Published in final edited form as:

J Acquir Immune Defic Syndr. 2017 December 01; 76(4): 438–444. doi:10.1097/QAI.0000000000001524.

Macrophage Activation and the Tumor Necrosis Factor Cascade in Hepatitis C Disease Progression among HIV-Infected Women Participating in the Women's Interagency HIV Study (WIHS)

Audrey L French, MD^{1,2}, Jonathan W Martin, DO^{1,2}, Charlesnika T Evans, PhD³, Marion Peters, MD⁴, Seble G Kessaye, MD⁵, Marek Nowicki, PhD⁶, Mark Kuniholm, PhD⁷, Elizabeth Golub, PhD⁸, Michael Augenbraun, MD⁹, and Seema N Desai, PhD² for the WIHS

¹CORE Center/Stroger (Cook County) Hospital

²Rush University Medical Center, Chicago, IL

³Hines VA Medical Center/Northwestern University Medical Center, Chicago, IL

⁴University of California, San Francisco, SF, CA

⁵Georgetown University Medical Center, Washington, DC

⁶Mendez National Institute of Transplantation Foundation

⁷University at Albany, State University of New York, Rensselaer NY

⁸Johns Hopkins Bloomberg School of Public Health, Baltimore, MD

⁹State University of New York Downstate, Brooklyn, NY, USA

Abstract

Background—HIV/hepatitis C coinfecting persons experience more rapid liver disease progression than HCV monoinfected persons, even in the setting of potent antiretroviral therapy.

Methods—We sought to articulate the role of macrophage activation and inflammation in liver disease progression by measuring serial soluble markers in HIV/HCV coinfecting women. We compared markers measured during retrospectively defined time periods of rapid liver disease progression to time periods where little or no liver disease progression occurred. Liver disease progression was defined by liver biopsy, liver-related death or the serum markers APRI and FIB-4. Soluble CD14, sCD163, lipopolysaccharide (LPS), TNF receptor II, interleukin-6 (IL-6) and chemokine ligand 2 (CCL 2) were measured at 3 timepoints over 5 years

Results—One hundred six time intervals were included in the analysis: including 31 from liver disease progressors and 75 from non-progressors. LPS, sCD14, IL-6 and CCL2 levels did not differ in slope or quantity over time between rapid liver disease progressors and non-progressors. TNFR2 and sCD163 were significantly higher in liver disease progressors at (p=0.002 and

Correspondence and Reprints: Audrey French, Division of Infectious Diseases, Stroger Hospital of Cook County, 1900 W Polk, Chicago, IL 60612, phone: 312-864-4578, facsimile: 312-864-9496, afrench@cookcountyhhs.org.

Data were presented in part at Conference on Retroviruses and Opportunistic Infections 2015, Seattle WA

Conflicts of Interest: None of the authors have a conflict of interest.

<0.0001 respectively) and preceding ($p=0.01$ and 0.003 respectively) the liver fibrosis outcome in unadjusted models, with similar values when adjusted for HIV RNA and CD4 count.

Conclusions—In women with HIV/HCV coinfection, higher sCD163 levels, a marker of macrophage activation, and TNFR11 levels, implying activation of the TNF- α system, were associated with liver disease progression. Our results provide an addition to the growing body of evidence regarding the relationship between macrophage activation, inflammation and liver disease progression in HIV/HCV coinfection.

Keywords

HIV; hepatitis C; hepatic fibrosis; macrophage activation; tumor necrosis factor receptor; inflammation; microbial translocation

Introduction

Hepatitis C virus (HCV) infection is highly prevalent in HIV-infected persons and a significant cause of morbidity and mortality^{1,2,3}. Liver disease progression is accelerated in persons coinfecting with HIV/HCV compared to those monoinfected with HCV^{4,5,6}. This accelerated disease progression occurs even among those on antiretroviral therapy^{7,8}. The mechanism for accelerated liver disease progression is not completely understood and is likely multifactorial.

Microbial translocation is increased in HIV infection and our group and others have found that markers of microbial translocation are associated with rapid liver disease progression in HIV/HCV co-infected patients^{9,10}. Hepatic macrophage activation by endotoxin is postulated to be a mechanism by which microbial translocation contributes to hepatic fibrosis in hepatitis C infection. We sought to characterize the role of macrophage activation and inflammation by comparing soluble marker levels over time in HIV/HCV co-infected women with varied trajectories of liver disease progression.

Despite the availability of highly effective HCV antiviral therapies, many persons in the United States and other countries do not have access to these drugs due to limited resources and high cost^{11,12,13}. Therefore, understanding the mechanisms of and markers for accelerated liver disease progression in HIV/HCV coinfection remains important in order to identify patients most at risk of liver disease outcomes who can be targeted for earlier therapy. Also, because the deleterious effects of microbial translocation, theoretically, may be mitigated by life-style changes such as diet, probiotics or abstinence from alcohol^{14,15,16}, understanding how microbial translocation affects liver disease remains relevant.

