January 2017

# The Natural History And Predictors Of Liver Fibrosis Progression Using The Fib-4 Score Among Hiv/hcv Co-Infected Adults In An Outpatient Clinic

Bianca Yuh
*Yale University*

# The Natural History and Predictors of Liver Fibrosis Progression Using the FIB-4 Score Among HIV/HCV co-infected Adults in an Outpatient Clinic

A Thesis Submitted to the Yale University School of Medicine in Partial Fulfillment of the Requirements for the Degree of Doctor of Medicine

By

Bianca Yuh

2017

## Abstract:

**Background:** Studies have documented more rapid progression of HCV-associated liver fibrosis in patients co-infected with HIV. However, the natural history of HCV infection in both mono-infected and HIV co-infected patients remains highly variable. The patterns and predictors of fibrosis progression in the HIV/HCV co-infected population are not fully characterized. Given the invasiveness of serial liver biopsies, Fibrosis-4 score (FIB-4), a composite of serum biomarkers that correlate well with fibrosis stage, is increasingly used. We used FIB-4 to study the natural history of liver fibrosis progression among co-infected patients and evaluated predictors of progression to cirrhosis over 5 years prior to treatment with direct acting agents (DAAs).

**Methods:** Study subjects were selected from HIV/HCV co-infected patients receiving care at Yale-New Haven Hospital from February 2014 through April 2016 without advanced fibrosis 5 years prior to study entry. Annual FIB-4 scores dating back 5 years were calculated from the most recent FIB-4 score or the last FIB-4 prior to DAA treatment initiation. Baseline demographics and clinical characteristics including HCV genotype, antiretroviral regimen, HIV viral loads and CD4 counts were collected. Patients were further categorized based on FIB-4 progression over the course of 5 years. Univariate and multivariable logistic regression models were used to examine factors associated with FIB-4 progression and a p-value of 0.05 was chosen as the threshold for statistical significance.

**Results:** There were 93 patients evaluated including 65 men and 28 women; mean age of 56.7 years; 32.3% were white, 53.8% were black. Injection drug use (IDU) was the major risk factor for HCV acquisition (63.4%) and the most common genotype was genotype 1 (81.2%). The median CD4+ count was 564 cells/$mm^3$, and the majority (88.4%) had HIV viral loads <50 copies/mL. Over 5 years, 25 (26.9%) had FIB-4 progress to >3.25 and 68 (73.1%) had FIB-4 remain <3.25. Demographic variables (age, gender, race/ethnicity, BMI, substance use), clinical variables (HIV viral load, CD4 count, ART use, HIV duration, HCV duration and viral load) and co-morbid conditions such as diabetes and hyperlipidemia did not differ significantly between those whose FIB-4 stayed below 3.25 and those whose FIB-4 progressed to above 3.25 in univariate and multivariable logistic regression models.

**Conclusion:** In this study of 93 HIV/HCV co-infected patients without baseline advanced fibrosis, 26.9% progressed to advanced fibrosis over the course of 5 years. We did not identify any statistically significant factors that predicted those who were more likely to progress, although clinically relevant factors such as absence of HIV virologic control, low CD4 count, and lack of statin use showed a trend towards significance and should be assessed in future studies in a larger cohort. Our findings highlight the importance of prioritizing all patients with HCV/HIV co-infection for HCV treatment.

**Acknowledgements:**

I would like to acknowledge my family for their support, Dr. Merceditas Villanueva for her guidance and mentorship throughout this process, Dr. Onyema Ogbuagu, Tassos Kyriakides of the Yale Center for Analytical Sciences, and grant support from the Yale School of Medicine Office of Student Research.

**Table of Contents:**

**Introduction:**

With the advent of combination antiretroviral therapy (cART), HIV infection has been transformed from a rapidly fatal disease to a manageable chronic illness. However, the threat of opportunistic infections, which were the most frequent cause of mortality in the pre-cART era, has been replaced by complications associated with aging and long-term HIV infection, including cardiovascular disease, lung disease, certain cancers, and liver disease. Chronic liver disease is the 2nd leading cause of mortality in the HIV-infected population, of which the majority are secondary to hepatitis C virus (HCV) infection. (1) Liver disease-related deaths have increased markedly in the HIV-infected population in the post-cART era, coinciding with the peak of HCV-related disease burden epidemiologically. (2)

It is estimated that of over 33.3 million people living with HIV infection globally, approximately 20-30% are co-infected with HCV due to shared routes of disease transmission, including parenteral (injection drug use, blood transfusion), perinatal, and sexual. (3) The incidence of HIV/HCV coinfection ranges from 10% to over 80% among those who acquire HIV by intravenous drug use. (4) Chronic HCV infection is not only associated with liver-specific health outcomes such as cirrhosis, liver failure, and hepatocellular carcinoma (HCC), but has also been associated with increased cardiovascular morbidity, renal dysfunction, insulin resistance, and cancer. (2) Given the wide array of adverse health outcomes associated with HCV and its potential as a major driver of mortality, treating HIV/HCV coinfected patients should be a critical priority.

*HIV and Liver Disease*

Although HCV co-infection accounts for much of the liver-related morbidity and mortality in HIV-infected individuals, an excess burden of liver disease has been reported in HIV mono-infected patients. (1) Liver enzyme elevations are frequently noted in patients with HIV, and can not only be attributed to viral hepatitis, but also other causes including drug-induced liver injury and both alcoholic and non-alcoholic fatty liver disease (NAFLD). Classically, the nucleoside reverse transcriptase inhibitor antiretrovirals (NRTIs), have been well studied for their association with hepatotoxicity, likely due to direct toxic effect on the hepatocyte mitochondria and resultant break in the generation of adenosine triphosphate (ATP) and accumulation of lactic acid. (4) NRTIs cause a greater rise in lactic acid and hepatotoxicity compared to HIV protease inhibitors (PIs), and are associated with a syndrome of hepatic steatosis. (5, 6) Among PIs, ritonavir and ritonavir-boosted regimens have been identified as independent risk factors for the development of hepatotoxicity. (4)

HIV-positive individuals also demonstrate a significant prevalence of NAFLD, which is viewed as a hepatic manifestation of metabolic syndrome in the general population. Risk factors of obesity, hypertension, insulin resistance, dyslipidemia, and diabetes mellitus are prevalent within the HIV population as well. However, HIV-infected patients are even more prone to NAFLD due to HIV itself, viral hepatitis, or ART toxicity. HIV itself can affect lipid profiles, with a higher viral load associated with lower LDL cholesterol and higher triglyceride concentrations. (1) Moreover, increased intestinal permeability through villous effacement and depletion of CD4 cells increases the amount of bacterial lipopolysaccharide reaching the liver and upregulates inflammation, accelerating NAFLD. (4) Exposure to ART is an independent risk factor

for the development of NAFLD, and insulin resistance and lipodystrophy are associated with the use of nucleoside inhibitors and other ART drugs. (4)

*Impact of HCV on HIV Disease*

The impact of HCV infection on the natural history of HIV progression is unclear. Prior to the introduction of cART, there was no difference observed in progression to AIDS, death, or decline in CD4+ count between HIV mono-infected and HIV/HCV co-infected patients. Progression to AIDS did not differ between HCV-positive patients acquiring HIV and those with HIV infection alone. (1) However, in the current cART era, there is some evidence suggesting that HCV co-infection may affect HIV progression. In the Swiss HIV Cohort Study which consisted of patients receiving potent antiretroviral therapy, HIV and HCV co-infection were associated with faster progression to AIDS and slower CD4+ recovery than in patients with HIV alone. (7) Subsequent meta-analysis found decreased CD4+ count recovery after 48 weeks of cART in HIV/HCV co-infected patients compared to HIV mono-infected patients. (8) Mechanisms of impaired CD4+ count recovery may include chronic immune activation driven by HCV infection leading to CD4+ T-cell apoptosis, suppression of CD4+ cell proliferation, and Fas-mediated apoptosis due to HCV replication in peripheral blood mononuclear cells and lymphoid tissue. (9)

*Impact of HIV on HCV Disease*

It is well-established that HIV modulates the natural history of HCV disease, and HIV infection has been associated with higher HCV viral loads and increased risk of chronic HCV infection. (10) In a longitudinal cohort of initially HCV mono-infected patients, some of whom HIV seroconverted, HCV RNA levels after HIV seroconversion

increased 58-fold over the study period, compared with an approximately threefold increase in patients who remained HIV negative. (11) Multiple studies have documented an association between higher HCV viral load and lower CD4 T cell count in co-infected patients, suggesting that cellular immune responses play an important role in control of HCV viral replication during chronic HCV infection. (9) HIV is also a prominent risk factor for failing to spontaneously clear HCV after acute infection, likely due to the impact of HIV infection on effective HCV specific T-cell responses. (12, 13) HIV infection may also impair innate immune responses that affect HCV clearance through diminished CD4+ stimulation of B-cell responses and antibody production. (9)

It is well-established that HIV accelerates progression to hepatic fibrosis and cirrhosis in HCV co-infected patients. (14) A meta-analysis of 8 separate studies that investigated the role of HIV in liver disease in HCV-infected patients found that co-infected patients had approximately 2 times the risk of cirrhosis diagnosed on liver biopsy and approximately 6 times the risk of decompensated liver disease (severe liver disease accompanied by clinical conditions including ascites, varices, or encephalopathy) when compared with HCV mono-infected patients. (15) Another meta-analysis of 27 natural history studies found a relative risk (RR) of 1.72 of cirrhosis among patients coinfected with HIV/HCV compared to HCV mono-infected patients. (15, 16) An unusually rapid progression to cirrhosis was also demonstrated in a multicenter cross-sectional study, where mean interval from estimated time of HCV infection to cirrhosis was significantly longer in HIV-negative than HIV-positive patients (23.2 vs 6.9 years, $p<0.001$). (17)

Accelerated fibrosis progression in the co-infected population has also been validated in multiple studies. In 282 HIV/HCV co-infected patients with 435 paired liver biopsies, fibrosis progression by at least 1 METAVIR stage after 2.5 years of follow-up was seen in 34% of the study population. (18) Two studies have demonstrated that while the majority of HIV/HCV co-infected patients did not show evidence of histologic progression over the course of 3 years, progression did occur in 16-24%. In a study of 174 co-infected patients, significant fibrosis defined as two Ishak units or greater between biopsies occurred in 24% over a 3-year interval. (19) In another study, which was a retrospective review of 135 co-infected individuals with a median time of 3.3 years (2.0-5.2) between repeat biopsies, 44% demonstrated fibrosis progression, with 16% having a 2+ METAVIR stage increase. (14, 19)

*Mechanisms of Fibrogenesis in HIV/HCV Co-infection*

Liver fibrosis occurs when extracellular matrix (ECM) deposition exceeds the rate of ECM clearance and produces a net increase of ECM proteins that initiate the cascade of nodule development and cirrhosis. Hepatic stellate cells (HSC) have been implicated as the primary instigators of fibrogenesis and when activated by hepatocytes or Kupffer cells, HSCs become myofibroblast-like cells that produce ECM and tissue inhibitors of metalloproteinases (TIMPs) which further downregulate matrix degradation. (9)

The mechanisms associated with accelerated fibrosis progression rates (FPR) among HIV/HCV co-infected patients may include direct viral effect on the hepatocytes and/or stellate cells, as direct activation of HSCs via HIV gp120 leads to increased expression and secretion of monocyte chemoattractant protein (MCP-1), a proinflammatory cytokine and stimulant of Type 1 collagen production. (20)

