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The Association of HIV Viral Load with Indirect Markers of Liver Injury

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SUMMARY

This study assessed the association of HIV RNA with indirect markers of liver injury including FIB-4 index, liver enzymes and platelet counts in a high-risk Hispanic population. The data were derived from a prospective study that included 138 HIV/hepatitis C (HCV) co-infected and 68 HIV-infected participants without hepatitis C or B co-infection (mono-infected). In unadjusted analyses, detectable HIV viral load (vs. undetectable, <400 copies/ml) was associated with a 40% greater odds (OR 1.4, 95% CI: 1.1 – 1.9, $p=0.016$) of FIB-4 > 1.45 in the HIV/HCV co-infected group and 70% greater odds of FIB-4 >1.45 (OR 1.7, 95% CI: 1.0–2.8; $p=0.046$) in the HIV mono-infected group. In multivariable analyses a 1 log₁₀ increase in HIV RNA was associated with a median increase in FIB-4 of 12% in the HIV/HCV co-infected group and 11% in the HIV mono-infected group ($p<0.0001$). Among the HIV/HCV co-infected, the elevating effect of HIV RNA on FIB-4 was strongest at low CD4 counts ($p=0.0037$). Among the HIV mono-infected, the association between HIV RNA and FIB-4 was independent of CD4 cell counts. HIV RNA was associated with alterations in both liver enzymes and platelet counts. HIV antiretroviral therapy was not associated with any measure of liver injury examined. This study suggests that HIV may have direct, injurious effects on the liver and that HIV viral load should be considered when these indirect markers are used to assess liver function.

Keywords

liver disease; HIV; hepatitis C; FIB-4

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INTRODUCTION

Liver disease is now a leading cause of morbidity and mortality in HIV-infected persons in the USA and Europe [1–6], due, principally, to the high rates of hepatitis C (HCV) co-infection in these populations. In the presence of HIV, the course of HCV-related liver disease is accelerated [7], and though immune suppression is thought to be an important factor [8–11], the underlying mechanisms that accelerate liver disease in co-infection are still poorly understood. HIV may play a direct role in this, and therefore the aim of this study was to describe the association of HIV RNA with liver damage, using indirect markers, while accounting for immune suppression.

Current evidence suggests that HIV is able to damage the liver directly by mechanisms that are independent of immune function (reviewed in [12]) through direct and indirect actions on a variety of hepatic cell types [13–17]. One proposed mechanism, based on *in vitro* studies, is signaling that promotes apoptosis activated through HIV receptors present on hepatocytes. This pro-apoptotic signaling may be enhanced by interactions between HIV and HCV envelope proteins [14]. In addition, HIV may promote hepatic fibrogenesis by activation of hepatic stellate cells through CXCR4 receptors [15].

Although these data suggest that HIV is capable of directly inducing liver damage, among many clinical studies only a few have found HIV RNA levels *per se* to be predictive of liver injury or liver disease progression [18–23]. Much of the epidemiologic evidence is indirect. For example, in HIV/HCV co-infected persons, effective antiretroviral therapy (ART) is associated with less fibrosis and slower fibrosis progression [24–26]. Cumulative ART exposure and undetectable viral load are associated with less necroinflammatory activity [19], and liver-related mortality is less frequent in persons on ART [27, 28]. On the other hand, ART is also associated with liver injury (reviewed in [29]). Thus, it is possible that competing effects of HIV and ART can explain why an injurious effect of HIV on the liver has not been seen in many clinical studies.

The paucity of data on the factors promoting liver disease in high-risk HIV-infected populations may also be related to the inability to collect liver biopsies in persons in whom it is not medically indicated, or the impracticality of conducting biopsies at intervals frequent enough to capture changes in risk factor profiles. Indirect measures of liver function are currently the only feasible alternative to liver biopsy for longitudinal studies with frequent assessment intervals. The FIB-4 index is a non-invasive marker of liver fibrosis that has been validated in HIV/HCV and HCV-infected populations [30, 31], and has performed well in subsequent validation studies [32, 33]. The factors used to calculate the FIB-4 index, platelet count and liver enzymes, are readily accessible in clinical laboratories throughout the world. These features make the FIB-4 index an attractive alternative for the assessment of liver function in longitudinal studies for which liver biopsy is not possible.

