver biopsy

Most liver biopsies were carried out to evaluate the indication of treatment for HCV infection. Percutaneous liver biopsies were performed under ultrasonographic guidance. Histological evaluation was made on sections stained with haematoxylin–eosin and Masson’s trichrome. Liver fibrosis was scored following the Knodell histological activity index modified by Scheuer. According to this classification, fibrosis is categorized into five categories, from F0 (absence of fibrosis) to F4 (liver cirrhosis). Liver fibrosis was scored following the Knodell histological activity index modified by Scheuer. According to this classification, fibrosis is categorized into five categories, from F0 (absence of fibrosis) to F4 (liver cirrhosis). Liver fibrosis progression rate (FPR) was defined as the ratio between the liver fibrosis stage and the estimated duration of HCV infection in years.

Statistical analysis

For purposes of analysis, the study population was divided into patients who developed mild LEEs (defined as grade 0 or 1), moderate LEEs (defined as grade 2) and severe LEEs (defined as grade 3 or 4). Length of LEEs was defined as the percentage of time
presenting every grade of LEEs in relation to the whole period of follow-up.

The outcome variable of this study was liver fibrosis, which was considered both as fibrosis stage and as FPR. The stages of liver fibrosis were categorized into advanced fibrosis, stages F3 (portal-central bridging fibrosis without cirrhosis) and F4 (definitive cirrhosis), and non-advanced fibrosis, stages F0–F2. FPR was categorized by the cut-off level of 0.2, as patients with an FPR > 0.2 could progress at least one stage of fibrosis every 5 years of HCV infection.

Categorical variables are expressed as numbers (percentages). They were compared using the χ² test with the Yate’s correction or the Fishers’ test, where appropriate, and using RxC tables when more than three different groups were compared. Continuous variables are expressed as median (Q1–Q3) and were compared using the Kruskall–Wallis test.

The statistical analysis was carried out using the SPSS 11 statistical software package (SPSS, Chicago, IL, USA).

Results

Characteristics of the study population

One-hundred and sixteen patients met the inclusion criteria of the study. The characteristics of the study population are shown in Table 2. Most of the patients were middle-aged males who previously had been injecting drug users. Forty-two (36%) patients showed advanced liver fibrosis and 19 (16%) patients showed cirrhosis. The different patterns of LEEs are shown in Table 3. In the same way, the frequency and the length of use of the third drug in HAART regimens are shown in Table 4.

LEEs during antiretroviral treatment and liver fibrosis

Forty-three (37%) patients did not develop ALT increases or showed only grade 1 LEEs. Forty-one (35%) patients showed grade 2 LEEs and 32 (28%) subjects severe LEEs during the follow-up. Advanced liver fibrosis was observed in similar percentages of patients who presented different degrees of LEEs (Table 5). Variables that could have influenced the development of advanced fibrosis did not show significant differences among patients with mild, moderate or severe LEEs (Table 5).
Table 5. Advanced fibrosis and potential confounders by severity of LEEs

<table>
<thead>
<tr>
<th>Variables</th>
<th>Grade of LEE</th>
<th></th>
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<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>minimal</td>
<td>moderate</td>
<td>severe</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(grades 0–1)</td>
<td>(grade 2)</td>
<td>(grades 3–4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(n = 43)</td>
<td>(n = 41)</td>
<td>(n = 32)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Advanced fibrosis, n (%)</td>
<td>18 (42)</td>
<td>14 (34)</td>
<td>11 (34)</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>Daily alcohol intake &gt;50 g, n (%)</td>
<td>15 (35)</td>
<td>11 (28)</td>
<td>9 (28)</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>Median (Q1–Q3) length of HCV infection (years)</td>
<td>15 (13–18)</td>
<td>17 (15–19)</td>
<td>15 (12–18)</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>Median (Q1–Q3) CD4 cell count at liver biopsy (cells/mm³)</td>
<td>450 (278–723)</td>
<td>514 (424–716)</td>
<td>499 (242–743)</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Median (Q1–Q3) HCV RNA level at liver biopsy (log₁₀ U/mL)</td>
<td>2.1 (1.7–4.6)</td>
<td>3.1 (1.6–4.4)</td>
<td>3.8 (1.9–5)</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>Median (Q1–Q3) duration of HAART exposure (days)</td>
<td>1170 (720–1891)</td>
<td>1440 (1019–1818)</td>
<td>1430 (909–2125)</td>
<td>0.3</td>
<td></td>
</tr>
</tbody>
</table>

The FPR could be calculated in 101 patients. The median FPR (Q1–Q3) was 0.12 (0.06–0.2). Eight (31%) of 26 patients with severe LEEs, 10 (26%) of 38 with moderate LEEs and 9 (24%) of 37 patients with mild LEEs showed FPR > 0.2 (P = 0.6).

Patterns of ALT level increase and liver fibrosis

The most frequent patterns of ALT level increases were both plateau and peak patterns (Table 3). Peaks were found in 31 (27%) patients and plateaus in 42 (36%) patients, whereas persistent and severe LEEs were found in 7 (6%) patients.