Methods

This is a nested study of HIV/HCV infected women participating in the Women's Interagency HIV Study. The WIHS is a longitudinal study of HIV infected and at-risk uninfected women that enrolled 2054 HIV-infected women and 569 uninfected women at six sites: Chicago, San Francisco Bay Area, Brooklyn and Bronx/Manhattan, New York, Washington, DC and Los Angeles from October 1994 through November 1995. From

October 2001 through September 2002, an additional 737 HIV-infected women and 406 uninfected women were enrolled. Informed consent was obtained from all participants in accordance with the US Department of Health and Human Services (DHHS) guidelines, the institutional review boards of participating institutions and the Helsinki Declaration of 1975, revised in 2000. Women are seen semiannually for interview, physical exam and collection of blood and genital specimens. The WIHS cohort was designed to reflect the demographics of the HIV epidemic among US women. Details of cohort recruitment, retention and demographics are published elsewhere^{17,18}.

Study subjects were HIV/HCV coinfecting women with the available data and specimens required for the study. Hepatitis C infection was defined as hepatitis C antibody and HCV RNA positivity. Soluble markers of microbial translocation and monocyte/macrophage activation (lipopolysaccharide (LPS), soluble CD163 and CD14), markers of inflammation/immune activation (interleukin-6, IL-6 and tumor necrosis factor (TNF) receptor II), and a profibrogenic chemokine, chemokine ligand 2 (CCL2) were measured from banked specimens, frozen uninterrupted at -80C since collection.

We retrospectively defined intervals of time during which women experienced rapid liver disease progression (LDP) and intervals during which there was no or minimal liver disease progression and compared serial soluble markers from these intervals. Liver disease progression intervals with available longitudinal specimens were first identified using the definitions below. After the liver disease progression intervals were identified, non-progression intervals from the same calendar time were identified in approximately a 2:1 ratio. We chose a 5 year span in consultation with academic hepatologists as a reasonable definition of rapid disease progression.

Liver disease was ascertained by liver biopsy, death related to complications of decompensated liver disease or by the non-invasive markers, APRI (AST to platelet ratio index: $\text{AST}/\text{upper limit of normal}/\text{platelet count } (10^9/\text{L}) \times 100$) and FIB-4 ($\text{age} \times \text{AST } (\text{U/L})/(\text{platelets } (10^9/\text{L}) \times \text{ALT } (\text{U/L}))^{1/2}$)^{19,20}. Liver death was ascertained by review of death certificates reviewed by two clinicians to come to a consensus cause of death. Death was ascribed to liver disease if cirrhosis or decompensated liver disease were the primary cause of death or the main underlying cause with a non-specific primary cause (e.g. when sepsis was primary cause and cirrhosis the first underlying cause, or GI bleed with esophageal varices and cirrhosis).^{21, 22,23} In the case of liver death, the soluble markers were measured on a specimen collected during a routine study visit approximately 1 year prior to liver death to assure that liver disease was present but avoid measuring markers that may reflect pre-mortem infectious or inflammatory conditions.

Soluble markers were measured at 3 timepoints for each interval studied; timepoints were approximately 2.5 years apart. Time 1 (T1) for all women was a point when there was no or minimal fibrosis by liver biopsy or non-invasive markers (APRI <0.5 and FIB-4 <1.45) at T1 or a visit within 6 months of T1 and no clinical evidence of end stage liver disease by self-report. For liver disease progressors, T3 was a point 5 years later where there was a liver biopsy with cirrhosis or bridging fibrosis, liver death within approximately 1 year or both APRI and FIB-4 consistent with cirrhosis (APRI >1.5 and FIB-4 >3.25 respectively). For

non-progressors, T3 was a point where there was no clinical evidence of end stage liver disease and biopsy or both markers were consistent with no or minimal fibrosis (APRI <0.5 and FIB-4 <1.45). T2 was equidistant between T1 and T3, approximately 2.5 years from each, with a range of 2–3 years.

To avoid confounders, the study included time intervals from HIV/HCV co-infected women who were hepatitis B surface antigen negative, had no hepatitis C therapy during the time interval and had no hazardous drinking (defined by the National Institute of Alcohol Abuse and Alcoholism criteria of ≥ 7 drinks/week) during the time interval.

APRI and FIB-4 are the most commonly used non-invasive markers in the WIHS because data are available at most WIHS visits to calculate these scores. Both have been found to be correlated with liver fibrosis and predict liver disease outcomes in HIV-infected persons and in WIHS²³. APRI >1.5 has an area under the receiver operating curve (AUROC) of 0.76 for biopsy-proven significant fibrosis (METAVIR F2–4) and 0.82 for cirrhosis in a large meta-analysis including HIV infected and uninfected HCV-infected patients²⁴ and a range of 0.71–0.82 for significant fibrosis and 0.81–0.92 and cirrhosis in large series²⁵. The negative predictive values of APRI <0.5 for lack of significant fibrosis has been found to be 80–91% in populations with prevalence of fibrosis similar to WIHS^{26,18}. Several reviews have found that the accuracy of APRI for cirrhosis was the same or better for studies of HIV/HCV co-infected persons compared to HCV mono-infected^{18,20,27,28}. FIB-4 >3.25 has been found to correlate with severe fibrosis (METAVIR F3–4) with AUROC 0.72–0.85 and FIB-4 <1.45 has been found to have a negative predictive value for significant fibrosis of 95% in one large study of HIV/HCV co-infected persons²⁹ with similar values (81 to 92) in more recent studies^{30,23,31}.