In this longitudinal study, we examined the independent association of HIV RNA and related factors with the FIB-4 index and other indirect markers of liver injury in a high-risk population of Hispanics participating in a study of HIV co-morbidities associated with drug abuse.

METHODS

Study subjects

The data were derived from the BIENESTAR study, a community-based prospective cohort study in Boston, designed to examine the role of injection and non-injection drug use in

HIV-associated co-morbidities in Hispanic adults. Details of the study participants and recruitment methods have been described elsewhere [34]. Briefly, eligible participants were Hispanic adults (≥ 18 years of age) who spoke Spanish fluently and were HIV seropositive or at risk of HIV through current illicit drug use. The exclusion criteria were: pregnant at the time of recruitment, the presence of a non-HIV associated malignancy or refusal to sign a consent form to release medical records. A total of 512 participants were recruited to the BIENESTAR cohort between September 1999 and March 2009, including a control group of 93 healthy Hispanics that was recruited beginning in January 2004. Of the 512 participants, 266 were HIV-infected. Beginning in April 2002, serum amino transferase levels were measured as part of the study protocol. For this analysis, we included all 203 study participants who were HIV-infected (with or without HCV) and had at least one visit with aminotransferase data. To focus the results on HCV, we further excluded six participants who were also infected with hepatitis B. The Institutional Review Board at Tufts Medical Center approved the study, and written, informed consent was provided by all participants.

Clinical data

Participants were seen twice-yearly at the Clinical Translational Research Center of the Tufts Medical Center. Study interviews were conducted in Spanish by trained, bilingual, Hispanic personnel. Fasting blood (> 5 hours) was collected for laboratory analyses and processed immediately in the clinical laboratories of the Tufts Medical Center, or frozen at -70 degrees centigrade for later assay (HIV RNA quantification, HCV serology). Body mass index (BMI) was calculated as weight in kilograms divided by height in square meters. Information on the demographic profile, HIV history, and history of current and past use of antiretroviral agents was collected at each visit by standardized interview. At the first study visit, participants were asked to provide a detailed history on past alcohol and drug use, which was updated at each follow-up visit. Alcohol use was measured at each clinic visit as the average grams consumed over the previous six months as well as recent daily consumption. The participants were asked if they had ever been told by their health care provider that they had liver damage due to the abuse of alcohol.

Laboratory data

With the exception of HCV testing, all assays were conducted in the clinical laboratories of the Tufts Medical Center. Self-reported HIV status was confirmed by enzyme immunoassay (Genetic Systems™ HIV1/HIV2 Plus O EIA, Biorad Laboratories, Redmond, WA). HIV RNA levels were measured by reverse transcriptase polymerase chain reaction (RT-PCR) using a Roche Amplicor Monitor system (Roche Molecular Systems, Somerville, NJ), with a lower detection limit of 400 copies/mL. An undetectable viral load was given a value of 200 copies/mL, the midpoint between zero and the limit of detection. The numbers of CD4 cells were determined using a specific monoclonal antibody and fluorescence-activated cell sorting (FACS) analysis. The levels of aspartate amino transferase (AST) and alanine amino transferase (ALT) were measured on a Beckman Coulter Synchron Clinical Systems Analyzer (Beckman Coulter Inc. Fullerton, CA, USA). Specifically, AST was measured using an oxaloacetate/malate dehydrogenase method, and ALT was measured using a pyruvate/lactate dehydrogenase method. Qualitative HCV testing was conducted at the Massachusetts Department of Public Health and was based on the presence of HCV RNA in serum (AMPLICOR Hepatitis C Virus test, Version 2.0 Roche Molecular Systems Inc., Branchburg, NJ, USA).

The primary outcome variable was the FIB-4 index calculated as: $(\text{Age [year]} \times \text{AST [U/L]}) / (\text{Platelet } [10^9/\text{L}] \times \text{ALT [U/L]}^{1/2})$ [30]. The index increases with increasing probability of advanced fibrosis in HCV and HIV/HCV-infected populations [30, 31], and with other histology abnormalities seen on biopsy, including necroinflammatory activity [30]. The

FIB-4 index was used as a categorical variable with three levels < 1.45 ; 1.45 to 3.25 , > 3.25 , corresponding to the recommended cutoffs used to identify the presence ($\text{FIB-4} > 3.25$) or absence ($\text{FIB-4} < 1.45$) of advanced fibrosis [30]. It was also used as a continuous variable, to enhance statistical power. Due to its skewed distribution, continuous FIB-4 values were expressed on the natural log scale.