Additional mechanisms include diminished HCV-specific T cell responses, altered levels of matrix metalloproteinases and fibrosis biomarkers, increased oxidative stress and induction of hepatocyte apoptosis via CXCR4, HIV-associated gut depletion of CD4 cells, and immune/cytokine dysregulation. (21, 22) Markers of microbial translocation such as lipopolysaccharide, lipopolysaccharide binding protein, CD14, and fucose-binding lectin are raised in co-infected individuals and show strong correlation with HIV-related depletion of CD4+ cells and progressive HCV-related liver disease. (23) The reduced ratio of CD4+ to CD8+ cells associated with HIV infection may also play a role because CD8+ cells are more fibrogenic than CD4+ cells. (24) HIV/HCV co-infected patients have increased intrahepatic IFN-gamma and TNF-alpha levels with higher IFN-gamma mRNA levels, which correlate with higher levels of fibrosis. (25) Moreover, HIV has been shown to accentuate an HCV-driven pro-fibrogenic program in hepatocyte and HSC lines through reactive oxygen species (ROS), NFkB, and TGFB1 upregulation. (26)

*Increased risk of decompensation, mortality, and HCC*

Several studies have demonstrated an association between HIV/HCV coinfection and elevated rates of hepatic decompensation and mortality. One meta-analysis found an adjusted RR of 6.14 for decompensated cirrhosis, and similar increases in RR were found in a recent study where the incidence of decompensated cirrhosis was 7.4% compared to 4.8% ($p<.001$) at 10 years in HIV/HCV co-infected compared to HIV mono-infected patients. (27, 28) There is also a significant increase in the risk of liver-related and all-cause mortality in the co-infected population. In one study, mortality rates were higher in HIV/HCV co-infected patients with 1-year, 2-year, and 5-year survival estimates of 54,

40, and 25%, respectively, compared with 74, 61, and 44% in HCV mono-infected patients, highlighting that co-infection reduces the survival of patients with HCV-related end-stage liver disease and should be taken into consideration when establishing appropriate timing of liver transplantation. (29) The prevalence of HCC is also increased in HIV/HCV co-infection compared to HCV mono-infection, and HCC is estimated to occur after an average of 17.8 years compared with 28.1 years in HCV mono-infection. (30, 31)

*Factors affecting Fibrosis Progression*

Recent studies have begun to examine the factors affecting fibrosis progression in the co-infected population. A multivariate analysis identified 4 independent predictors of progression to cirrhosis in HIV/HCV co-infected patients: absence of PI therapy, heavy alcohol consumption, low CD4 count, and age at HCV acquisition. (32) Poorly controlled HIV mono-infection has also been found to be an independent risk factor for liver fibrosis. (10, 33) Another study found that fibrosis progression correlated with HIV RNA levels in a HIV/HCV co-infected population, further supporting the direct role of HIV in liver fibrogenesis. (34)

Co-infected patients with undetectable HIV RNA through ART, in one study, had a slower fibrosis progression rate (FPR) than those with any detectable HIV RNA level and a FPR similar to HCV mono-infected patients, suggesting that HIV viremia and not CD4 cell count independently predicted FPR. (35) However, not all studies have supported a definitive role of poorly controlled HIV as indicated by viral load and CD4 count in fibrosis progression. In a prospective cohort of 184 HIV/HCV co-infected individuals in which 24% demonstrated significant fibrosis progression over 3 years by

liver biopsy, measures of HIV disease and its treatment (CD4 cell count, HIV-RNA level, and ART exposure) were not significantly different compared to non-progressors. (19) After multivariate adjustment, only the serum AST level between biopsies was significantly associated with fibrosis progression, while covariates such as age, sex, race, HCV-RNA level, HCV genotype, ART, HCV therapy, CD4 cell count, and HIV-RNA level were not significantly associated with fibrosis progression.

The accelerated fibrogenesis seen in HIV/HCV co-infection may be partially mitigated by ART. In an analysis of 2646 co-infected patients, the relative risk for cirrhosis for those not receiving ART was 2.49 (95% CI, 1.81-3.42), compared to 1.72 (95% CI, 1.06-2.80) for those receiving ART, suggesting a protective but incomplete effect of ART on HCV-associated liver disease. (15) Another study found similar progression on liver histology between co-infected and HCV mono-infected patients where most patients were on effective ART, suggesting the benefits of ART on progression of HCV. (36) It is possible that HIV virologic suppression through ART lessens fibrogenic pathways through reductions in CD8+ T-cell activation. (37)

Spontaneous clearance of HCV infection has been described occasionally in co-infected patients with the IL28B CC genotype after initiating antiretroviral therapy and regaining immune competence. (13) Antiretroviral therapy interruption has also been found to be associated with increased risk of liver fibrosis progression in HIV/HCV co-infected patients, with a hazard ratio for ART interruption of 2.52 (95% Confidence Interval [CI] 1.20-5.28). (38)

The type of antiretroviral therapy may also play a role in modulating fibrogenesis. Recent studies have reported conflicting findings about the impact of protease inhibitor

(PI)- based therapy on fibrosis progression—one study reported that the use of PI-based ART in HIV/HCV co-infected patients was associated with less severe fibrosis and slower fibrosis progression, while another study found that the use of protease inhibitors, mainly lopinavir, was associated with increased liver fibrosis progression. (39, 40) Fibrosis progression in co-infected persons may be influenced to a greater degree by the antiretroviral therapy backbone more so than the class of anchor agent, as both PI- and NNRTI-based regimens were associated with increases in APRI score, another measure of hepatic fibrosis, over time when combined with abacavir/lamivudine (ABC/3TC). (41)

The presence of metabolic derangements such as obesity, diabetes, and hyperlipidemia may also accelerate the progression to advanced fibrosis in the co-infected population by generating a proinflammatory and high oxidative stress environment. Steatosis is a frequent histologic finding among patients with chronic Hepatitis C infection, and is significantly associated with body mass index as well as fibrosis. Thus, increasing body mass index (BMI) may play a role in the pathogenesis of steatosis in chronic Hepatitis C infection, and steatosis may contribute to fibrosis. (42) Insulin resistance and type 2 diabetes are also tightly linked to severe fibrosis, likely due to the ability of insulin to stimulate hepatic stellate cells and induce tumor necrosis-factor alpha and connective growth factor production. (43, 44) Moreover, hyperglycemia leads to enhanced formation and deposition of advanced glycation products that activate hepatic stellate cells, which may induce liver collagen production and upregulation of pro-fibrogenic cytokines. (44) In a cohort of patients with genotype 1 chronic HCV infection, not only was the presence of insulin resistance linked to advanced fibrosis, but overt type 2 diabetes also further increased the risk of severe fibrosis. (45) Diabetes has

been associated with a 2-3 fold increase in the risk of HCC, regardless of the presence of HCV, HBV, alcoholic liver disease, or non-specific cirrhosis. (46) Dyslipidemia may also be an important risk factor, as low HDL and diabetes were significantly associated with development of cirrhosis in a cohort of co-infected veterans. (47)

A growing body of evidence suggests that the concurrent use of statins, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-coA) reductase inhibitors, in HIV/HCV co-infected patients may slow fibrosis progression due to their anti-inflammatory, immunomodulatory, and antineoplastic properties. In animal models, statins have demonstrated antifibrogenic effects by blocking the activation of hepatic myofibroblasts and preventing proliferation of hepatic stellate cells and collagen production. (48) Recent studies have reported an association between statin use and reduced risk of cirrhosis and HCC in the HCV mono-infected population. (48-50) In a large cohort of HIV/HCV co-infected veterans, statin use >= 30% time was significantly associated with reduced risk of development of cirrhosis compared with those with less time on statin drugs. (47) Therefore, the benefits of statin use may extend beyond its cardioprotective properties to include reduction in cirrhosis progression in patients with chronic liver disease.

Prior treatment with interferon-based therapy may also play a role in fibrosis progression. The antifibrotic effects of interferon α may be due to repression of the collagen gene (COL1A2) promoter sequence and collagen gene transcription. (51) A study of 74 co-infected patients found that those that received Pegylated-Interferon α-2a based therapy demonstrated significant decreases in fibrosis progression rate (FPR) and stabilization or regression of cirrhosis compared to those who received no treatment or interferon-based therapy. (52) Moreover, Peg-IFN α-2a/RBV therapy improved hepatic

histology and led to a decrease in fibrosis progression not only in patients who achieved sustained virologic response (SVR) but also in non-responders, suggesting that the benefits of treatment with Peg-IFN α-2a/RBV may extend beyond viral clearance to histologic improvement and delayed fibrosis progression.

The order of acquisition of HIV and HCV may be an important consideration. Historically, most co-infected patients acquired HCV first through parenteral routes and then subsequently became infected with HIV. This order of infection is the rule in patients with parenteral exposures because HCV is much more infectious than HIV via parenteral routes. (53) However, this paradigm has shifted in recent years with an emerging epidemic of sexually acquired HCV infection among HIV-infected men who have sex with men (MSM), where the order of infection is reversed because HIV is more infectious than HCV through sexual transmission. (54) Available evidence shows that these HIV-infected men rapidly progress to moderate levels of fibrosis within the first year of HCV infection, and some with the most severe immunocompromise experience further rapid progression to cirrhosis. (55) Additional prospective studies are necessary to more clearly define the long-term outcomes of these HIV-infected men after HCV infection.

*Methods for Assessing Fibrosis Progression*

The accurate evaluation of severity of liver fibrosis in chronic hepatitis C is critical for determining therapeutic indications and prognosis. Among the available methods for assessing liver fibrosis, liver biopsy is currently considered the gold standard but its use is limited by cost, invasiveness, and potential complications such as pain, bleeding, peritonitis, and bowel perforation. Due to limited sampling of the liver

parenchyma, liver biopsy is demonstrated to carry a diagnostic error rate of 20% for fibrosis stage. (56) Increasingly, non-invasive tools such as serologic-based scores are being utilized, including the AST-to-platelet ratio (APRI), FIB-4, FibroTest, FibroSpect II, HepaScore, as well as imaging-based methods such as vibration controlled liver elastography. However, routine use of many of these noninvasive tests can still be hampered by cost, false negative or positive results, or the need for standardization assays.

FIB-4, the assessment tool used in this study, is a simple index which was specifically developed in 832 co-infected patients enrolled in the AIDS Pegasys Ribavirin International Coinfection Trial (APRICOT). In many respects, the FIB-4 serves as a highly useful clinical tool—it is based on simple calculations and easy to use, results are available in a timely fashion, and it is inexpensive, relying only on readily available clinic parameters such as age, ALT, AST, and platelet count without additional costs for equipment. FIB-4 <1.45 was associated with a sensitivity of 70% and a NPV of 90% to exclude advanced fibrosis, while a score >3.25 had a specificity of 97%, and a PPV of 65%. Values between 1.45 to 3.25 were classified as indeterminate. (57)

Since the APRICOT trial, FIB-4 has subsequently been validated in multiple studies as a simple, accurate, and inexpensive method for assessing liver fibrosis for values outside 1.45-3.25, and shows promise for application in emerging countries where more expensive and invasive methods of assessing fibrosis are not accessible. (58-60) Moreover, it is not only predictive of advanced fibrosis, but also of liver-related clinical outcomes and overall mortality. (61, 62)

*Treatment of HIV/HCV Co-Infection*

For many years, the standard therapy for treatment of chronic hepatitis C has been pegylated interferon α (Peg-IFN) and ribavirin (RBV), administered for 24- to 48-weeks depending on the genotype. Under this regimen, viral eradication rates were suboptimal. In patients with genotype 1 HCV, sustained virologic response (SVR) rates were 40% following 48 weeks of Peg-IFN/RBV and even lower in those with African descent, HIV co-infection, or high viral loads or advanced fibrosis. (63-65) In addition, significant side effects associated with interferon and ribavirin therapy have also hindered patient tolerance. Interferon treatment is associated with myelosuppression, flu-like symptoms, neuropsychiatric symptoms, and also lowers the seizure threshold and exacerbates immune-mediated conditions. (66) The addition of ribavirin confers additional risks of hemolytic anemia, rash, and insomnia.