Laboratory Methods

Plasma LPS levels were quantified in duplicate by dilution of plasma specimens to 10% with endotoxin-free water using a Limulus Amebocyte Assay (Lonza Group Ltd., Basel, Switzerland). Background was subtracted and LPS levels were calculated by following the manufacturer's recommended protocol. Commercially available enzyme-linked immunosorbent assays (ELISAs) were used to measure soluble CD14 (R & D Systems, Minneapolis, MN), sCD163 (Trillium Diagnostics, Bangor, ME), TNF-RII (R&D Systems), IL-6 (R& D Systems), and CCL2 (R&D Systems). All assays were performed according to manufacturer's instructions. Laboratory investigators were blinded to the liver disease progression status of participants.

Statistical Methods

Demographic and clinical characteristics were assessed at time periods (T1–3), which were contemporaneous with measures of the soluble plasma markers. At the initial study visit (T1), age, median CD4+ T-lymphocyte count (CD4 count), median HIV RNA level, antiretroviral therapy use, and alcohol, injection drug or cocaine use ever and in the last 6 months (by self-report) were assessed. Over the duration of the study period, we calculated median CD4 count and median HIV RNA level, use of highly active antiretroviral therapy (HAART) and alcohol use (by self-report) at each visit. The unadjusted association between

progression and categorical covariates were assessed using Fisher's exact tests and continuous variables were compared using the Wilcoxon rank-sum test. Unadjusted associations between progression and each plasma marker were also assessed at T1, T2, and T3 using Wilcoxon-rank sum tests. Because HIV RNA levels have been associated with inflammatory and activation markers, the association between progression status and each plasma marker was calculated controlling for level of HIV RNA. Similarly, the associations were calculated controlling for CD4 count. Because of a suspected relationship between the chemoattractant protein CCL2 and fibrosis, we further investigated the relationship between CCL2 and FIB-4 values using Wilcoxon rank sum. Repeated measures analysis using Proc GLM with the link function Identity were fit to assess associations and slopes for each serum marker by progression status and time controlling for log HIV RNA level. P-values from repeated measures analysis are reported for demonstrating differences in slopes between progressors and non-progressors for each plasma marker. All analyses were performed in SAS software, version 9.3 (SAS Institute Inc, Cary NC). Graphs were produced using STATA 14 (StataCorp LLC, College Station, TX).

Results

A total of 106 time intervals from 106 women were included in the analysis; 31 of them were categorized as progressors and 75 were categorized as non-progressors. Reflecting the WIHS as a whole, the majority of study patients were African-American. The median age at T1 was 41.0 in non-progressors and 42.3 in progressors. The groups were similar in terms of race, age, HIVRNA, CD4 count, nadir CD4 and HAART use (Table 1) as well as illicit drug use. By design, alcohol use was low in both groups. Median CD4+ T-lymphocyte count was 462 in liver disease progressors and 498 in non-progressors, though only about 40% were on HAART at T1. In addition, HAART use, mean CD4 count and HIV RNA values were very similar between progressors and non-progressors over the course of the study (data not shown). Progressors did have a higher mean FIB4 score at baseline than non-progressors which was statistically significant. APRI was similar between progressors and non-progressors at baseline.

Association of Soluble Marker Levels with Liver Disease Progression

The six soluble markers were measured in the liver disease progressors and non-progressors at 3 time points. There were no significant differences in the level of soluble markers at T1 between progressors and non-progressors. The median time between T1 and T3 was 4.89 years in progressors and 4.84 years in non-progressors. LPS, sCD14, IL-6 and CCL-2 levels did not differ in slope or quantity over time between women who had rapid liver disease progression and non-progressors (Table 2 and Figure). TNFR2 levels were significantly higher in progressors at T2, (4372.5pg/mL vs 3100.6 pg/mL for non-progressors, $p=0.01$) and T3 (4215.9 pg/mL vs 3007.1 pg/mL for non-progressors $p=0.002$) in unadjusted and analyses adjusted for HIV RNA ($p=0.003$ and 0.0003 respectively) and CD4 count ($p=0.007$ and 0.002 respectively) (Table 2). Similarly sCD163 showed a significant difference between progressors and non-progressors with higher levels in progressors at T2 (4321.6 ng/mL vs 3006 ng/mL for non-progressors $p=0.008$) and T3 ($p<0.0001$) in unadjusted analyses, with similar values in analyses adjusted for HIV RNA and CD4 count. Progressors

had a steeper slope of change over time in sCD163 (742 ng/mL over 5 years vs -38.4 ng/mL) compared to non-progressors in unadjusted models and models adjusted for HIV RNA level ($p=0.01$ and 0.02 , respectively); however TNFR2 showed no significant difference in slope between progressors and non-progressors ($p=0.17$) (Figure). We performed an additional analysis to determine if CCL2 was correlated with fibrosis by FIB-4. There was a significant relationship between this chemoattractant protein and FIB-4 >3.25 (consistent with fibrosis): mean CCL2 235.6 pg/mL (sd 101.9) in those with FIB-4 >3.25 and 141.1 pg/mL (sd 101.9) in those with FIB-4 ≤ 3.25 ($p=0.03$).