Statistical Analyses

Statistical analyses were done using SAS version 9.1 (SAS Inc., Cary NC). The 203 study participants contributed 1194 person-visits, the primary unit of observation, to the study. Longitudinal data analyses were conducted in which the baseline visit was deemed to be the first time point for each participant at which aminotransferase levels were available [35]. The number of months since baseline was used as the time metric. The median follow-up for the HIV/HCV co-infected group was 24 months (range: 0 to 76 months) or 4 follow-up visits. For the HIV mono-infected group, the median follow-up was 30 months (range: 0 to 80 months) or 5 follow-up visits. The characteristics of the participants at the baseline visit (Table 1) are reported as mean and standard deviation (SD) for normally distributed continuous data as determined by formal testing, or median (25th, 75th percentile) for non-normally distributed continuous data. The differences in baseline characteristics of the HIV/HCV co-infected and HIV-infected participants were tested with the Student's t-test for normally distributed variables and the Wilcoxon Rank Sum test for skewed variables, using either the raw or log transformed data, where appropriate. Chi-square or Fisher's exact test was used to test between-group differences for categorical variables. The longitudinal, repeated measures regression analyses in Tables 2–4 in which FIB-4 was treated as a continuous outcome were conducted using mixed models in SAS PROC MIXED. For analyses in which FIB-4 was treated as a categorical outcome, such as for the calculation of odds ratios, repeated measures regression for binary outcomes using generalized estimating equations was conducted in SAS using PROC GENMOD. The association of HIV RNA with the FIB-4 index was evaluated using separate multivariate analyses for the HIV/HCV co-infected and HIV mono-infected groups. HIV RNA and potential confounders were assessed in three ways: as cumulative averages up to and including baseline values; as the value at baseline; or as time-varying variables. The models shown in Tables 3–5 are based on time-varying values of HIV RNA, CD4 counts, FIB-4, AST, ALT and platelets.

Potential confounding factors evaluated in the analyses included gender, estimated duration of HIV infection (self-report), estimated duration of HCV infection (defined as date of first reported injection drug use plus 6 months), BMI, alcohol use, and current/past use of illicit drugs. Potential confounding by ART was evaluated using a variable that described ART use (yes, no), or variables describing the duration of ART use, the class of ART (PI or NRTI) used, or the duration of use of each class of ART. Each definition of ART was assessed as a covariate in separate models. Additional analyses were done to evaluate as a class NRTI types associated with mitochondrial toxicity (zidovudine, didanosine, zalcitabine, and stavudine) compared to NRTI types without known mitochondrial toxicity (other NRTI types including lamivudine and tenofovir). Variables deemed not to be confounders, by a criterion of $< 10\%$ change in the estimated effect of HIV RNA on the FIB-4 index, were dropped from the model unless they were significant predictors of the outcome. The exception was the variable describing alcohol use, which was left in the final models. The final model for each of the HIV/HCV co-infected and HIV mono-infected groups was used to evaluate the association of HIV RNA with the components that comprise the FIB-4 index (AST, ALT and platelet counts). AST and ALT were analyzed on the natural log scale due to their skewed distributions. To facilitate the interpretation of results derived from outcomes expressed on the natural log scale, the results in Tables 3–5 are

expressed as the percent difference in the median value of the outcome for a one unit difference in the predictor variable.