However, the advent of revolutionary new interferon-free direct-acting antiviral agents (DAA) has radically changed the face of HCV therapy. DAAs are designed to inhibit viral proteins involved in the HCV life cycle, with numerous potential targets such as the NS3/4A serine protease, NS5A replication complex protein, NS5B RNA-dependent RNA polymerase, and NS4B and NS3 helicase proteins. (67) With the proliferation of DAAs that offer the potential of highly effective and well-tolerated treatment, regimen selection varies depending on patient genotype and factors such as the presence of cirrhosis and prior treatment history.

In contrast to the previous paradigm in the interferon era, the DAA era has seen comparable HCV cure rates of over 90% in HIV/HCV co-infected patients, eliminating the need for distinguishing between mono- and co-infected patients. (68) The efficacy of DAAs was first demonstrated in trials of the protease inhibitors telaprevir and boceprevir

added to peginterferon and ribavirin, and further confirmed in trials of the newer DAAs, even with regimens that do not contain interferon. It is notable, however, that most clinical trial data on the efficacy of HCV therapy derives from patients on antiretroviral therapy with suppressed HIV viral loads and CD4 counts greater than 200 cells/$mm^3$ and may lack generalizability in the real world. (69-74) Thus, curative all-oral treatment is a possibility for all HIV/HCV co-infected patients.

A major consideration is monitoring drug-drug interactions between antiretroviral therapy and DAAs. For example, because ledipasvir (an NS5a inhibitor) and sofosbuvir (an NS5b inhibitor) are both substrates of the P-glycoprotein transporter, concomitant use of ritonavir boosted tipranavir is not recommended because it may decrease levels due to induction of the transporter. Simeprevir (HCV protease inhibitor) is oxidatively metabolized by the CYP3A subfamily and not recommended for use with HIV protease inhibitors boosted by ritonavir. (75)

Theoretically, all patients with virologic evidence of chronic HCV infection should be considered for treatment; however, there are clinical characteristics that are taken into account when deciding when to initiate HCV therapy. Per the American Association of the Study of Liver Diseases (AASLD) and Infectious Disease Society of America (IDSA) guidelines, treatment should not be withheld from those who currently use illicit drugs or those who are in an opioid treatment program, provided that they wish to be treated, are willing and able to maintain close monitoring, and practice contraception; however, many healthcare providers remain reluctant to treat this at-risk population. (76) Although history of alcohol abuse is not an absolute contraindication to treatment, continued alcohol use has been shown to decrease the response to interferon-

based therapy and accelerate disease progression. (77, 78) Limited data is available on the effectiveness of DAAs in patients who continue to drink.

Financial constraints continue to prevent the widespread availability of novel DAA regimens. Sofosbuvir is an example of a new oral HCV medication that, when combined with other therapies, has a therapeutic efficacy (cure) greater than 90% across 4 genotypes, limited adverse effects, and a shorter treatment window than its interferon-based predecessors; however, its cost forces payers to ration this lifesaving treatment. One study in Massachusetts found that the mean drug cost per patient per SVR was $123,559 for ledipasvir/sofosbuvir and $251,550 for sofosbuvir + simeprevir. (79) It is estimated that third-party payers would need $136 billion to cover medication costs for all eligible patients with HCV from 2015-2020, of which $61 billion would need to be paid by the government. (80) For this reason, some payers restrict access only to patients in more advanced stages of disease, in some cases requiring fibrosis scores of F3 or F4 before covering a DAA medication. (81) In addition, many prior authorization criteria require abstinence from the use of alcohol, illicit drugs, or both in the months leading up to treatment approval, presenting another barrier to treatment. The immense budgetary impact on private and government providers that would be incurred from treating all eligible patients with HCV in the United States continues to preclude widespread access to treatment, making knowledge of the factors that predict fibrosis progression in the HIV/HCV co-infected population all the more critical for effective resource allocation.

Current guidelines suggest a high priority for treating the HIV/HCV co-infected population given the more rapid progression to advanced liver disease in the setting of HIV infection. (82) Achieving SVR has been associated with improved overall survival

in co-infected patients even in lower stages of fibrosis (F0-F2), suggesting that HCV therapy may provide benefits beyond the cure of HCV and may prevent the progression of liver disease. (83) However, it seems reasonable to adopt a "wait and see" policy for patients with little or no fibrosis (F0-F1) since they are unlikely to progress while taking stable cART, while for patients with advanced fibrosis, treatment should be prioritized because fibrosis has already developed and cirrhosis may occur. (68) Therefore, determination of the presence of significant fibrosis or liver cirrhosis is vital for resource allocation and treatment prioritization purposes. As a corollary, predicting who is likely to progress would be important for resource allocation as well. Finally, longitudinal natural history studies in an established cohort are uncommon, but provide critical information about the time course of illness and fibrosis progression in the HIV/HCV co-infected population.

**Hypothesis/Aims:**

The purpose of this study is to elucidate the natural history and identify potential predictors of fibrosis progression in the HIV/HCV co-infected population. We hypothesized that in our co-infected cohort, elevated BMI, history of alcohol abuse, low CD4 count, and elevated HIV viral load would be associated with faster fibrosis progression. We analyzed data collected retrospectively and examined (a) patient demographics and clinical characteristics within this group of patients; (b), the non-invasive score FIB-4 in order to describe patterns of fibrosis progression over 5 years**;** and (c), the association of various factors such as BMI, diabetes, hyperlipidemia, history of alcohol abuse, CD4 count, and HIV viral load with FIB-4 progression.

**Methods:**

Study subjects were selected from HIV/HCV co-infected patients receiving care at the Nathan Smith Clinic of Yale-New Haven Hospital from June 2002 through April 2016. The date of study entry was the date of last laboratory values prior to treatment with DAAs or the most recent laboratory values for patients not treated with DAAs. Data going back 5 years was chosen because a 5-year time interval has been postulated as the average time to progression between stages of liver fibrosis. Patients who did not have cirrhosis (as defined by FIB-4>3.25) at 5 years prior to study entry were eligible for the study.

On a retrospective review of medical records, data on the following variables were collected: demographics such as gender and race, clinical characteristics related to HCV and HIV such as year of HIV and HCV diagnosis as recorded in chart notes (if known), mode of HCV acquisition, HCV genotype, antiretroviral therapy type and backbone, prior treatment for HCV with interferon-based therapy, liver biopsy stage if performed, and DAA treatment course if treated. The body mass index (BMI) used in the analysis was the value recorded closest to the date of study entry. Diagnosis of diabetes was based on the presence of diabetes mellitus on the patient's problem list, or at least 2 Hemoglobin A1c values $\geq$ 6.5. Diagnosis of hyperlipidemia was captured as charted in patients' medical record, or at least 2 values of elevated total cholesterol over the previous 5 years. Statin use was recorded if a statin was on the patient's medication list at the time of study entry. History of smoking was positive if the patient was a current or former smoker as recorded in clinic notes at the time of study entry. The Alcohol Use

Disorders Identification Test (AUDIT)- C score was recorded as the highest AUDIT-C score recorded at a patient visit during the 5 years prior to study entry; the AUDIT-C score is routinely calculated at each clinic visit, with scores of 4 or more in men and 3 or more in women considered a positive screen and optimal for identifying hazardous drinking or active alcohol use disorders. History of alcohol abuse was determined by any prior history of alcohol abuse recorded in visit notes. Active drug use was determined as documented in visit notes or positive urine toxicology screen for cocaine or opiates within the previous 6 months before study entry. Hepatitis B co-infection was indicated by positive Hepatitis B surface antigen (HBsAg). The VACS score (Veterans Aging Cohort Study) at time of study entry was calculated using the patient's age, sex, race, CD4 count, HIV viral load, hemoglobin, AST, ALT, platelet count, FIB-4, Creatinine, eGFR, and Hepatitis C status at the time of study entry, using the calculator provided at https://vacs.med.yale.edu/calculator/IC. VACS scores typically range from 0 to 100, with a higher score indicating worse prognosis and increased risk of mortality.

Routine laboratory values including AST, ALT and platelet count were extracted to calculate the FIB-4 score, which is computed as follows: FIB-4 = age [years] × AST [IU/L]/platelet count [platelets $\times 10^9$/L] × ($ALT^{1/2}$[IU/L]). Yearly FIB-4 scores dating back 5 years were calculated from the last FIB-4 prior to treatment initiation for patients treated with DAAs, or the most recent FIB-4 for patients not treated with DAAs. The mean of 3 values closest to the chosen time point was used to calculate the annual FIB-4 score. The closest CD4 count, CD4/CD8 ratio, HIV viral load, and HCV viral load obtained within 6 months of the annual FIB-4 score was recorded. In order to obtain 3 year and 5 year log viral loads and CD4 counts, all log viral loads or CD4 counts for each

patient over the 3 year or 5 year period prior to study entry were extracted and the mean of all the available values was obtained.

The primary outcome measure of the study was progression to advanced fibrosis, indicated by FIB-4 levels exceeding the value of 3.25, and patients were categorized based on FIB-4 progression over the course of 5 years. Progressors were defined as those whose FIB-4 progressed from below 3.25 to above 3.25 while non-progressors were defined as those whose FIB-4 remained below 3.25. Descriptive statistics were used to assess baseline characteristics. In order to compare progressors vs non-progressors, parametric (t-tests) or non-parametric (Wilcoxon scores) methods were used for continuous variables as appropriate; Chi-square or Fisher's exact test were used for categorical variables as appropriate. Univariate logistic regression models were used to examine factors associated with progression to FIB-4 >3.25: such models were used for age, BMI, diabetes, hyperlipidemia, statin use, alcohol abuse, injection drug use, HIV viral load, CD4+ cell count, among others. Variables were chosen for inclusion in a multivariable model based on a univariate model p-value of $\leq 0.20$. A p value less than 0.05 was considered statistically significant. Statistical analysis was performed using SAS v9.4 (SAS Institute Inc., Cary, NC). The Yale University Institutional Review Board approved the study protocol.

**Results:**

**Patient Demographics**

Of 126 HIV/HCV co-infected patients, 93 started out with initial FIB-4 scores below 3.25, indicating lack of advanced fibrosis. This subgroup of 65 males and 28

females had mean age of 56.7 years and average BMI of 27.1; 32.3% were white, 53.8% were black, 11.8% identified as Hispanic (**Table 1**). 15.0% of patients had diabetes, 22.6% had a history of hypercholesterolemia, and 17.2% were prescribed a statin. 90.3% were current or former smokers, and 25% demonstrated active drug use. 50.5% of patients had documented history of alcohol abuse within the chart. The mean AUDIT-C score was 1.1 and 56.0% had an AUDIT-C score of 0. The mean VACS score was 38.5, corresponding to a 5-year mortality rate of approximately 18%, and only 2 patients were also co-infected with hepatitis B.

**Table 1. Baseline characteristics of 93 HIV/HCV co-infected patients**

| Characteristic | Total (**N=93**) |
|---|---|
| Age, mean years | 56.7 |
| Gender | |
| Male | 65 (69.9%) |
| Female | 28 (30.1%) |
| Race | |
| White | 30 (32.3%) |
| Black | 50 (53.8%) |
| Hispanic | 11 (11.8%) |
| Other | 2 (2.2%) |
| Body mass index, mean kg/m$^2$ | 27.1 |
| History of diabetes | 14 (15.1%) |
| History of hyperlipidemia | 21 (22.6%) |
| Statin use | 16 (17.2%) |
| Ever smoker | 84 (90.3%) |
| History of alcohol abuse | 46 (49.5%) |
| Active drug use | 23 (25.0%) |
| AUDIT-C Score (highest recorded) | |
| 0-3 | 80 (87.9%) |
| 4+ | 13 (12.1%) |
| VACS Index Score, mean (range)[1] | 38.5 (5-107) |
| Hepatitis B co-infection | 2 (2.2%) |

*AUDIT, Alcohol Use Disorders Identification Test; VACS, Veterans Aging Cohort Study*

1. *The VACS score ranges from 0-164, with higher scores predicting higher risk of 5 year mortality.*

**HCV-Specific Characteristics**

Injection drug use was the predominant risk factor for HCV acquisition (63.4%), followed by both injection drug use and heterosexual transmission (12.9%), heterosexual transmission alone (6.5%), and men who have sex with men (MSM) (3.2%) (**Table 2**). The duration of HCV infection was available for 27 patients and the mean was 19.8 years. Genotypes included: genotype 1 (81.2%), genotype 3 (9.4%), genotype 2 (5.9%) and genotype 4 (3.5%). 15 patients (16.1%) had prior treatment with interferon-based therapy. Of 38 patients who had ever received a liver biopsy, 47.4% (18) showed no to mild fibrosis (F0-F1), 36.8% (14) showed moderate fibrosis (F2), 7.9% (3) showed severe fibrosis (F3), and 7.9% (3) showed evidence of cirrhosis (F4). Of the three patients with biopsy proven cirrhosis, FIB-4 showed concordance with liver biopsy in two patients.