The frequency of advanced liver fibrosis according to the different patterns of ALT level increase is shown in Table 3. LEEs grade ≥ 3 for equal or more than 30% of the follow-up on HAART were associated with more severe liver fibrosis. After excluding patients who showed LEEs grade ≥ 3, patients who developed non-severe LEEs were dichotomized as developing or not grade 2 LEEs for 30% or more of the duration of HAART. Eight (10%) of 84 patients showed long-term grade 2 LEEs. Advanced liver fibrosis was observed in 28 (37%) of 76 subjects who did not develop long-term grade 2 LEEs and in three (38%) of eight patients who developed them (P = 0.9).

Five (71%) of seven subjects who developed severe LEEs for at least 30% of the follow-up showed an FPR > 0.2, compared with 23 (24%) of 94 patients without long-term severe LEEs (P = 0.007). Seventeen (25%) of 67 patients who did not develop long-term grade 2 LEEs showed an FPR > 0.2, and two (25%) of eight patients who developed grade 2 LEEs for 30% or more of the follow-up showed an FPR > 0.2 (P = 0.4).

Discussion

In this study, we did not find that non-severe LEEs, whether transient or persistent, were associated with liver fibrosis progression. Different patterns of LEEs were also not related to liver fibrosis. In contrast, persistent severe LEEs, grade 3 or 4 hepatotoxicity observed for one-third of the follow-up or longer, were associated with advanced liver fibrosis.

We did not find any evidence of an increased risk of developing advanced fibrosis in patients with non-severe LEEs. This may be hard to understand in the case of persistent low-level LEEs. However, liver enzyme levels are not fully correlated with liver damage. Indeed, only high levels of ALT by the date of liver biopsy are associated with a modest increase in the probability of advanced fibrosis. Moreover, patients with normal ALT levels do show precirrhosis and cirrhosis, though at lower frequency than patients with elevated ALT levels. In HIV/HCV-coinfected patients, a combination of two non-invasive indexes, based on routine measurements, predicted significant fibrosis reliably. However, fibrosis could only be predicted in 41% of the patients applying both indexes sequentially. Thus, using two combinations of five blood determinations still leaves 59% of the patients with indeterminate results, in whom it is not possible to predict liver fibrosis. Thus it is not surprising that a single parameter, as is the presence of non-severe LEEs, was not predictive of liver fibrosis.

We found that persistent severe LEEs were predictors of advanced liver fibrosis. This result is in agreement with the study by Mehta et al. We also found that liver fibrosis progression was faster among patients with persistent and severe LEEs. The reason for this probably is that grade 3 or higher hepatotoxicity could be better correlated with liver necroinflammatory changes. In the study by Mehta et al., ALT levels at liver biopsy were associated with necroinflammatory activity, but ALT elevations before liver biopsy were not. Thus, severe LEEs during the follow-up could be more closely related to inflammation and necrosis at each time point. Persistent extensive hepatocyte necrosis, reflected by continued severe ALT enzyme elevations, would finally lead to increasing fibrosis. Thus, persistent severe LEEs should be taken into account in HIV/HCV-coinfected patients on HAART. On the other side, persistent severe LEEs were not very frequent in the study population, as they were observed in 6% of the patients.

This study has some limitations. First, our sample was limited to patients selected to undergo a liver biopsy. Most liver biopsies were performed as part of the evaluation to indicate hepatitis C treatment. These patients are usually more adherent with their previous follow-up and HAART than patients not selected for liver biopsy. They are less likely to be engaged in active drug or alcohol use. However, these patients represent better the effect of HAART on LEEs. Thus, the influence of confounders of liver...
enzyme levels, such as alcohol intake, is less likely. Moreover, adequate HAART exposure is more frequent. Second, patients did not undergo a liver biopsy at baseline, before starting HAART. Thus, we could only estimate liver fibrosis progression retrospectively from a single point in time. Furthermore, severe LEEs have been observed more frequently in coinfected patients with advanced liver fibrosis at baseline.\(^\text{15}\)\(^\text{16}\) Hence, the association of historical severe LEEs before liver biopsy with advanced fibrosis could be due to the pre-existence of precirrhosis or cirrhosis at baseline. So, patients with advanced liver damage would show persistent severe LEEs after starting HAART, and the stage of liver fibrosis is uncovered later in the follow-up of the patient. This is unlikely. Studies with paired liver biopsies have shown that a high proportion of coinfected patients under HAART progress at least one stage of fibrosis over a median of 3 years.\(^\text{15}\)\(^\text{16}\) The median follow-up of the study patients was 5 years, which is a period of time long enough to observe progression of fibrosis. In addition, 71% of HIV/HCV-coinfected patients with compensated cirrhosis develop decompensated cirrhosis and 29% die of liver failure within 5 years of diagnosis.\(^\text{17}\) Thus, if patients with cirrhosis at liver biopsy would have showed this stage of fibrosis at starting HAART, most of them should have shown signs of liver failure during their follow-up.

HIV/HCV-coinfected patients frequently have elevated liver enzyme levels during HAART, but many LEEs resolve spontaneously without changes in HAART regimens. However, persistent severe LEEs should not be underestimated. Coinfected patients with this pattern of LEEs could be progressing to more severe liver fibrosis. Probably, the best approach for HIV/HCV-coinfected patients would be to treat hepatitis C. If this is not feasible or HCV is not eradicated after treatment, it would be advisable for these patients to choose the less hepatotoxic HAART regimen.

**Transparency declarations**

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**References**