RESULTS

Participant characteristics

The majority of study participants were born in Puerto Rico (140/203, 69%) or born in the USA (40/203, 20%). Other demographic and clinical characteristics of the 138 HIV/HCV co-infected and 65 HIV mono-infected participants are shown in Table 1. Thirty-seven percent of the HIV/HCV co-infected group and 25% of HIV mono-infected group reported no current use of ART. HIV/HCV co-infected participants were less likely to be using a treatment regimen that included a non-nucleoside reverse transcriptase inhibitor (NNRTI) compared to the HIV mono-infected group. The use of NRTI agents known to be associated with mitochondrial toxicity (zidovudine, didanosine, zalcitabine, and stavudine) was 21% in the HIV/HCV co-infected and 26% in the HIV mono-infected participants. As expected, the HIV/HCV co-infected group had higher average FIB-4, AST and ALT values, and lower platelet counts than the HIV mono-infected group. In the HIV mono-infected group, elevated AST (>40 IU/L) was observed in 12% and elevated ALT (>42 IU/L) was observed in 22% of participants. The average FIB-4 value increased in the HIV/HCV co-infected participants by 5% per year (95% CI: 1.1% to 8.9%, $p=0.028$) and by 1.5 % per year (95% CI: 1.49% to 1.51%, $p=0.038$) in the HIV mono-infected participants. However, the difference in the yearly rate of increase in FIB-4 between the HIV/HCV co-infected group and the HIV mono-infected group did not achieve significance at the $\alpha=0.05$ level. The prevalence at baseline of FIB-4 values >3.25 , consistent with advanced fibrosis [30], was 16% in the HIV/HCV co-infected group and 0% in the HIV mono-infected group. However, over follow-up, two participants in the HIV mono-infected group developed FIB-4 >3.25 that persisted over 3 visits each, thereby contributing six person-visits of FIB-4 >3.25 to the longitudinal analyses (Table 2).

Participants who reported active illicit drug use at the baseline visit were less likely to be on ART (55% vs. 76%, $p=0.0016$), and had higher alcohol intake compared to non-drug users (7.2 g/d vs. 2.7 g/d, $p=0.048$). However, alcohol intake was not related to the probability of ART use ($p=0.49$), and was not correlated with HIV RNA ($p=0.72$). Five percent (3/62) of HIV mono-infected participants, and 18% (25/138) HIV/HCV co-infected participants reported at the baseline visit that they had been told by their health care provider that they had liver damage due to alcohol abuse.

The association of HIV RNA with FIB-4 in the HIV/HCV co-infected participants

The crude (unadjusted) association of HIV viral load with categories of FIB-4 in the HIV/HCV co-infected group is shown in Table 2. Higher average HIV RNA was associated with higher category of FIB-4 ($P < 0.0001$). In analyses that accounted for the repeated measures design, person-visits with a detectable HIV viral load (≥ 400 copies/ml) had 40% greater odds (OR 1.4, 95% CI: 1.1 to 1.9; $p=0.016$) of FIB-4 > 1.45 compared to person-visits with an undetectable HIV viral load.

In fully adjusted multivariate analyses HIV RNA at the same visit was a significant predictor of FIB-4 (Table 3). However, the average HIV RNA up to baseline did not predict either the baseline FIB-4 ($p=0.41$) or the increase in FIB-4 over follow-up ($p=0.39$). Lower CD4 count at the same visit was also a significant predictor of higher FIB-4, with a significant two-way interaction effect of concurrent HIV RNA and CD4 cell count on FIB-4 such that the elevating effect of HIV RNA on FIB-4 was strongest at low CD4 counts (Table 3: -2%, 95% CI: -4% to -1%, $p=0.0037$).

The association of HIV RNA with FIB-4 in HIV mono-infected participants

As was the case for the HIV/HCV co-infected group, increasing HIV RNA was associated with increasing category of FIB-4 index ($P<0.0001$) in the HIV mono-infected group (Table 2). A detectable viral load (vs. undetectable) was associated with a 70% higher odds of FIB-4 >1.45 (OR 1.7, 95% CI 1.0 to 2.8; $p=0.046$). Similar to the results in HIV/HCV co-infected participants, HIV RNA and CD4 cell counts at the same visit were significant predictors of FIB-4, while HIV RNA or CD4 from other time points were not. However, in contrast to the HIV/HCV co-infected participants, in the HIV mono-infected participants, there was no evidence of an interaction between HIV RNA and CD4 count on FIB-4 (p for the interaction=0.82).

Alcohol and drug use were not confounders of the association of HIV RNA with FIB-4. By any definition, ART was not a significant predictor of FIB-4 or confounder in these analyses.