52 patients were subsequently treated with DAAs while 41 remained untreated at the time of data collection. Of those treated with DAAs, the majority (65.4%) were treated with ledipasvir/sofosbuvir, 15.4% were treated with sofosbuvir/ribavirin, 9.6% with sofosbuvir/simeprevir, 7.7% with sofosbuvir/daclatasvir, and 1.9% with other regimens.

**Table 2. HCV-Specific Characteristics**

| HCV-Specific Characteristic | Total (**N=93**) |
|---|---|
| Risk mode of HCV acquisition | |
| IDU alone | 59 (63.4%) |
| IDU and heterosexual | 12 (12.9%) |
| Heterosexual | 6 (6.5%) |
| MSM | 3 (3.2%) |
| Blood Transfusion | 3 (3.2%) |
| IVDU, heterosexual, | 1 (1.1%) |

| | |
|---|---|
| tattoos, blood transfusion | |
| Unknown | 12 (12.9%) |
| Duration of HCV infection, mean years[1] | 19.8 |
| HCV Genotype | |
| 1 | 69 (81.2%) |
| 2 | 5 (5.9%) |
| 3 | 8 (9.4%) |
| 4 | 3 (3.5%) |
| Liver biopsy performed | 38 (40.9%) |
| F0-1 | 12 |
| F2 | 14 |
| F3 | 3 |
| F4 | 3 |
| Subsequent treatment with DAA | 52 (55.9%) |
| DAA treatment regimen: | |
| Ledipasvir/sofosbuvir | 34 |
| Sofosbuvir/ribavirin | 8 |
| Sofosbuvir/simeprevir | 5 |
| Sofosbuvir/daclatasvir | 4 |
| Elbasvir/grazoprevir | 1 |

*1.Data on year of HCV diagnosis available in 27 patients*

**HIV-Specific Characteristics**

The mean duration of HIV infection based on year of HIV diagnosis was available for 82 patients and was 22.3 years (**Table 3**). At the time of study entry, the median CD4 count was 564 cells/mm$^3$ (Interquartile Range [IQR] 368, 891) the median CD4/CD8 ratio was 0.7 (IQR 0.4, 1.1), and the median historical nadir CD4 count was 308 cells/mm$^3$ (IQR 186, 498). The majority (88.4%) had HIV viral loads <500 copies/mL.

The vast majority of patients (97.9%) were on antiretroviral therapy; 29% were on a protease inhibitor-based regimen, 28.0% were on a non-nucleoside reverse transcriptase inhibitor (NNRTI)- based regimen, and 28.0% were on an integrase strand transfer inhibitor (INSTI)-based regimen. The antiretroviral regimen backbone consisted of tenofovir disoproxil fumarate/emtricitabine (TDF/FTC) for 67.7%, and abacavir/lamivudine (ABC/3TC) for 20.4%.

**Table 3. HIV-Specific Characteristics**

| HIV-Specific Characteristic | Total (**N=93**) |
|---|---|
| Duration of HIV infection, mean years | 22.3 |
| CD4+ Cell count, median (IQR) | 564 (368, 891) |
| CD4/CD8 ratio, median (IQR) | 0.7 (0.4, 1.1) |
| Nadir CD4+ count, median (IQR) | 308 (186, 498) |
| HIV-1 RNA <500 copies/mL at study entry n (%) | 76 (88.4%) |
| On ART n (%) | 91 (97.9%) |
| ART treatment regimen (n): | |
| PI-based | 27 |
| NNRTI-based | 26 |
| INSTI-based | 26 |
| (INSTI and PI) NRTI sparing | 4 |
| Other[1] | 8 |
| ART backbone (n): | |
| TDF/FTC | 63 |
| ABC/3TC | 19 |
| Other | 9 |

1. *Other regimens included: raltegravir(RAL)/tenofovir disoproxil fumarate(TDF)/emtricitabine(FTC)/darunavir(DRV)/ritonavir(r) (3), TDF/FTC/DRV/r/DTG(dolutegravir), RAL/3TC/ATZ(atazanavir)/r, RAL/TDF/FTC/ATZ/r, RPV(rilpivirine)/TDF/FTC/DRV/c(cobicistat), RAL/TDF/FTC/ETR(etravirine)/DRV/r*

**Progression to Advanced Fibrosis**

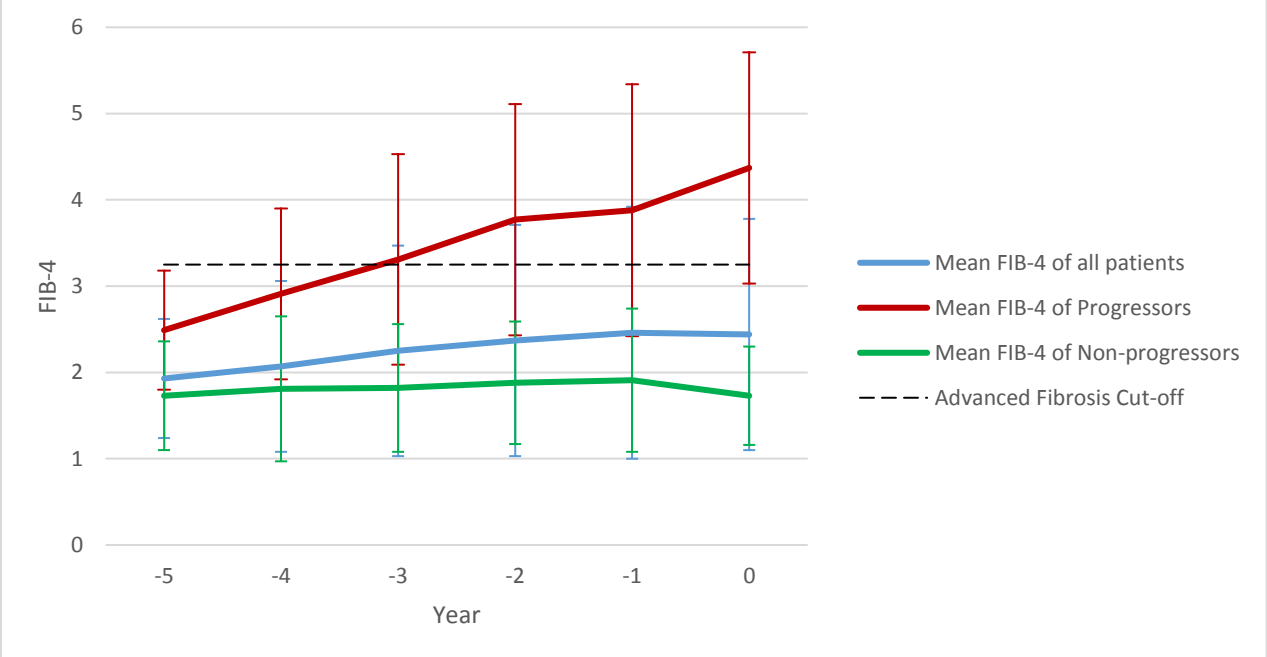
Over the course of 5 years, 68 patients (73.1%) had FIB-4 remain below 3.25, while 25 (26.9%) had FIB-4 progress to >3.25 (**Table 4, Figure 1**). Of the 68 non-progressors, 23 patients started out below 1.45, and 14 remained below 1.45 after 5 years. Five years prior to study entry, the annual mean FIB-4 score of the full cohort started at 1.93, trending upward to 2.07 at 4 years, 2.25 at 3 years, 2.37 at 2 years, 2.46 at 1 year, and 2.44 at the time of study entry. In patients whose FIB-4 remained below 3.25 (non-progressors), the mean annual FIB-4 scores from 5 years to time of study entry were 1.73, 1.81, 1.82, 1.88, 1.91, and 1.73. In patients whose FIB-4 progressed to above 3.25

(progressors), the mean annual FIB-4 scores from 5 years to time of study entry were 2.49, 2.91, 3.31, 3.77, 3.88 and 4.37. The difference between lowest and highest FIB-4 scores was 1.39 in progressors and 0.18 in non-progressors.

**Table 4.** Trends in FIB-4 Progression over 5 years

| | Total (N=93) | Mean (SD) FIB-4 5 years prior t = -5 | Mean (SD) FIB-4 4 years t = -4 | Mean (SD) FIB-4 3 years t = -3 | Mean (SD) FIB-4 2 years t = -2 | Mean (SD) FIB-4 1 year t = -1 | Mean FIB-4 (SD) at time 0 t = 0 |
|---|---|---|---|---|---|---|---|
| Progressed to >3.25 | 25 (26.9%) | 2.49 (0.51) | 2.91 (1.00) | 3.31 (1.51) | 3.77 (1.69) | 3.88 (1.76) | 4.37 (0.81) |
| Remained <3.25 | 68 (73.1%) | 1.73 (0.63) | 1.81 (0.84) | 1.82 (0.74) | 1.88 (0.71) | 1.91 (0.83) | 1.73 (0.57) |

**Figure 1.** FIB-4 Progression Over 5 Years



**Comparison of Progressors to Non-Progressors**

We hypothesized that markers of HIV and HCV disease severity such as duration of disease and quantitative markers would differ between progressors and non-progressors. Duration of HIV and HCV infection (when available), mean HIV and HCV viral load, nadir CD4 count, CD4 count, and CD4/8 ratio (over the 3 or 5 years prior to study entry) did not differ significantly between patients who progressed to >3.25 and patients who remained <3.25 (**Table 5**).

**Table 5.** Correlates of Fibrosis Progression

| Characteristic | Non-progressors (N = 68) Mean +/- S.D. | Progressors (N = 25) Mean +/- S.D. | P value[1] |
|---|---|---|---|
| HCV duration, years (*N=27) | 20.0 +/- 8.9 | 19.0 +/-12.5 | 0.95 |
| HIV duration, years (N = 82) | 22.6 +/- 6.6 | 21.4 +/- 8.4 | 0.51 |
| Mean log HCV viral load (from t= 0 to t=-3) | 6.3 +/- 1.1 | 5.7 +/- 1.0 | **0.02** |
| Mean log HCV viral load (from t=0 to t=-5) | 6.3 +/- 1.1 | 5.9 +/- 1.1 | 0.08 |
| Mean log HIV viral load (from t=0 to t=-3) | 2.0 +/- 0.7 | 2.2 +/- 0.9 | 0.14 |
| Mean log HIV viral load (from t=0 to t=-5) | 2.0 +/- 0.6 | 2.2 +/- 0.8 | 0.21 |
| Mean CD4 count (from t=0 to t=-3) | 633 +/- 342 | 551 +/-336 | 0.31 |
| Mean CD4 count (from t=0 to t=-5) | 618 +/- 336 | 550 +/- 323 | 0.38 |
| Mean CD4/CD8 ratio (from t=0 to t=-3) | 0.7 +/- 0.5 | 0.9 +/-0.7 | 0.22 |
| Mean CD4/CD8 ratio (from t=0 to t=-5) | 0.7 +/- 0.4 | 0.9 +/- 0.7 | 0.13 |

1. *P values were obtained from t-tests or the Wilcoxon Two-Sample test (non-parametric) as appropriate.*

**Predictors of Fibrosis Progression**

In univariate logistic regression models, age, gender, race, BMI, alcohol abuse, active injection drug use, diabetes, hyperlipidemia, statin use, HIV and HCV duration and viral load, and CD4 count did not significantly predict progression to advanced fibrosis (**Table 6**). A number of variables were suggestive of a protective trend (such as hyperlipidemia, statin use, AUDIT score <4, HIV viral load <500 copies/mL), however, they did not achieve statistical significance. Similarly, the use of non-NNRTI-based regimen appeared protective but was not statistically significant. The 3 year mean log HCV viral load (defined as the mean log HCV viral load in the 3 years prior to study entry for whom data was available) showed a statistically significant effect favoring low HCV viral loads predictive of fibrosis progression. Conversely, a CD4 count <100 cells/mL at the time of study entry appeared to correlate with higher risk of progression, but this was not statistically significant.