In addition, GLM models confirmed that progression of liver disease and HIV viral load were associated with higher levels of TNFR2 ($p<0.0001$ for both) and soluble CD163 ($p<0.0001$ and $p=0.02$, respectively). IL-6 levels increased over time ($p=0.001$) and with increasing HIV viral load ($p=0.002$) for both progressors and non-progressors. While not associated with liver disease progression, sCD14, as expected, increased with increasing HIV RNA level ($p<0.0001$) as did CCL-2 ($p=0.0002$).

Discussion

In this longitudinal study of HIV/HCV co-infected women we demonstrated that liver disease progressors had significantly higher levels of sCD163 and TNFR2 than non-progressors and that elevated levels of these soluble markers preceded the defined fibrosis outcome.

Soluble CD163, a marker of macrophage activation, was higher in progressors and this difference was apparent at T2, prior to the fibrosis outcome. CD163 is a cell-surface glycoprotein receptor that is highly expressed on most tissue macrophages including hepatic Kupffer cells. CD163 is cleaved from the cell surface in response to LPS and sCD163 is thus considered a marker of translocation of microbial products across the gastrointestinal mucosa³². Cleavage of CD163 from Kupffer cells triggers the release of proinflammatory proteins^{33,34}, thus promoting fibrosis formation by stellate cells^{35,36}. Several investigators have found sCD163 to be a marker of hepatic fibrosis in cross sectional studies and some have proposed using sCD163 levels alone or in combination with HOMA-IR and platelet count as non-invasive markers of HCV-induced hepatic fibrosis^{30,37}. Our findings support the use of sCD163 as a marker of liver disease progression and support a central role of macrophage activation in hepatitis C disease progression.

We found that tumor necrosis factor receptor II (TNFR2) was elevated in liver disease progressors at and prior to the liver fibrosis endpoint. TNFR2 is part of the tumor necrosis factor cascade that is involved in the immune response to infection, and typically signals cell survival³⁸. Expressed on various cell types including monocytes/macrophages, the receptor is induced by a number of mediators such as LPS and TNF- α . Soluble TNFR2 is produced by cleavage of the cell-surface receptor and can be used as a surrogate marker for activation of the TNF- α system. In HIV disease, sTNFR-II levels have been shown to be elevated and associated with poor clinical outcomes^{39,40}. Significantly elevated levels of sTNFR-II were reported in HCV-infected patients with cirrhosis and hepatocellular carcinoma compared to controls and may reflect degree of hepatic inflammation^{41,42}. A

recent study showed that HCV-induced endothelial inflammatory response depends on the functional expression of TNFR-II⁴³. Exactly how the TNF cascade affects HCV disease progression and what triggers of activation of the TNF-cascade in HIV/HCV coinfection are not fully articulated.

We found that classic markers of microbial translocation, LPS and sCD14, were not higher in women with more rapid liver disease progression. This is in contrast to an early cross sectional study that showed that elevated LPS was independently associated with cirrhosis¹⁰ as well as several studies examining sCD14 levels (including our own) which reported that elevated levels of sCD14 were associated with a increased risk of liver disease progression in patients with and without HIV^{9,10,44,45}. The difference in outcomes is perhaps due to difference in study design, population, or the endpoints defining liver disease progression, or related to the low proportion of patients in the current study that reported hazardous alcohol consumption. Our earlier study included women with heavy alcohol use, and there are some data that alcohol use, itself, without significant liver disease, increases sCD14 levels in HIV-infected persons^{40,46}.

CCL2 (also known as monocyte chemoattractant protein 1 or MCP 1) is an inflammatory cytokine that is a principal chemoattractant for inflammatory monocytes and has been implicated in hepatic cell ischemia and inflammatory liver injury^{47,48}. We had hypothesized that CCL2 may be on the pathogenetic pathway of HCV-associated liver disease progression in HIV and that levels of CCL2 may be associated with fibrosis progression. Though in our primary analysis, we found that CCL2 levels were not significantly different in progressors and nonprogressors, an additional analysis revealed that CCL2 was correlated with FIB-4 >3.25 (which is consistent with cirrhosis). This implies that soluble CCL2 should continue to be explored as a marker of hepatic injury in chronic viral liver disease and supports the central role of inflammatory monocytes in hepatic injury in HIV/HCV infected persons⁴⁹.

Our findings should be considered in light of the limitations of our study. For many patients we used non-invasive markers of fibrosis stage to define our progressors and non-progressors. While we used levels of APRI and FIB-4 at the extremes that have been found to correlate highly with minimal or severe fibrosis, these measures are not the gold standard and may not accurately reflect liver histology. We dichotomized women as either liver disease progressors or non-progressors for the timepoints studied; liver disease progression, in vivo, is a continuum, women who we labeled non-progressors may have experienced some liver disease progression during the 5 year study period. While all our subjects had minimal or no liver fibrosis at T1 by our definitions, liver disease progressors had significantly higher median FIB-4 levels at T1 than non-progressors, implying some difference in stage of liver disease at baseline. However, given that the trajectory of progression was markedly different between the two groups, the correlations between progression and trends in soluble markers should be valid despite this difference at baseline. An obvious limitation is sample size, while the significant associations we found should be robust, we do not have power to assert lack of association. Also, because of sample size, we were unable to adjust for all variables that could potentially affect inflammation or liver disease progression.