The association of HIV RNA and CD4 cell counts with the components of the FIB-4 index: AST, ALT and platelets

To determine which of the factors used in the calculation of the FIB-4 index could explain the association of HIV RNA and CD4 cell counts with FIB-4, we used the components of the index, AST, ALT and platelet count as the dependent variables of interest. The FIB-4 index is calculated with age and AST in the numerator, and platelet count and the square root of ALT in the denominator. We used the final models without interaction shown in Tables 3 and 4, substituting FIB-4 with AST, ALT, AST/ALT ratio or platelet count. The associations of HIV RNA and CD4 cell counts with the components of FIB-4 are shown in Table 5. In the HIV/HCV co-infected participants, HIV RNA was associated with lower ALT, higher AST/ALT ratio and lower platelets. In the HIV mono-infected participants, HIV RNA was associated with higher AST, higher ALT, and lower platelets. Lower CD4 counts were associated with higher AST and lower platelets.

DISCUSSION

In this study of HIV-infected persons, among whom many had poorly controlled viral load, we found an independent association of HIV RNA with the FIB-4 index, an indirect measure of liver damage. This was explained by the association of HIV RNA with both liver enzyme levels and platelet counts. The FIB-4 index has age and AST in the numerator and platelets and the square root of ALT in the denominator. Thus, in persons with HIV mono-infection, the increase in FIB-4 values with increasing HIV RNA was due to the association of HIV RNA with lower platelets and higher AST values. In HIV/HCV co-infected persons, the increase in FIB-4 values with increasing HIV RNA was due to the association of HIV RNA with lower platelets and lower ALT values and higher AST/ALT ratio. The AST to ALT ratio increases in cirrhosis, whether through an increase in AST or a decrease in ALT [36], and this may be a driving factor for the increase in FIB-4 with progressive liver disease.

Our data, which suggest that HIV itself may play a role in liver injury, are consistent with two previous reports in HIV/HCV co-infected patients that were based on liver histology. Brau and colleagues [18] reported that higher HIV RNA was associated with faster fibrosis progression, especially among those with low CD4 counts. Mehta and colleagues found that a detectable level of HIV RNA (>400 copies/ml) was associated with a 3.8-fold higher odds of necroinflammatory activity, but not fibrosis [19]. Our results also agree with previous studies in HIV-infected persons without hepatitis C or B virus co-infection, which have demonstrated an independent association of HIV RNA with elevated AST and ALT [20, 21]. The Swiss HIV Cohort study found that HIV RNA levels greater than 100,000 copies/mL,

but not lower levels of HIV RNA, were associated with chronic, elevated ALT, independently of ART [22]. The percent of HIV mono-infected participants with elevated AST and ALT in our study is similar to that reported in another, clinic-based population [20]. A recent cross-sectional study found significant fibrosis by AST-to-platelet ratio index (APRI) scores in 8.3% of HIV-monoinfected patients [23]. None of the HIV-monoinfected participants in our study had FIB-4 indicative of significant fibrosis perhaps because they were recruited from the community and not a clinic setting.

Our results also agree with a recently published cross-sectional analysis of the HIV Epidemiologic Research (HER) Study data (1993–2000) [37]. That study found a significant association of HIV RNA with FIB-4 at the same visit in a subset of HIV mono-infected women who were hepatitis B surface antigen negative, ART naïve and who reported no alcohol intake during the past six months. However, HIV RNA was not independently associated with FIB-4 when HIV mono-infected women who were on ART were included in the analyses. This supports our view that deleterious effects of HIV RNA on the liver may be obscured in the presence of ART-related liver injury.

Mechanisms that can explain the data from these epidemiologic clinic and field studies have been described in several *in vitro* works showing that hepatocytes express co-receptors for HIV that permit cell damage by an innocent bystander mechanism [13, 14, 38]. HIV viral envelope protein, gp120 binds to CXCR4 causing hepatocyte death [13], and sensitizes hepatocytes to apoptosis mediated by TRAIL (TNF-related apoptosis-inducing ligand) by inducing hepatocyte expression of TRAIL receptor 2 [38]. It is thus plausible that higher levels of HIV RNA would lead to greater rates of apoptosis and necroinflammatory activity. Such apoptosis and necroinflammatory activity would be expected to elevate liver enzymes in a manner dependent on HIV viral load. This would be an acute effect, and, since FIB-4 is elevated in necroinflammation [30], may explain why we observed an effect of HIV RNA at concurrent time points. HIV may also cause liver damage by interactions with other cell types in the liver. For example, hepatic Kupffer and endothelial cells can be infected with HIV [15, 16]. Hepatic stellate cells express CXCR4, and activation of this receptor induces fibrogenesis [15]. Another possible mechanism of liver injury is through HIV damage to the intestinal mucosa resulting in microbial translocation and hepatic inflammation [39].