In a multivariable logistic regression model, age, race, gender, CD4 count, viral load, hemoglobin, and estimated glomerular filtration rate (eGFR) were not found to be significant, in addition to AUDIT score, ART class or backbone, statin use, and 3 year mean HIV viral load (**Table 7**).

**Table 6.** Univariate Analysis of Correlates of Fibrosis Progression

| **Demographic characteristic** | Odds Ratio >1 (95% CI)<br>'Elevated' Risk | Odds Ratio <1 (95% CI)<br>'Reduced' Risk |
|---|---|---|
| Age | 1.01 (0.94, 1.08) | |
| Female vs male | 1.13 (0.42, 3.04) | |
| Black vs non-black | | 0.73 (0.29, 1.83) |
| BMI <18.5 vs >=30 kg/$m^2$ | 1.91 (0.26, 13.87) | |
| BMI 18.5-<25 vs >=30 kg/$m^2$ | 1.43 (0.48, 4.24) | |

| | | |
|---|---|---|
| BMI 25-29 vs >=30 kg/m$^2$ | | 0.45 (0.10, 2.00) |
| **Comorbid conditions** | | |
| History of alcohol abuse | | 0.92 (0.37, 2.31) |
| Active drug use | | 0.93 (0.32, 2.71) |
| Diabetes | 1.64 (0.49, 5.47) | |
| Hyperlipidemia | | 0.57 (0.17, 1.90) |
| Statin use | | 0.34 (0.07, 1.60) |
| Ever smoker | | 0.71 (0.16, 3.08) |
| AUDIT score <4 vs 4+ | | 0.59 (0.12, 2.93) |
| **HIV-specific characteristic** | | |
| PI-based ART | 1.21 (0.45, 3.28) | |
| Non-NNRTI based ART | | 0.56 (0.19, 1.69) |
| ABC/3TC ART | 1.34 (0.45, 4.01) | |
| HIV duration in years | | 0.98 (0.91, 1.05) |
| HIV viral load <500 vs >=100,000 copies/mL at study entry | | 0.36 (0.05, 2.71) |
| HIV viral load 500-9999 vs >=100,000 copies/mL at study entry | | 0.50 (0.02, 11.09) |
| 3-year mean log HIV viral load | 1.52 (0.86, 2.68) | |
| 5-year mean log HIV viral load | 1.48 (0.79, 2.79) | |
| CD4 count <100 vs >=500 cells/mL at study entry | 6.91 (0.57, 83.5) | |
| CD4 count 100-199 vs >=500 cells/mL at study entry | 3.46 (0.61, 19.59) | |
| CD4 count 200-349 vs >=500 cells/mL at study entry | 1.48 (0.33, 6.70) | |
| CD4 count 350-500 vs >=500 cells/mL at study entry | 1.15 (0.34, 3.88) | |
| 3-year mean CD4/CD8 ratio | 1.66 (0.73, 3.76) | |
| 5-year mean CD4/CD8 ratio | 1.96 (0.81, 4.76) | |
| **HCV-specific characteristic** | | |
| HCV duration in years | | 0.99 (0.89, 1.10) |
| HCV duration, <10 vs 10+ years | | 0.15 (0.02, 1.50) |
| 3-year mean log HCV viral load | | 0.62 (0.38, 1.00) |
| 5-year mean log HCV viral load | | 0.70 (0.46, 1.08) |
| Prior HCV treatment | 1.45 (0.44, 4.76) | |

*For continuous variables, the odds ratio refers to the per unit increase in risk.*

**Table 7**. Multivariate Analysis of Correlates of Fibrosis Progression

| **Demographic Characteristic** | Odds Ratio (95% CI) |
|---|---|
| Age | 0.99 (0.90, 1.09) |
| Female sex | 0.80 (0.20, 3.13) |

| | |
|---|---|
| Black race | 1.03 (0.28, 3.72) |
| **Comorbid conditions** | |
| AUDIT-C score level | 0.53 (0.10, 2.71) |
| Statin use | 0.40 (0.08, 1.96) |
| Hemoglobin <10 vs >=14 g/dL | 1.85 (0.04, 87.17) |
| Hemoglobin 10-11.9 vs >14 g/dL | 1.14 (0.19, 6.80) |
| Hemoglobin 12-13.9 vs >=14 g/dL | 3.11 (0.89, 10.82) |
| eGFR <30 vs >=60 mL/min | 1.47 (0.07, 31.61) |
| eGFR 30-44.9 vs >=60 mL/min | 6.40 (0.65, 63.49) |
| eGFR 45-59.9 vs >=60 mL/min | 3.71 (0.69, 19.90) |
| **HIV-specific characteristics** | |
| CD4 count <100 vs >=500 cells/mL at study entry | 19.01 (0.28, 1000) |
| CD4 count 100-199 vs >=500 cells/mL at study entry | 12.13 (0.67, 221.30) |
| CD4 count 200-349 vs >=500 cells/mL at study entry | 1.40 (0.22, 8.86) |
| CD4 count 350-500 vs >=500 cells/mL at study entry | 0.92 (0.21, 3.99) |
| Log HIV viral load at study entry | 0.72 (0.29, 1.82) |
| 3-year mean log HIV viral load | 1.50 (0.85, 2.66) |
| PI-based ART | 1.01 (0.34, 3.01) |
| Non-NNRTI based ART | 0.52 (0.15, 1.82) |
| ABC/3TC ART | 0.98 (0.30, 3.23) |
| **HCV-specific characteristics** | |
| Prior HCV treatment with interferon | 1.67 (0.49, 5.71) |

**Discussion:**

Patients with chronic hepatitis C and HIV co-infection exhibit more rapid progression to advanced liver disease, making coinfection a compelling reason to prioritize a patient for HCV antiviral therapy, particularly in the era of highly effective and well-tolerated DAAs. However, in the current era, DAAs are not widely accessible for those in need due to prohibitive costs and resource constraints, making the prioritization of patients at greatest risk of fibrosis progression an important consideration.

In this study of 93 HIV/HCV co-infected patients without baseline advanced fibrosis based on FIB-4, we found that 26.9% progressed to advanced fibrosis over 5 years of follow-up. This observed incidence of fibrosis progression over a relatively short

interval is consistent with prior studies of HIV/HCV co-infected patients where progression to advanced fibrosis also occurred in as few as 3 to 5 years. (14, 18, 84)

Fibrosis progression of two Ishak fibrosis stages has been observed in only 8-12% of HCV mono-infected patients over median intervals of 30-44 months, and a recent study of nearly 14,000 HIV-infected patients found that 10% of HIV mono-infected patients progressed to advanced fibrosis compared to 24% of HCV co-infected patients in a median of 3 years ($p<0.01$). (33, 85, 86) Since our patients had likely been infected with HCV for many years prior to the study period, our findings may also suggest that fibrosis progression in the co-infected population does not always follow a linear trajectory of inevitable progression, necessitating serial monitoring to facilitate detection of individuals with progressive disease prior to the onset of clinical liver disease. (18)

We did not find history of alcohol abuse, hyperlipidemia, diabetes, BMI, CD4 count, and HIV viral load to be statistically significant predictors of fibrosis progression (Table 6), although there appeared to be an association between HIV viral load and CD4 count at time of study entry and risk of fibrosis progression. There was a consistent trend towards increased risk of fibrosis progression with increasing viral load—HIV viral load <500 vs >=100,000 copies/mL showed OR 0.36, and the mean log HIV viral loads over 3 years and 5 years showed OR of 1.52 and 1.48, respectively. The CD4 level at study entry also showed an increasing OR with each decreasing level, with OR of 1.15 for 350-500 vs >=500 cells/mL, OR of 1.48 for 200-349 vs >=500 cells/mL, OR of 3.46 for 100-199 vs >=500 cells/mL, and OR of 6.91 for <100 vs >500 cells/mL, although these differences did not achieve statistical significance. The evidence implicating these variables is conflicting in the literature. In a small study of 30 co-infected patients, heavy

alcohol consumption, metabolic disorder, CD4 count, and HIV viral load were not significantly associated with fibrosis progression, and only elevated ALT ($p<0.001$) and AST ($p<.0340$) higher than 3 times the upper limit of normal were associated with fibrosis progression. (87) This was consistent with the findings of another study of 174 patients in which only the serum AST level was significantly associated with fibrosis progression while other covariates such as age, sex, race, CD4 cell count, and HIV-RNA did not show a significant association. (19)

In contrast, other studies have reported an association between low CD4 count and fibrosis progression. (47, 88, 89) This was supported by increasing odds ratios for each decreasing level of CD4 count at time of study entry in Table 6. One could assert that the majority of our patients had clinically stable HIV infection with undetectable HIV viral loads due to ART, making it difficult to detect any association between CD4 count and liver fibrosis progression.

We also examined the CD4/CD8 ratio because the CD4/CD8 ratio is emerging as a biomarker of immune activation and systemic inflammation in HIV positive patients. Our data showed that increasing CD4/CD8 ratio may have been associated with increased risk of fibrosis progression, which was unexpected because chronic HCV infection has been associated with low CD4/CD8 ratios in HIV positive women in a recent study. (90) However, this same study observed low CD4/CD8 ratios in women with cleared HCV infection, highlighting that T-cell dynamics are not well elucidated in the HIV/HCV co-infected population.

In our study, age did not predict risk of fibrosis progression, although some studies have identified older age as an independent predictor of fibrosis progression. (91,

92) Black race did not confer a protective effect on the development of fibrosis in our patient population (p = 0.59), in contrast to previous findings that black race was associated with lower rates of hepatic decompensation. (28) Despite studies reporting improved liver histology and slower fibrosis progression rate among treated non-responders compared to non-responders, we did not detect an effect of prior interferon-based HCV treatment on fibrosis progression in our cohort, of which 16.1% had received prior treatment (p = 0.54). (52) AUDIT score less than 4 may have been protective (OR 0.59), though this finding was not statistically significant (0.51).

Multiple studies have supported the lack of correlation between HCV viral load and fibrosis progression in the HCV mono-infected population (93-95) In the setting of co-infection, HCV RNA levels increase after HIV seroconversion and continue to increase over time compared to patients with HCV alone. (53) The level of HCV viremia is inversely correlated with lower CD4 counts in most studies; however, overall increases in the HCV viral load do not appear associated with severity of liver disease. (96, 97) In our results, the 3 year mean log HCV viral load appeared to differ significantly between progressors and non-progressors (p = 0.02, Table 5), however statistical significance was not seen for the 5 year mean log HCV viral load (p = 0.08) and the univariate logistic regression model showed borderline significance (p = 0.05), suggesting that any difference in HCV viral load between progressors and non-progressors was either confounded by other parameters or was a random chance finding.