Conclusions

In women with HIV/HCV co-infection sCD163 levels, a marker of macrophage activation, were associated with liver disease progression. In addition, TNFR2 levels correlated with liver disease progression. Our results provide an addition to the growing body of evidence regarding the relationship between macrophage activation, inflammation and liver disease progression in women with HIV/HCV co-infection.

Acknowledgments

Source of Funding: Data in this manuscript were collected by the Women's Interagency HIV Study (WIHS). The contents of this publication are solely the responsibility of the authors and do not represent the official views of the National Institutes of Health (NIH). WIHS (Principal Investigators): U01-AI-103408; Bronx WIHS (Kathryn Anastos), U01-AI-035004; Brooklyn WIHS (Howard Minkoff and Deborah Gustafson), U01-AI-031834; Chicago WIHS (Mardge Cohen and Audrey French), U01-AI-034993; Metropolitan Washington WIHS (Seble Kassaye), U01-AI-034994; Connie Wofsy Women's HIV Study, Northern California (Ruth Greenblatt, Bradley Aouizerat, and Phyllis Tien), U01-AI-034989; WIHS Data Management and Analysis Center (Stephen Gange and Elizabeth Golub), U01-AI-042590; Southern California WIHS (Joel Milam), U01-HD-032632 (WIHS I – WIHS IV). The WIHS is funded primarily by the National Institute of Allergy and Infectious Diseases (NIAID), with additional co-funding from the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), the National Cancer Institute (NCI), the National Institute on Drug Abuse (NIDA), and the National Institute on Mental Health (NIMH).

References

1. Lewden C, Salmon D, Morlat P, et al. Causes of death among human immunodeficiency virus(HIV)-infected adults in the era of potent antiretroviral therapy: emerging role of hepatitis and cancers, persistent role of AIDS. In *J Epidemiol*. 2005; 34(1):121–130.
2. Weber R, Sabin C, Friis-Muller N, et al. Liver-related deaths in persons infected with the human immunodeficiency virus: The Data Collection on Adverse Events of Anti-HIV drugs Study Group. *Arch Intern Med*. 2006; 166:1632–1641. [PubMed: 16908797]
3. French AL, Gaweel S, Hershow R, et al. Trends in Mortality and Causes of Death for Women with HIV in the US: A Ten Year Study. *J Acquir Immune Defic Syndr*. 2009; 51:399–406. [PubMed: 19487953]
4. Lo Re V, Kallan M, Tate J, et al. Hepatic decompensation of antiretroviral-treated patients co-infected with HIV and hepatitis C virus compared with hepatitis C virus mono-infected patients: a cohort study. *Ann Intern Med*. 2014; 160(6):369–379. [PubMed: 24723077]
5. Chen T, Ding E, Seage Lii G, et al. Meta-analysis: increased mortality associated with hepatitis C in HIV-infected persons is unrelated to HIV disease progression. *Clin Infect Dis*. 2009; 49(10):1605–1615. [PubMed: 19842982]
6. Fierer D, Dieterich D, Fiel M, et al. Rapid progression to decompensated cirrhosis, liver transplant, and death in HIV-infected men after primary hepatitis C infection. *Clin Infect Dis*. 2013; 56(7): 1038–1043. [PubMed: 23264364]
7. de Ledinghen V, Barreiro P, Foucher J, et al. Liver fibrosis on account of chronic hepatitis C is more severe in HIV-positive than HIV-negative patients despite antiretroviral therapy. *J Viral Hepat*. 2008; 15(6):427–433. [PubMed: 18221303]
8. Thein H, Yi Q, Dore G, et al. Natural history of hepatitis C virus infection in HIV-infected individuals and the impact of HIV in the era of highly active antiretroviral therapy: a meta-analysis. *AIDS*. 2008; 22(15):1979–1991. [PubMed: 18784461]
9. French AL, Evans C, Agniel D, et al. Microbial translocation and liver disease progression in women coinfected with HIV and hepatitis C virus. *J Infect Dis*. 2013; 208(4):670–689.
10. Balogopal A, Philip F, Asemborski J, et al. HIV-related microbial translocation and progression of hepatitis C. *Gastroenterology*. 2008; 135:226–233. [PubMed: 18457674]