Among the HIV/HCV co-infected participants, the magnitude of the effect of HIV RNA on FIB-4 was greatest in those with low CD4 counts. In contrast, the magnitude of the effect of HIV RNA on FIB-4 was independent of CD4 cell counts in HIV mono-infected persons. This may reveal something about the interaction of HCV and HIV in the liver. Munshi et al. [14] reported a cooperative induction of hepatocyte apoptosis by HCV and HIV envelope proteins that did not occur when the cells were exposed to either protein alone. Thomas et al. [40] found that HCV RNA levels increased with increasing HIV RNA levels and decreasing CD4 counts. Thus, it is possible that in the absence of effective ART, high HIV viral load and declining CD4 counts lead to elevations in HCV RNA, which then permit high levels of cooperative hepatocyte apoptosis by HCV and HIV envelope proteins, and greater liver damage. We did not have measures of HCV RNA and therefore cannot report on the relations among HCV RNA, HIV RNA, CD4 cell counts and the FIB-4 index.

If HIV has the potential to cause liver damage, it is not clear why an association of HIV viral load and liver damage has not been reported more often. We were unable to identify more than seven previous studies demonstrating an association of HIV viral load with markers of liver damage [18–23, 37], among numerous studies that have examined predictors of liver damage in HIV-infected persons. A common factor among the studies demonstrating an association of HIV RNA with liver damage, including our own, was the high proportion of enrolled participants who were not currently on ART or had high HIV viral loads. Since

most HIV clinical studies are conducted in academic medical centers, serving populations with good quality, continuous care, it is possible that the association of HIV viral load with liver damage has been veiled by high adherence and good HIV viral load suppression in these clinic-based populations. As HIV RNA and ART are opposing causes of liver damage, ART-induced damage would be most apparent in a population with well-suppressed HIV viral load, while HIV RNA-induced liver damage would be most apparent in a population poorly-controlled HIV viral load. As ART has improved over time and mitochondrial toxicity has waned with use of later generation agents (e.g. tenofovir), our ability to see the benefits of viral suppression on liver health compared to uncontrolled HIV disease may have improved.

It is highly probable that some portion of the observed association between HIV RNA and FIB-4 is driven by HIV effects on platelet numbers unrelated to liver injury. Uncontrolled HIV viral load is a risk factor for thrombocytopenia [41], due to reductions in platelet production and survival [42]. Since platelet count is in the denominator of the FIB-4 index, HIV-associated reductions in platelet counts by these mechanisms would lead to higher FIB-4 levels. There is no means to separate the independent effects of HIV and liver damage on platelet counts. However, FIB-4 was first developed and validated as a measure of liver injury in HIV/HCV co-infected persons [30], though the effect of HIV RNA levels on the validity of FIB-4 is not described. The association of HIV RNA with alterations in AST and ALT seen in our study demonstrates that the association of HIV RNA with FIB-4 is not solely explained by the effect of HIV RNA on platelet counts. Our data should be interpreted in the context of previous studies and our current limited understanding of the interaction of HIV with all types of cellular elements.

The chronic elevations of aminotransferase levels in our cohort are of concern since Hispanics are at high risk of steatosis [43]. Steatosis by itself may not necessarily lead to fibrosis, however it may sensitize the liver, and, with an additional insult that increases inflammatory mediators, steatosis can lead to steatohepatitis, with significant implications for liver disease progression [44]. Uncontrolled HIV is associated with the expression of proinflammatory cytokines which could serve as the necessary additional insult [45].