Suppression of HIV infection through ART has been associated with slower liver fibrosis regression rate; however, there is conflicting data regarding the impact of type of ART on fibrosis progression. The use of protease inhibitors, mainly lopinavir, was

associated with increased liver fibrosis progression in one study, while another reported that PI-based ART was a protective factor against fibrosis progression. (39, 40) Moreover, a recent study asserts that the choice of antiretroviral backbone influences fibrosis progression more than the class of anchor agent, with abacavir/lamivudine containing regimens associated with fibrogenesis. (41) However, we failed to find a significant association between fibrosis progression and ART class ($p = 0.70$ for PI, $p=0.30$ for NNRTI) or ABC/3TC backbone ($p = 0.61$). This could be because in our cohort of primarily immunocompetent patients, the effect of ART on fibrogenesis may be subtle and require observation for a duration longer than a 5 year period.

Statin therapy in HCV mono-infection has been shown to reduce cirrhosis risk by 69-87%, and recently, has also been demonstrated to mitigate the risk of liver disease progression in HIV/HCV co-infected patients. (47, 48, 50) However, we did not find a statistically significant association between statin use and fibrosis progression ($p = 0.17$) although there seemed to be a protective trend (OR 0.34). This may be because in our study, only a few patients (17.2%) received statin prescriptions although 22.6% had a recorded history of hyperlipidemia. Thus, our ability to detect any protective effects associated with statin therapy is limited. Although there are safety concerns about the potential hepatotoxicity associated with statins, statin use in the presence of liver disease or enzyme elevation is generally safe and well tolerated. (98) Further prospective studies would be needed to better understand the efficacy of statin drugs as adjunct therapy in the care of HIV/HCV co-infected patients. It is worth noting that HIV-infected patients have a greater prevalence of dyslipidemia, earlier incidence and progression of atherosclerosis, and a nearly twofold increased risk for myocardial infection compared to those without

HIV, making a compelling case for increased statin use in the HIV-infected population for cardiovascular disease prevention. (99)

We did not find a significant association between BMI, diabetes, and hyperlipidemia and risk of fibrosis progression, in contrast to previous studies which have identified that metabolic risk factors such as obesity, low HDL, and diabetes are associated with development of cirrhosis. (18, 33, 47) Conversely, we found that the diagnosis of hyperlipidemia was associated with an odds ratio of 0.57 (0.17, 1.90), which may be attributed to the fact that of the 21 patients with hyperlipidemia, 14 were on a statin and may have exhibited the potentially protective effects of statin treatment on fibrosis progression. Moreover, we found that an overweight BMI in the range of 25-29 $kg/m^2$ may have protective effects compared to the reference group with BMI of 30+ (OR 0.45), while an underweight or average BMI may have detrimental effects on fibrosis progression compared to those with BMI of 30+. Although these results were not statistically significant, they may support the obesity paradox in chronic disease, which refers to an inverse association between excess adiposity and mortality. It has been demonstrated in a group of HIV-infected men who have sex with men, where those who were overweight possessed higher CD4 cell counts and lower viral loads than those of normal weight (100). Prospective studies will be needed to further assess the impact of metabolic derangements on the risk of fibrosis progression in the HIV/HCV co-infected population.

We hypothesized that a composite score of disease severity might be predictive of progression. The VACS Index generates a weighted score based on age, routinely monitored indicators of HIV disease (CD4 count and HIV-1 RNA) and general indicators

of organ system injury (hemoglobin, platelets, AST and ALT, FIB-4, creatinine, and HCV infection) to indicate increasing risk of all-cause mortality with increasing score (99). Not only does the VACS Index discriminate mortality risk more effectively than traditional indices restricted to CD4 count, HIV-1 RNA, and age, but it also predicts many other health outcomes in people living with HIV infection and has been validated in several European and North American cohorts (101, 102). The VACS Index has been shown to predict outcomes after admission for bacterial pneumonia, medical intensive care unit admission and fragility fractures, weight gain in the first 12 months after ART initiation, and acute myocardial infarction (103-109). We found that the VACS Index score differed significantly between progressors and non-progressors; however, the presence of Hepatitis C and the FIB-4 score which was the basis for our outcome variable, are included in the VACS Index calculation. A multivariate model that included all of the elements of the VACS Index except for Hepatitis C infection and FIB-4 score did not achieve statistical significance.

There are several limitations to our study. The relatively small sample size might not have afforded us enough power to achieve statistical significance for the observed differences for variables that one would expect to show significance based on prior studies, including low CD4 count, high HIV viral load, diabetes, hyperlipidemia, and alcohol use disorder. An alternative explanation is that the HIV/HCV co-infected population is already at higher risk of fibrosis progression and cannot be further subdivided into increasing risk categories. Although FIB-4 has been well validated in HIV/HCV co-infected populations as an accurate method of assessing liver fibrosis, FIB-4 values may fluctuate due to other comorbid conditions, both intrahepatic (hepatic injury

due to medications, alcohol, infections) and extrahepatic (sepsis, thrombocytopenia due to immune dysregulation or immune suppression), which may not truly reflect fibrosis severity. (33) In addition, our laboratory results were not restricted to those obtained in the outpatient setting and included some tests such as transaminases obtained during acute illness. However, we tried to mitigate the impact of FIB-4 fluctuations by taking the mean of 3 FIB-4 scores to obtain the annual FIB-4 score. Although it is possible that relying on a single non-invasive marker such as FIB-4 score to assess fibrosis severity may have influenced our findings, we found that the FIB-4 score showed strong concordance with liver biopsy stages in differentiating cirrhotic and non-cirrhotic patients out of the 38 patients who had ever received a liver biopsy. There were only 3 patients in our study with biopsy proven cirrhosis, and in 2 out of the 3 patients, the liver biopsies were obtained during the 5 year study period and corresponded to annual FIB-4 scores of 4.10 and 7.31. The study was also limited by missing data. For example, the duration of HCV infection was only documented in a minority of patients, but this information would have been useful for determining whether those who progressed faster had been infected with HCV for a longer duration.

Our study could also have been influenced by disease spectrum bias; those who progressed had higher mean FIB-4 scores at 5 years prior to study entry compared to non-progressors (2.49 vs 1.73; Figure 1), and may have represented individuals with a longer duration of HCV infection. However, there was no statistically significant difference in age between the 2 groups which would make this bias less likely. Although most patients had been infected with HCV for well over a decade, the study duration was limited to 5 years and may not have fully captured the natural history of fibrosis progression in this

population. Therefore, further observation over a longer duration of 10-15 years in a larger pool of patients may have detected smaller and more long-term effects.

Data extracted from problem lists and chart review may not have completely captured variables such as alcohol use disorder; therefore, we also extracted information about AUDIT scores, which were routinely obtained at every outpatient visit. Yet it is possible that we underestimated the degree of alcohol intake. Our study was limited to patients receiving care at a single outpatient clinic and patients who were initially cirrhotic (FIB-4>3.25) were excluded, further reducing the sample size (N=93).

In conclusion, in this cohort of HIV/HCV-infected patients receiving care at a single clinic, based on FIB-4 score measurements, a quarter had significant fibrosis progression over a 5-year interval. There were no significant predictors of progression, although certain measures of HIV virologic control such as HIV viral load and CD4 count at study entry tended to correlate with fibrosis progression.

Our study cannot fully exclude the impact of these factors, and further research is needed to identify the factors that influence fibrogenesis through large, prospective natural history studies. It is notable that information from this study provides valuable insight into which variables should be utilized for testable hypotheses in future studies. What are the potential implications of our findings for prioritizing treatment with DAAs? While others might consider a “wait and see” approach feasible given that many patients in our study did not progress to cirrhosis based on FIB-4 scoring over 5 years, our findings did not identify any reliable predictors of fibrosis progression. Thus, universal treatment with DAAs would likely be the best approach and may be more readily implementable as HCV medication prices decline in the future.

## References:


- 1. Joshi D, O'Grady J, Dieterich D, Gazzard B, Agarwal K. Increasing burden of liver disease in patients with HIV infection. Lancet (London, England). 2011;377(9772):1198-209.
- 2. Klein MB, Rockstroh JK, Wittkop L. Effect of coinfection with hepatitis C virus on survival of individuals with HIV-1 infection. Current opinion in HIV and AIDS. 2016;11(5):521-6.
- 3. Sherman KE, Rouster SD, Chung RT, Rajicic N. Hepatitis C Virus prevalence among patients infected with Human Immunodeficiency Virus: a cross-sectional analysis of the US adult AIDS Clinical Trials Group. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2002;34(6):831-7.
- 4. Acharya C, Dharel N, Sterling RK. Chronic liver disease in the human immunodeficiency virus patient. Clinics in liver disease. 2015;19(1):1-22.
- 5. Carr A. HIV protease inhibitor-related lipodystrophy syndrome. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2000;30 Suppl 2:S135-42.
- 6. Brivet FG, Nion I, Megarbane B, Slama A, Brivet M, Rustin P, et al. Fatal lactic acidosis and liver steatosis associated with didanosine and stavudine treatment: a respiratory chain dysfunction? Journal of hepatology. 2000;32(2):364-5.
- 7. Greub G, Ledergerber B, Battegay M, Grob P, Perrin L, Furrer H, et al. Clinical progression, survival, and immune recovery during antiretroviral therapy in patients with HIV-1 and hepatitis C virus coinfection: the Swiss HIV Cohort Study. The Lancet. 2000;356(9244):1800-5.
- 8. Miller MF, Haley C, Koziel MJ, Rowley CF. Impact of hepatitis C virus on immune restoration in HIV-infected patients who start highly active antiretroviral therapy: a meta-analysis. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2005;41(5):713-20.
- 9. Chew KW, Bhattacharya D. Virologic and immunologic aspects of HIV-HCV coinfection. AIDS (London, England). 2016.
- 10. Hernandez MD, Sherman KE. HIV/hepatitis C coinfection natural history and disease progression. Current opinion in HIV and AIDS. 2011;6(6):478-82.
- 11. Eyster ME, Fried MW, Di Bisceglie AM, Goedert JJ. Increasing hepatitis C virus RNA levels in hemophiliacs: relationship to human immunodeficiency virus infection and liver disease. Multicenter Hemophilia Cohort Study. Blood. 1994;84(4):1020-3.
- 12. Schnuriger A, Dominguez S, Guiguet M, Harfouch S, Samri A, Ouazene Z, et al. Acute hepatitis C in HIV-infected patients: rare spontaneous clearance correlates with weak memory CD4 T-cell responses to hepatitis C virus. AIDS (London, England). 2009;23(16):2079-89.
- 13. Stenkvist J, Nystrom J, Falconer K, Sonnerborg A, Weiland O. Occasional spontaneous clearance of chronic hepatitis C virus in HIV-infected individuals. Journal of hepatology. 2014;61(4):957-61.
- 14. Macías J, Berenguer J, Japón MA, Girón JA, Rivero A, López-Cortés LF, et al. Fast fibrosis progression between repeated liver biopsies in patients coinfected with human immunodeficiency virus/hepatitis C virus. Hepatology. 2009;50(4):1056-63.
- 15. Thein HH, Yi Q, Dore GJ, Krahn MD. 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 (London, England). 2008;22(15):1979-91.
- 16. Rosen HR. Clinical practice. Chronic hepatitis C infection. The New England journal of medicine. 2011;364(25):2429-38.