11. Chhatwal J, Kanwal F, Roberts M, et al. Cost-effectiveness and budget impact of hepatitis C virus treatment with sofosbuvir and ledipasvir in the United States. *Ann Intern Med*. 2015; 162(6): 397–406.
12. Najafzadeh M, Andersson K, Shrank W, et al. Cost-effectiveness of novel regimens for the treatment of hepatitis C virus. *Ann Intern Med*. 2015; 162(6):407–419. [PubMed: 25775313]
13. Rein D, Wittenborn J, Smith B, et al. The cost-effectiveness, health benefits, and financial costs of new antiviral treatments for hepatitis C virus. *Clin Infect Dis*. 2015; 61(2):157–168. [PubMed: 25778747]
14. D'Ettorre G, Ceccarelli G, Giustini N, et al. Probiotics reduce inflammation in antiretroviral treated, HIV-infected individuals: results of the Probio-HIV Clinical Trial. *PLoS One*. 2015; 10:e0132700. [PubMed: 26196395]
15. Weber AR, Sattar A, Funderburg NT, et al. Alcohol and dietary factors associated with gut integrity and inflammation in HIV-infected adults. *HIV Med*. 2016 Nov 9. Epub ahead of print. doi: 10.1111/hiv.12442
16. Kelly CJ, Colgan SP, Frank DN. Of microbes and meals: the health consequences of dietary endotoxemia. *Nutr Clin Pract*. 2012; 27:215–25.
17. Barkan SE, Melnick SL, Preston-Martin S, et al. The Women's Interagency HIV Study. *Epidemiology*. 1998; 9(2):117–125. [PubMed: 9504278]
18. Hessol NA, Schneider M, Greenblatt RM, et al. Retention of women enrolled in a prospective study of HIV infection: impact of race, unstable housing and use of HIV therapy. *Am J Epidemiol*. 2001; 154(6):563–573. [PubMed: 11549562]
19. Sterling R, Lissen E, Clumeck N, et al. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. *Hepatology*. 2006; 43(6):1317–1325. [PubMed: 16729309]
20. Kim BK, Kim DY, Park JY, et al. Validation of FIB-4 and comparison with other simple noninvasive indices for predicting liver fibrosis and cirrhosis in hepatitis B virus-infected patients. *Liver Int*. 2010; 30(4):546–553. [PubMed: 20074094]
21. French AL, Gaweel SH, Hershow RC, Benning L, Hessol NA, Levine AM, Anastos K, Augenbraun M, Cohen MH. Trends in Mortality and Causes of Death for Women with HIV in the US: A Ten-Year Study. *Journal of the Acquired Immune Deficiency Syndrome*. 2009; 51:399–409.
22. Sarkar M, Aouzierat B, Bacchetti P, Prokunina-Olsson L, French A, Seaberg E, O'Brien T, Kuniholm M, Minkoff H, Plankey M, Strickler HD, Peters M. Association of IFN13 and IFNL4 polymorphisms with liver-related mortality in a multiracial cohort of HIV/HCV co-infected women. *J Viral Hepat*. 2015; 22:1055–60. [PubMed: 26115445]
23. Bambha K, Pierce C, Cox C, French AL, Tien PC, Sharp GB, Augenbraun M, Glesby MJ, Villacres MC, Plankey M, Strickler HD, Gange SJ, Peters MG. Assessing mortality with hepatitis C virus and HIV using indirect markers of fibrosis. *AIDS*. 2012; 26(5):599–607. [PubMed: 22156972]
24. Shaheen AA, Myers RP. Diagnostic accuracy of the aspartate aminotransferase to platelet ratio index for the prediction of hepatitis C related fibrosis: a systematic review. *Hepatology*. 2007; 46:912–921. [PubMed: 17705266]
25. Stauber RE, Lackner C. Noninvasive diagnosis of hepatic fibrosis in chronic hepatitis C. *World J Gastroenterol*. 2007; 13:4287–4294. [PubMed: 17708599]
26. Carvalho-Filho RJ, Schiavon LL, Narciso-Schiaivon JL, et al. Optimized cutoffs improve performance of aspartate aminotransferase to platelet ratio index for predicting significant liver fibrosis in HIV/HCV co-infection. *Liver Inter*. 2008; 1478:486–492.
27. Nunes D, Fleming C, Offner G, et al. HIV infection does not affect the performance of noninvasive markers of fibrosis for the diagnosis of hepatitis C related liver disease. *J Acquir Immune Defic Syndr*. 2005; 40:538–544. [PubMed: 16284529]
28. Merli M, Castagna A, Salpietro S, et al. Diagnostic accuracy of APRI, FIB-4 and Forns for the detection of liver cirrhosis in HIV/HCV-coinfected patients. *New Microbiol*. 2016; 39(2):110–113. [PubMed: 27196548]