Strengths of this study include the prospective design and longitudinal analysis, which allowed us to assess the predictors of FIB-4 at different time points and to prospectively collect data on important potential confounders and modifiers, as well as collect detailed data on current ART use. The cohort was recruited from the community, rather than being clinic-based and therefore participants represent of a wide range of HIV-infected persons that includes both those in and not in regular HIV care. The study was conducted in Hispanics and this may limit its generalizability. While FIB-4 has been validated as a measure of advanced fibrosis in HCV infected and HIV/HCV co-infected patients [30, 31], as well as patients with non-alcoholic fatty liver disease [33], its validity has not been assessed in HIV mono-infected patients, and the meaning of incremental increases in FIB-4 is not known. Other limitations of this study include its observational design and relatively few participants in some groups.

In conclusion, HIV RNA is independently associated with indirect measures of liver injury including FIB-4, liver enzymes and platelet counts in both HIV/HCV co-infected and HIV-infected persons without hepatitis co-infection. Clinicians should consider HIV RNA levels when investigating elevated liver enzymes in HIV mono-infected persons, and when using FIB-4 to assess the probability of advanced fibrosis. Further studies are needed to examine the validity of FIB-4 as a marker of liver fibrosis in persons with varying levels of HIV RNA. Prospective studies of possible sub-clinical liver disease in HIV-infected patients without chronic viral hepatitis are also needed. Reductions in HIV viral load should be

considered a goal in HIV/HCV co-infected persons due to their high risk of liver-related mortality.

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Table 1Characteristics of the 203 study participants at the baseline visit ^a

	HIV/HCV co-infected n=138	HIV mono-infected n=65
Age, years	42 (37–47)	40 (35–45)
Men	115 (83)	35 (54) [‡]
FIB-4	1.5 (1.1–2.5)	0.90 (0.66–1.18) [‡]
FIB-4 Category:		
<1.45	65 (47)	52 (80) [‡]
1.45–3.25	51 (37)	13 (20)
>3.25	22 (16)	0 (0)
AST (IU/L)	49 (31–77)	26 (22–31) [‡]
AST > 40 IU/L	88 (64)	8 (12) [‡]
ALT (IU/L)	52 (32–81)	25 (20–37) [‡]
ALT > 42 IU/L	82 (59)	14 (22) [‡]
Platelet count ($\times 10^3/\text{mm}^3$)	182 (149–244)	233 (189–278) [‡]
Platelet count < 150 ($\times 10^3/\text{mm}^3$)	36 (26)	5 (8) 0.0023 [‡]
HIV RNA, $\log_{10}$ copies/ml	3.0 (2.3–4.4)	2.7 (2.3–3.8)
HIV RNA < 400 (copies/ml)	62 (45)	31 (48)
CD4 cells/uL	381 (228–526)	412 (246–662)
CD4 < 200 cells/uL	31 (23)	12 (18)
Years with HIV infection	11 (7–14)	8 (5–11) [‡]
Years with HCV infection	22 (16–30)	NA
Currently on antiretroviral therapy	87 (63)	49 (75)
Currently on PI	51 (37)	19 (29)
Currently no NRTI	85 (62)	48 (74)
Currently on NNRTI	24 (17)	24 (37) [‡]
Current drug user	78 (57)	11 (17) [‡]
Alcohol (g/day)	0.014 (0.002–0.86)	0.11 (0 – 2.26)

	HIV/HCV co-infected n=138	HIV mono-infected n=65
Body mass index (kg/m ²)	26.0 (24.0–28.5)	25.9 (22.5 – 30.8)
<25	50 (36)	25 (38)
>=25 and <30	63 (46)	21 (32)
>=30	25 (18)	19 (29)

^a Values are mean (SD), number (%) or median (25th, 75th percentile)

HIV, human immunodeficiency virus; HCV, hepatitis C; PI, protease inhibitors, NRTI, nucleoside reverse transcriptase inhibitors; NNRTI, non-nucleoside reverse transcriptase inhibitors; AST, aspartate aminotransferase; ALT, alanine aminotransferase;

[†] p<0.01

[‡] p<0.001

Table 2

Relation of HIV viral load with categories of FIB-4 (unadjusted)

	FIB-4 category		
	<1.45	1.45 to 3.25	>3.25
	HIV/HCV co-infected		
Number of Observations ^a	n=319	n=325	n=153
Detectable HIV RNA ^b (%)	142 (45)	158 (49)	73 (48)
Mean HIV RNA ^c	3.1	3.3	3.4 [‡]
	HIV mono-infected		
Number of Observations ^a	n=315	n=76	n=6 ^d
Mean HIV RNA ^c	3.0	3.4	4.1 [‡]
Detectable HIV RNA ^b (%)	135 (43)	40 (53)	5 (83)