- 17. Soto B, Sanchez-Quijano A, Rodrigo L, del Olmo JA, Garcia-Bengoechea M, Hernandez-Quero J, et al. Human immunodeficiency virus infection modifies the natural history of chronic parenterally-acquired hepatitis C with an unusually rapid progression to cirrhosis. Journal of hepatology. 1997;26(1):1-5.
- 18. Konerman MA, Mehta SH, Sutcliffe CG, Vu T, Higgins Y, Torbenson MS, et al. Fibrosis progression in human immunodeficiency virus/hepatitis C virus coinfected adults: prospective analysis of 435 liver biopsy pairs. Hepatology. 2014;59(3):767-75.
- 19. Sulkowski MS, Mehta SH, Torbenson MS, Higgins Y, Brinkley SC, de Oca RM, et al. Rapid fibrosis progression among HIV/hepatitis C virus-co-infected adults. AIDS (London, England). 2007;21(16):2209-16.
- 20. Bruno R, Galastri S, Sacchi P, Cima S, Caligiuri A, DeFranco R, et al. gp120 modulates the biology of human hepatic stellate cells: a link between HIV infection and liver fibrogenesis. Gut. 2010;59(4):513-20.
- 21. Tuyama AC, Hong F, Saiman Y, Wang C, Ozkok D, Mosoian A, et al. Human immunodeficiency virus (HIV)-1 infects human hepatic stellate cells and promotes collagen I and monocyte chemoattractant protein-1 expression: implications for the pathogenesis of HIV/hepatitis C virus-induced liver fibrosis. Hepatology. 2010;52(2):612-22.
- 22. Mastroianni CM, Lichtner M, Mascia C, Zuccala P, Vullo V. Molecular mechanisms of liver fibrosis in HIV/HCV coinfection. International journal of molecular sciences. 2014;15(6):9184-208.
- 23. Balagopal A, Philp FH, Astemborski J, Block TM, Mehta A, Long R, et al. Human immunodeficiency virus-related microbial translocation and progression of hepatitis C. Gastroenterology. 2008;135(1):226-33.
- 24. Safadi R, Ohta M, Alvarez CE, Fiel MI, Bansal M, Mehal WZ, et al. Immune stimulation of hepatic fibrogenesis by CD8 cells and attenuation by transgenic interleukin-10 from hepatocytes. Gastroenterology. 2004;127(3):870-82.
- 25. Sitia G, De Bona A, Bagaglio S, Galli L, Paties CT, Uberti-Foppa C, et al. Naive HIV/HCV-coinfected patients have higher intrahepatic pro-inflammatory cytokines than coinfected patients treated with antiretroviral therapy. Antiviral therapy. 2006;11(3):385-9.
- 26. Salloum S, Holmes JA, Jindal R, Bale SS, Brisac C, Alatrakchi N, et al. HIV/HCV in hepatic and stellate cell lines reveals cooperative profibrotic transcriptional activation between viruses and cell types. Hepatology. 2016.
- 27. Graham CS, Baden LR, Yu E, Mrus JM, Carnie J, Heeren T, et al. Influence of human immunodeficiency virus infection on the course of hepatitis C virus infection: a meta-analysis. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2001;33(4):562-9.
- 28. Lo Re V, 3rd, Kallan MJ, Tate JP, Localio AR, Lim JK, Goetz MB, et al. Hepatic decompensation in antiretroviral-treated patients co-infected with HIV and hepatitis C virus compared with hepatitis C virus-monoinfected patients: a cohort study. Annals of internal medicine. 2014;160(6):369-79.
- 29. Pineda JA, Romero-Gomez M, Diaz-Garcia F, Giron-Gonzalez JA, Montero JL, Torre-Cisneros J, et al. HIV coinfection shortens the survival of patients with hepatitis C virus-related decompensated cirrhosis. Hepatology. 2005;41(4):779-89.
- 30. Giordano TP, Kramer JR, Souchek J, Richardson P, El-Serag HB. Cirrhosis and hepatocellular carcinoma in HIV-infected veterans with and without the hepatitis C virus: a cohort study, 1992-2001. Archives of internal medicine. 2004;164(21):2349-54.

- 31. Brau N, Fox RK, Xiao P, Marks K, Naqvi Z, Taylor LE, et al. Presentation and outcome of hepatocellular carcinoma in HIV-infected patients: a U.S.-Canadian multicenter study. Journal of hepatology. 2007;47(4):527-37.
- 32. Benhamou Y, Di Martino V, Bochet M, Colombet G, Thibault V, Liou A, et al. Factors affecting liver fibrosis in human immunodeficiency virus–and hepatitis C virus–coinfected patients: Impact of protease inhibitor therapy. Hepatology. 2001;34(2):283-7.
- 33. Kim HN, Nance R, Van Rompaey S, Delaney JC, Crane HM, Cachay ER, et al. Poorly Controlled HIV Infection: An Independent Risk Factor for Liver Fibrosis. Journal of acquired immune deficiency syndromes (1999). 2016;72(4):437-43.
- 34. Sherman KE, Rockstroh J, Thomas D. Human immunodeficiency virus and liver disease: An update. Hepatology. 2015;62(6):1871-82.
- 35. Brau N, Salvatore M, Rios-Bedoya CF, Fernandez-Carbia A, Paronetto F, Rodriguez-Orengo JF, et al. Slower fibrosis progression in HIV/HCV-coinfected patients with successful HIV suppression using antiretroviral therapy. Journal of hepatology. 2006;44(1):47-55.
- 36. Sterling RK, Wegelin JA, Smith PG, Stravitz RT, Luketic VA, Fuchs M, et al. Similar progression of fibrosis between HIV/HCV-infected and HCV-infected patients: Analysis of paired liver biopsy samples. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2010;8(12):1070-6.
- 37. Jain V, Hartogensis W, Bacchetti P, Hunt PW, Hatano H, Sinclair E, et al. Antiretroviral therapy initiated within 6 months of HIV infection is associated with lower T-cell activation and smaller HIV reservoir size. The Journal of infectious diseases. 2013;208(8):1202-11.
- 38. Thorpe J, Saeed S, Moodie EE, Klein MB. Antiretroviral treatment interruption leads to progression of liver fibrosis in HIV-hepatitis C virus co-infection. AIDS (London, England). 2011;25(7):967-75.
- 39. Macias J, Mira JA, Lopez-Cortes LF, Santos I, Giron-Gonzalez JA, Gonzalez-Serrano M, et al. Antiretroviral therapy based on protease inhibitors as a protective factor against liver fibrosis progression in patients with chronic hepatitis C. Antiviral therapy. 2006;11(7):839-46.
- 40. Fernandez-Montero JV, Barreiro P, Vispo E, Labarga P, Sanchez-Parra C, de Mendoza C, et al. Liver fibrosis progression in HIV-HCV-coinfected patients treated with distinct antiretroviral drugs and impact of pegylated interferon/ribavirin therapy. Antiviral therapy. 2014;19(3):287-92.
- 41. Brunet L, Moodie EE, Young J, Cox J, Hull M, Cooper C, et al. Progression of Liver Fibrosis and Modern Combination Antiretroviral Therapy Regimens in HIV-Hepatitis C-Coinfected Persons. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2016;62(2):242-9.
- 42. Hourigan LF, Macdonald GA, Purdie D, Whitehall VH, Shorthouse C, Clouston A, et al. Fibrosis in chronic hepatitis C correlates significantly with body mass index and steatosis. Hepatology. 1999;29(4):1215-9.
- 43. Svegliati-Baroni G, Ridolfi F, Di Sario A, Casini A, Marucci L, Gaggiotti G, et al. Insulin and insulin-like growth factor-1 stimulate proliferation and type I collagen accumulation by human hepatic stellate cells: differential effects on signal transduction pathways. Hepatology. 1999;29(6):1743-51.
- 44. Paradis V, Perlemuter G, Bonvoust F, Dargere D, Parfait B, Vidaud M, et al. High glucose and hyperinsulinemia stimulate connective tissue growth factor expression: a potential

mechanism involved in progression to fibrosis in nonalcoholic steatohepatitis. Hepatology. 2001;34(4 Pt 1):738-44.

- 45. Petta S, Camma C, Di Marco V, Alessi N, Cabibi D, Caldarella R, et al. Insulin resistance and diabetes increase fibrosis in the liver of patients with genotype 1 HCV infection. The American journal of gastroenterology. 2008;103(5):1136-44.
- 46. Davila JA, Morgan RO, Shaib Y, McGlynn KA, El-Serag HB. Diabetes increases the risk of hepatocellular carcinoma in the United States: a population based case control study. Gut. 2005;54(4):533-9.
- 47. Oliver NT, Hartman CM, Kramer JR, Chiao EY. Statin drugs decrease progression to cirrhosis in HIV/hepatitis C virus coinfected individuals. AIDS (London, England). 2016;30(16):2469-76.
- 48. Simon TG, King LY, Zheng H, Chung RT. Statin use is associated with a reduced risk of fibrosis progression in chronic hepatitis C. Journal of hepatology. 2015;62(1):18-23.
- 49. Singh S, Singh PP, Singh AG, Murad MH, Sanchez W. Statins are associated with a reduced risk of hepatocellular cancer: a systematic review and meta-analysis. Gastroenterology. 2013;144(2):323-32.
- 50. Yang YH, Chen WC, Tsan YT, Chen MJ, Shih WT, Tsai YH, et al. Statin use and the risk of cirrhosis development in patients with hepatitis C virus infection. Journal of hepatology. 2015;63(5):1111-7.
- 51. Inagaki Y, Nemoto T, Kushida M, Sheng Y, Higashi K, Ikeda K, et al. Interferon alfa down-regulates collagen gene transcription and suppresses experimental hepatic fibrosis in mice. Hepatology. 2003;38(4):890-9.
- 52. Rodriguez-Torres M, Rodriguez-Orengo JF, Rios-Bedoya CF, Fernandez-Carbia A, Marxuach-Cuetara AM, Lopez-Torres A, et al. Effect of hepatitis C virus treatment in fibrosis progression rate (FPR) and time to cirrhosis (TTC) in patients co-infected with human immunodeficiency virus: a paired liver biopsy study. Journal of hepatology. 2007;46(4):613-9.
- 53. Thomas DL. Correlates of hepatitis C virus infections among injection drug users. Medicine (Baltimore). 1995;74(4):212-20.
- 54. Kaplan-Lewis E. Acute HCV in HIV-Infected MSM: Modes of Acquisition, Liver Fibrosis, and Treatment. Current HIV/AIDS reports. 2015;12(3):317-25.
- 55. Fierer DS. The Order of Addition of Immunocompromise: The Effects of HIV Infection on Fibrosis Progression Among Hepatitis C Virus–Infected Patients. Journal of Infectious Diseases. 2016;214(8):1134-6.
- 56. Afdhal NH. Diagnosing fibrosis in hepatitis C: is the pendulum swinging from biopsy to blood tests? Hepatology. 2003;37(5):972-4.
- 57. Sterling RK, Lissen E, Clumeck N, Sola R, Correa MC, Montaner J, et al. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. Hepatology. 2006;43(6):1317-25.
- 58. Vallet-Pichard A, Mallet V, Nalpas B, Verkarre V, Nalpas A, Dhalluin-Venier V, et al. FIB-4: an inexpensive and accurate marker of fibrosis in HCV infection. comparison with liver biopsy and fibrotest. Hepatology. 2007;46(1):32-6.
- 59. Amorim TG, Staub GJ, Lazzarotto C, Silva AP, Manes J, Ferronato Mda G, et al. Validation and comparison of simple noninvasive models for the prediction of liver fibrosis in chronic hepatitis C. Annals of hepatology. 2012;11(6):855-61.
- 60. Holmberg SD, Lu M, Rupp LB, Lamerato LE, Moorman AC, Vijayadeva V, et al. Noninvasive serum fibrosis markers for screening and staging chronic hepatitis C virus patients

in a large US cohort. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2013;57(2):240-6.