29. Vallet-Pichard A, Mallet V, Nalpas B, et al. FIB-4; an inexpensive and accurate marker of fibrosis in HCV infection: comparison with liver biopsy and fibrotest. *Hepatology*. 2007; 46:32–36. [PubMed: 17567829]
30. Tanwar S, Trembling PM, Hogan BJ, et al. Biomarkers of Hepatic Fibrosis in Chronic Hepatitis C: A Comparison of 10 Biomarkers using 2 Different Assays for Hyaluronic Acid. *J Clin Gastroenterol*. 2016 Epub ahead of print.
31. Amorin TG, Staub GJ, Lazzorato C, et al. Validation and comparison of simple noninvasive models for the prediction of liver fibrosis in hepatitis C. *Ann Hepatol*. 2012; 11:855–61. [PubMed: 23109448]
32. Fabrick BO, van Bruggen R, Deng DM, et al. The macrophage scavenger receptor CD163 function as an innate immune sensor for bacteria. *Blood*. 2009; 113(4):887–892. [PubMed: 18849484]
33. Etzerodt A, Moestrup SK. CD163 and inflammation: biological, diagnostic, and therapeutic aspects. *Antioxid Redox Signal*. 2013; 18(17):2352–2363. [PubMed: 22900885]
34. Etzerodt A, Maniecki MB, Moller K, Moller HJ, Moestrup SK. Tumor necrosis factor alpha-converting enzyme (TACE/ADAM17) mediates ectodomain shedding of the scavenger receptor CD163. *J Leukoc Biol*. 2010; 88(6):1201–1205.
35. Kazankov K, Barrera F, Holger JM, et al. Soluble CD163, a macrophage activation marker, is independently associated with fibrosis in patients with chronic viral hepatitis B and C. *Hepatology*. 2014; 60:521–530. [PubMed: 24623375]
36. Ramachandran P, Iredale JP. Macrophages: central regulators of hepatic fibrinogenesis and fibrosis resolution. *J Hepatol*. 2012; 56:1417–1419. [PubMed: 22314426]
37. Kuniholm MH, Hanna DB, Landay AL, Kaplan RC, Ley K. Soluble CD163 is associated with noninvasive measures of liver fibrosis in hepatitis C virus- and hepatitis C virus/human immunodeficiency virus-infected women. *Hepatology*. 2015; 61(2):734–735. [PubMed: 25044447]
38. Faustman DL, Davis M. TNF Receptor 2 and Disease: Autoimmunity and Regenerative Medicine *Front Immunol*. 2013; 4(478)
39. Morlat P, Periera E, Clayette P, et al. Early evolution of plasma soluble TNF-alpha p75 receptor as a marker of pregression in treated HIV-infected persons. *AIDS Res Human Retro*.
40. Tenorio AR, Zheng Y, Bosch RJ, et al. Soluble markers of inflammation and coagulation but not T-cell activation predict non-AIDS-defining morbid events during suppressive antiretroviral treatment. *J Infect Dis*. 2014; 210:1248–59. [PubMed: 24795473]
41. Kakuma S, Okumura A, Ishikawa T, et al. Serum levels of IL-10, IL-15 and soluble tumour necrosis factor alpha receptors in type C chronic liver disease. *Clin Expe Immunol*. 1997; 109:458–63.
42. Kallinowski B, Haseroth K, Marinos G, et al. Induction of tumour necrosis factor receptor type p55 and p75 in patients with chronic hepatitis C virus infection. *Clin Experimental Immunol*. 1998; 111:269–277.
43. Pircher J, Czernak T, Merkel M, et al. Hepatitis C virus induced endothelial inflammatory response depends on the function expression of TNF alpha receptor subtype @. *PLOS one*. 2014; 9:e113351. [PubMed: 25419735]
44. Pinzone MR, Celesia BM, Di Rosa M, et al. Microbial Translocation in Chronic Liver Diseases. In *K Microbiol*. 2012; 2012:694629.
45. Monnig MA, Kahler CW, Cioe PA, et al. alcohol use predicts elevation in inflammatory marker soluble CD14 in men living with HIV. *AIDS Care*. 2016; 28:1434–40. [PubMed: 27242060]
46. Carrico AW, Hunt PW, Emenyonu NI, et al. Unhealthy alcohol use is associated with monocyte activation prior to starting anti-retroviral therapy. *Alcohol Clin Exp Res*. 2015; 39:2422–2426. [PubMed: 26509359]
47. Zhang J, Xu P, Song P, et al. CCL2-CCR2 signaling promotes hepatic ischemia/reperfusion injury. *J Surg Res*. 2016; 202(2):352–362. [PubMed: 27229110]
48. Ramachandran P, Iredale JP. Liver fibrosis: a bidirectional model of fibrogenesis and resolution. *QJM*. 2012; 105(9):813–817. [PubMed: 22647759]
49. Tacke F. Targeting hepatic macrophages to treat liver diseases. *Journal of Hepatology*. 2017; 66:1300–12. [PubMed: 28267621]

Figure. Soluble markers over time comparing liver disease progressors and non-progressors

The differences in slope between progressors and non-progressors was calculated for each and presented as a p value. A. sCD163 in ng/ml ($p=0.01$); B. sCD14 in pg/ml ($p=0.11$); C. TNF receptor II in pg/ml ($p=0.1$); D. Lipopolysaccharide (LPS) in eu/ml ($p=1.0$); E. Chemokine ligand-2 (CCL2) in pg/ml ($p=0.67$); F. Interleukin-6 (IL-6) in pg/ml ($p=0.37$). For CCL2 and TNFR II graphs three outliers are eliminated for scale (for CCL2 three non-progressors with values >4000 pg/ml and for TNFR II two non-progressors and one progressor with values $>20,000$ ng/ml)

Table 1

Demographic Characteristics by Liver Disease Progression among HIV/HCV-infected Patients, N=106