^a Unit of observation is the person-visit^b Detectable HIV RNA = ≥ 400 copies/ml^c HIV RNA, log₁₀ copies/ml^d Two participants contributed 3 person-visits each for n=6[‡] P for trend <0.0001

Table 3
The association of HIV RNA and CD4 cell counts with FIB-4 in HIV/HCV co-infected participants

Predictor	Unadjusted model		Adjusted Model		Adjusted Model With Interaction	
	% Difference in median FIB-4 ^a (95% CI)	p	% Difference in median FIB-4 (95% CI)	P	% Difference in median FIB-4 (95% CI)	P
HIV RNA	14% (10% to 17%)	<0.0001	12% (8% to 16%)	<0.0001	18% (12% to 26%)	<0.0001
CD4 count $\times$ 100/mm ³	--	--	-5% (-7% to -2%)	<0.0001	2% (-3% to 7%)	0.45
HIV RNA $\times$ CD4	--	--	--	--	-2% (-4% to -1%)	0.0039
HCV duration, y	--	--	1% (0.003% to 2.5%)	0.017	1% (0% to 2.5%)	0.022
Male	--	--	39% (3% to 87%)	0.031	37% (2% to 85%)	0.041
BMI, kg/m ²	--	--	-41% (-54% to 3%)	0.069	-27% (-51% to 10%)	0.13
Alcohol, ln g/day	--	--	0% (-3% to 2%)	0.82	0% (-2% to 2%)	0.90

^a Interpreted as the % difference in median value of FIB-4 for a 1 unit difference in the predictor variable

HIV RNA, log 10 copies/ml; HCV, hepatitis C virus; BMI, body mass index

Table 4
The association of HIV RNA and CD4 cell counts with FIB-4 in HIV mono-infected participants

Predictor	Unadjusted model		Adjusted Model	
	% Difference in median FIB-4 ^a (95% CI)	p	% Difference in median FIB-4 (95% CI)	p
HIV RNA	11% (7% to 15%)	<0.0001	11% (6% to 14%)	<0.0001
CD4 count × 100/mm ³	--	--	-3% (-4% to 0%)	0.009
Male	--	--	19% (-4% to 48%)	0.12
BMI, kg/m ²	--	--	-2% (-3% to 0%)	0.0094
Alcohol, ln g/day	--	--	-3% (-6% to 0%)	0.027

^a Interpreted as the % difference in median value of FIB-4 for a unit difference in the predictor variable

HIV RNA, log 10 copies/ml; BMI, body mass index

Table 5

The association of HIV RNA and CD4 cell count with liver enzymes and platelet counts in HIV/HCV co-infected and HIV mono-infected participants

Predictor	Outcome					
	AST		ALT		Platelet count	
	% Difference in median ^a (95% CI)	p	% Difference in median ^a (95% CI)	p	% Difference in median ^a (95% CI)	p
HIV/HCV co-infected participants ^b						
HIV RNA ^c	0% (-3% to 4%)	0.87	-4% (-8% to -1%)	0.020	-8% (-10% to -6%)	<0.0001
CD4 cell count × 100/ mm ³ ^c	-2% (-4% to 0%)	0.052	0% (-2% to 2%)	0.99	2% (0% to 3%)	0.015
HIV mono-infected participants ^d						
HIV RNA ^c	5% (1% to 9%)	0.028	5% (-1% to 10%)	0.091	-7% (-5% to -9%)	<0.0001
CD4 cell count × 100/ mm ³ ^c	-2% (-4% to 0%)	0.086	-1% (-4% to 1%)	0.31	2% (0% to 3%)	0.01

^a Interpreted as the % difference in median value of the outcome for a 1 unit difference in the predictor variable

^b Adjusted for body mass index, gender, years with HCV infection, alcohol use as in Table 3

^c Values at same visit as FIB-4

^d Adjusted for body mass index, gender, alcohol use as in Table 4

HCV, hepatitis C virus; HIV RNA, log 10 copies/ml; AST, aspartate aminotransferase; ALT, alanine aminotransferase