- 61. Jain MK, Seremba E, Bhore R, Dao D, Joshi R, Attar N, et al. Change in fibrosis score as a predictor of mortality among HIV-infected patients with viral hepatitis. AIDS patient care and STDs. 2012;26(2):73-80.
- 62. Berenguer J, Zamora FX, Aldamiz-Echevarria T, Von Wichmann MA, Crespo M, Lopez-Aldeguer J, et al. Comparison of the prognostic value of liver biopsy and FIB-4 index in patients coinfected with HIV and hepatitis C virus. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2015;60(6):950-8.
- 63. McHutchison JG, Gordon SC, Schiff ER, Shiffman ML, Lee WM, Rustgi VK, et al. Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. Hepatitis Interventional Therapy Group. The New England journal of medicine. 1998;339(21):1485-92.
- 64. McHutchison JG, Lawitz EJ, Shiffman ML, Muir AJ, Galler GW, McCone J, et al. Peginterferon Alfa-2b or Alfa-2a with Ribavirin for Treatment of Hepatitis C Infection. New England Journal of Medicine. 2009;361(6):580-93.
- 65. Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales FL, Jr., et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. The New England journal of medicine. 2002;347(13):975-82.
- 66. Lam BP, Jeffers T, Younoszai Z, Fazel Y, Younossi ZM. The changing landscape of hepatitis C virus therapy: focus on interferon-free treatment. Therapeutic advances in gastroenterology. 2015;8(5):298-312.
- 67. Welsch C, Jesudian A, Zeuzem S, Jacobson I. New direct-acting antiviral agents for the treatment of hepatitis C virus infection and perspectives. Gut. 2012;61 Suppl 1:i36-46.
- 68. Rockstroh JK. Optimal therapy of HIV/HCV co-infected patients with direct acting antivirals. Liver international : official journal of the International Association for the Study of the Liver. 2015;35 Suppl 1:51-5.
- 69. Sulkowski MS, Sherman KE, Dieterich DT, Bsharat M, Mahnke L, Rockstroh JK, et al. Combination therapy with telaprevir for chronic hepatitis C virus genotype 1 infection in patients with HIV: a randomized trial. Annals of internal medicine. 2013;159(2):86-96.
- 70. Sulkowski M, Pol S, Mallolas J, Fainboim H, Cooper C, Slim J, et al. Boceprevir versus placebo with pegylated interferon alfa-2b and ribavirin for treatment of hepatitis C virus genotype 1 in patients with HIV: a randomised, double-blind, controlled phase 2 trial. The Lancet Infectious diseases. 2013;13(7):597-605.
- 71. Sulkowski MS, Naggie S, Lalezari J, Fessel WJ, Mounzer K, Shuhart M, et al. Sofosbuvir and ribavirin for hepatitis C in patients with HIV coinfection. Jama. 2014;312(4):353-61.
- 72. Osinusi A, Townsend K, Kohli A, Nelson A, Seamon C, Meissner EG, et al. Virologic response following combined ledipasvir and sofosbuvir administration in patients with HCV genotype 1 and HIV co-infection. Jama. 2015;313(12):1232-9.
- 73. Sulkowski MS, Eron JJ, Wyles D, Trinh R, Lalezari J, Wang C, et al. Ombitasvir, paritaprevir co-dosed with ritonavir, dasabuvir, and ribavirin for hepatitis C in patients co-infected with HIV-1: a randomized trial. Jama. 2015;313(12):1223-31.
- 74. Saeed S, Strumpf EC, Walmsley SL, Rollet-Kurhajec K, Pick N, Martel-Laferriere V, et al. How Generalizable Are the Results From Trials of Direct Antiviral Agents to People Coinfected With HIV/HCV in the Real World? Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2016;62(7):919-26.

- 75. Rockstroh JK. Treatment of hepatitis C virus infection in the HIV-infected patient [N/A]. UpToDate: N/A; 2016 [updated August 1, 2016. N/A:[N/A]. Available from: https://www.uptodate.com/contents/treatment-of-hepatitis-c-virus-infection-in-the-hiv-infected-patient#H870364631.
- 76. America JpftAAotSoLDatIDSo. Recommendations for Testing, Managing, and Treating Hepatitis C 2016 [Available from: http://www.hcvguidelines.org/.
- 77. Okazaki T, Yoshihara H, Suzuki K, Yamada Y, Tsujimura T, Kawano K, et al. Efficacy of interferon therapy in patients with chronic hepatitis C. Comparison between non-drinkers and drinkers. Scandinavian journal of gastroenterology. 1994;29(11):1039-43.
- 78. Nalpas B, Driss F, Pol S, Hamelin B, Housset C, Brechot C, et al. Association between HCV and HBV infection in hepatocellular carcinoma and alcoholic liver disease. Journal of hepatology. 1991;12(1):70-4.
- 79. Bach TA, Zaiken K. Real-World Drug Costs of Treating Hepatitis C Genotypes 1-4 with Direct-Acting Antivirals: Initiating Treatment at Fibrosis 0-2 and 3-4. Journal of managed care & specialty pharmacy. 2016;22(12):1437-45.
- 80. Chhatwal J, Kanwal F, Roberts MS, Dunn MA. Cost-effectiveness and budget impact of hepatitis C virus treatment with sofosbuvir and ledipasvir in the United States. Annals of internal medicine. 2015;162(6):397-406.
- 81. Canary LA, Klevens RM, Holmberg SD. Limited Access to New Hepatitis C Virus Treatment Under State Medicaid Programs. Annals of internal medicine. 2015;163(3):226-8.
- 82. Hepatitis C guidance: AASLD-IDSA recommendations for testing, managing, and treating adults infected with hepatitis C virus. Hepatology. 2015;62(3):932-54.
- 83. Berenguer J, Zamora FX, Carrero A, Von Wichmann MA, Crespo M, Lopez-Aldeguer J, et al. Effects of sustained viral response in patients with HIV and chronic hepatitis C and nonadvanced liver fibrosis. Journal of acquired immune deficiency syndromes (1999). 2014;66(3):280-7.
- 84. Labarga P, Fernandez-Montero JV, Lopez M, Barreiro P, de Mendoza C, Sierra-Enguita R, et al. Progression to advanced liver fibrosis in HIV-HCV-coinfected patients and prioritization of new hepatitis C therapies. Antiviral therapy. 2014;19(8):799-803.
- 85. Ghany MG, Kleiner DE, Alter H, Doo E, Khokar F, Promrat K, et al. Progression of fibrosis in chronic hepatitis C. Gastroenterology. 2003;124(1):97-104.
- 86. Ryder SD, Irving WL, Jones DA, Neal KR, Underwood JC. Progression of hepatic fibrosis in patients with hepatitis C: a prospective repeat liver biopsy study. Gut. 2004;53(3):451-5.
- 87. Leite AG, Duarte MI, Mendes-Correa MC. Fibrosis Progression in Paired Liver Biopsies from HIV/HCV-Coinfected Patients without Prior Treatment of Hepatitis C. Journal of the International Association of Providers of AIDS Care. 2015;14(5):463-8.
- 88. Mohsen AH, Easterbrook PJ, Taylor C, Portmann B, Kulasegaram R, Murad S, et al. Impact of human immunodeficiency virus (HIV) infection on the progression of liver fibrosis in hepatitis C virus infected patients. Gut. 2003;52(7):1035-40.
- 89. Martin-Carbonero L, Benhamou Y, Puoti M, Berenguer J, Mallolas J, Quereda C, et al. Incidence and predictors of severe liver fibrosis in human immunodeficiency virus-infected patients with chronic hepatitis C: a European collaborative study. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2004;38(1):128-33.
- 90. Kuniholm MH, O'Brien TR, Prokunina-Olsson L, Augenbraun M, Plankey M, Karim R, et al. Association of Hepatitis C Virus Infection With CD4/CD8 Ratio in HIV-Positive Women. Journal of acquired immune deficiency syndromes (1999). 2016;72(2):162-70.

- 91. Barreiro P, Martín-Carbonero L, Núñez M, Rivas P, Morente A, Simarro N, et al. Predictors of Liver Fibrosis in HIV-Infected Patients with Chronic Hepatitis C Virus (HCV) Infection: Assessment Using Transient Elastometry and the Role of HCV Genotype 3. Clinical Infectious Diseases. 2006;42(7):1032-9.
- 92. Schiavini M, Angeli E, Mainini A, Zerbi P, Duca PG, Gubertini G, et al. Risk factors for fibrosis progression in HIV/HCV coinfected patients from a retrospective analysis of liver biopsies in 1985–2002. HIV medicine. 2006;7(5):331-7.
- 93. Thomas DL, Astemborski J, Rai RM, Anania FA, Schaeffer M, Galai N, et al. The natural history of hepatitis C virus infection: host, viral, and environmental factors. Jama. 2000;284(4):450-6.
- 94. Poynard T, Ratziu V, Charlotte F, Goodman Z, McHutchison J, Albrecht J. Rates and risk factors of liver fibrosis progression in patients with chronic hepatitis c. Journal of hepatology. 2001;34(5):730-9.
- 95. de Torres M, Poynard T. Risk factors for liver fibrosis progression in patients with chronic hepatitis C. Annals of hepatology. 2003;2(1):5-11.
- 96. Daar ES, Lynn H, Donfield S, Gomperts E, Hilgartner MW, Hoots WK, et al. Relation between HIV-1 and hepatitis C viral load in patients with hemophilia. Journal of acquired immune deficiency syndromes (1999). 2001;26(5):466-72.
- 97. Ghany MG, Leissinger C, Lagier R, Sanchez-Pescador R, Lok AS. Effect of human immunodeficiency virus infection on hepatitis C virus infection in hemophiliacs. Digestive diseases and sciences. 1996;41(6):1265-72.
- 98. Bader T. Yes! Statins can be given to liver patients. Journal of hepatology. 2012;56(2):305-7.
- 99. Feinstein MJ, Achenbach CJ, Stone NJ, Lloyd-Jones DM. A Systematic Review of the Usefulness of Statin Therapy in HIV-Infected Patients. The American journal of cardiology. 2015;115(12):1760-6.
- 100. Blashill AJ, Mayer KH, Crane HM, Grasso C, Safren SA. Body mass index, immune status, and virological control in HIV-infected men who have sex with men. Journal of the International Association of Providers of AIDS Care. 2013;12(5):319-24.
- 101. VACS Index Information and VACS Calculator [updated July 2016. Available from: https://medicine.yale.edu/intmed/vacs/welcome/vacsindexinfo.aspx.
- 102. Justice AC, Modur SP, Tate JP, Althoff KN, Jacobson LP, Gebo KA, et al. Predictive accuracy of the Veterans Aging Cohort Study index for mortality with HIV infection: a North American cross cohort analysis. Journal of acquired immune deficiency syndromes (1999). 2013;62(2):149-63.
- 103. Tate JP, Justice AC, Hughes MD, Bonnet F, Reiss P, Mocroft A, et al. An internationally generalizable risk index for mortality after one year of antiretroviral therapy. AIDS (London, England). 2013;27(4):563-72.
- 104. Salinas JL, Rentsch C, Marconi VC, Tate J, Budoff M, Butt AA, et al. Baseline, Time-Updated, and Cumulative HIV Care Metrics for Predicting Acute Myocardial Infarction and All-Cause Mortality. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2016;63(11):1423-30.
- 105. Barakat LA, Juthani-Mehta M, Allore H, Trentalange M, Tate J, Rimland D, et al. Comparing clinical outcomes in HIV-infected and uninfected older men hospitalized with community-acquired pneumonia. HIV medicine. 2015;16(7):421-30.

- 106. Akgun KM, Gordon K, Pisani M, Fried T, McGinnis KA, Tate JP, et al. Risk factors for hospitalization and medical intensive care unit (MICU) admission among HIV-infected Veterans. Journal of acquired immune deficiency syndromes (1999). 2013;62(1):52-9.
- 107. Womack JA, Goulet JL, Gibert C, Brandt CA, Skanderson M, Gulanski B, et al. Physiologic frailty and fragility fracture in HIV-infected male veterans. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2013;56(10):1498-504.
- 108. Yin MT, Shiau S, Rimland D, Gibert CL, Bedimo RJ, Rodriguez-Barradas MC, et al. Fracture Prediction With Modified-FRAX in Older HIV-Infected and Uninfected Men. Journal of acquired immune deficiency syndromes (1999). 2016;72(5):513-20.
- 109. Yuh B, Tate J, Butt AA, Crothers K, Freiberg M, Leaf D, et al. Weight change after antiretroviral therapy and mortality. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2015;60(12):1852-9.