Characteristics	Progressor N=31	Non-Progressor N=75	p-value
Total study time in years			0.70
Mean (SD)	4.89 (1.01)	5.04 (1.20)	
Median (IQR)	4.89 (3.99–5.86)	4.84 (4.11–5.48)	
Race/Ethnicity			0.95
White	6 (19.4)	16 (21.3)	
Black	20 (64.5)	47 (62.7)	
Hispanic	5 (16.1)	10 (13.3)	
Other race	0 (0.0)	2 (2.7)	
Age at T1, median (IQR)	42.3 (39.5–45.4)	41.0 (36.9–46.0)	0.36
Calendar Month/Year T1 (IQR)	12/98 (5/96–10/01)	10/98 (7/95–11/02)	0.71
CD4 at study visit 1, median (IQR)	462 (289–661)	498 (326–663)	0.40
CD4 nadir, median (IQR)	214 (113.5–359.5)	226 (153–318)	0.98
Viral load, median (IQR)	1100 (130–7900)	600 (80–16000)	0.65
Method used to define degree of liver disease progression n(%)	Liver biopsy 14 (45.1) Liver death 6 (19.4) Serum markers 11 (35.5)	Liver biopsy 3 (4.0) Serum markers 72 (96.0)	
APRI, Median (IQR)^a	0.33 (0.27–0.49)	0.34 (0.27–0.42)	0.42
Fib-4, Median (IQR)^a	1.29 (0.88–1.45)	0.94 (0.73–1.15)	0.005
On HAART at T1			0.52
Yes	11 (35.5)	32 (42.7)	
No	20 (64.5)	43 (57.3)	
Average # of drinks/week during study Median (IQR)	0 (0.0–1.0)	0.50 (0.0–1.8)	0.12
Lifetime Injection Drug Use (IDU), T1^b	4 (12.9)	16 (21.1)	0.42
Lifetime Crack use, T1	7 (22.6)	22 (28.9)	0.63
Lifetime Cocaine use, T1	4 (12.9)	19 (25.0)	0.20
IDU in past 6 months at T1	4 (12.9)	11 (14.5)	1.00
Crack use in past 6 months at T1	5 (16.1)	15 (19.7)	0.79
Cocaine use in past 6 months at T1	1 (3.2)	6 (7.9)	0.67
Diabetes	7 (22.6)	14 (18.7)	0.79

Characteristics	Progressor N=31	Non-Progressor N=75	p-value
BMI			0.28 ^c
Underweight (<18.5)	0 (0)	0 (0)	
Normal (18.5–24.9)	11 (40.7)	36 (52.2)	
Overweight (25–29.9)	31 (44.4)	19 (27.5)	
Obese (>30)	4 (14.8)	14 (20.3)	
Obese (BMI ≥ 30)	4 (12.9)	14 (18.7)	0.58

^a N=88 due to missing data

^b Includes lifetime use, including history of use prior to WIHS enrollment and any use reported during WIHS up to and including Time 1

^c global chisquare

p-values by Fisher's exact tests or Wilcoxon test except as noted

Table 2

Soluble Markers over Time: Liver Disease Progressors vs Non-progressors

Soluble Marker Levels Mean (sd)	Overall	Progressor	Non-progressor	Wilcoxon Rank sum p-value	Model adjusted for HIV RNA p-value	Model adjusted for CD4 p-value
Lab data at Time one (T1)	N=106	N=31	N=75			
LPS	0.78 (0.30)	0.76 (0.34)	0.79 (0.29)	0.31	0.57	0.59
sCD14	2112.7 (509.9)	2055.1 (597.6)	2136.5 (471.3)	0.24	0.45	0.32
IL-6	2.02 (2.03)	1.88 (1.44)	2.08 (2.23)	0.90	0.61	0.60
TNFR II	3204.3 (1391.4)	3555.7 (1822.6)	3059.0 (1152.1)	0.32	0.09	0.13
CCL2	139.9 (95.3)	141.5 (90.2)	139.2 (97.9)	0.90	0.88	0.93
sCD163	3202.6 (1827.8)	3524.5 (1698.2)	3069.6 (1873.5)	0.11	0.25	0.28
Lab data at T2	N=106	N=31	N=75			
LPS	0.81 (0.33)	0.81 (0.41)	0.82 (0.29)	0.22	0.94	0.98
sCD14	2180.6 (503.2)	2172.9 (546.2)	2183.7 (488.2)	0.48	0.72	0.67
IL-6	2.51 (2.59)	2.86 (2.85)	2.36 (2.48)	0.43	0.43	0.38
TNFR II	3472.4 (2072.1)	4372.1 (2803.4)	3100.6 (1557.9)	0.01	0.003	0.007
CCL2	146.3 (105.7)	160.5 (97.9)	140.4 (108.8)	0.20	0.50	0.51
sCD163	3390.8 (2228.6)	4321.6 (2388.6)	3006.0 (2054.6)	0.004	0.008	0.008
Lab data at T3	N=102	N=29	N=73			
LPS (n=101)	0.80 (0.30)	0.78 (0.31)	0.80 (0.29)	0.67	0.75	0.81
sCD14 (n=102)	2168.8 (478.6)	2237.8 (510.0)	2141.4 (466.3)	0.57	0.46	0.77
IL-6 (n=102)	3.05 (3.18)	3.82 (3.34)	2.75 (3.09)	0.03	0.16	0.07
TNFR II (n=102)	3342.2 (1658.4)	4215.9 (2040.3)	3007.1 (1359.5)	0.002	0.0003	0.002
CCL2 (n=98)	154.0 (131.8)	182.7 (161.9)	142.7 (117.3)	0.15	0.22	0.31
sCD163 (n=101)	3481.1 (2179.3)	4914.3 (2537.3)	2931.4 (1754.1)	<0.0001	<0.0001	<0.0001

Abbreviations and units- T:Time; sd: standard deviation; CD4: CD4+ T-lymphocyte count; LPS: lipopolysaccharide in eu/mL; sCD14: soluble CD14 in pg/mL; IL6: Interleukin 6 in pg/mL; TNFR II: tumor necrosis factor receptor 2 in pg/mL; CCL2: chemokine ligand 2 in pg/mL; sCD163: soluble CD163 in ng/mL