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IMPACT OF HIV ON LIVER FIBROSIS IN MEN WITH HEPATITIS C INFECTION AND HEMOPHILIA

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Abstract

Introduction—Hepatitis C virus (HCV) is the major cause of liver disease in hemophilia. Few data exist on the proportion with liver fibrosis in this group after long-term HCV and HIV co-infection.

Aim—We conducted a cross-sectional multi-center study to determine impact of HIV on the prevalence and risk factors for fibrosis in hemophilic men with chronic hepatitis C.

Methods—Biopsies were independently scored by Ishak, Metavir, and Knodell systems. Variables were tested for associations with fibrosis by logistic regression and receiver operating curves (ROC).

Results—Of 220 biopsied HCV(+) men, 23.6% had Metavir ≥F3 fibrosis, with higher mean Metavir fibrosis scores among HIV/HCV co-infected than HCV mono-infected, 1.6 vs. 1.3 (p=0.044). Variables significantly associated with fibrosis included AST, ALT, APRI score (AST/ULN×100/platelet ×10⁹/L), alpha-fetoprotein (all p<0.0001), platelets (p=0.0003), and ferritin (p=0.0008). In multiple logistic regression of serum markers, alpha-fetoprotein, APRI, and ALT were significantly associated with ≥ F3 fibrosis, AUROC=0.77 (95%CI 0.69, 0.86). Alpha-fetoprotein, APRI, and ferritin were significant in HIV(−), (AUROC 0.82 (95%CI 0.72, 0.92), and alpha-fetoprotein and platelets in HIV(+) (AUROC=0.77 (95%CI 0.65, 0.88). In a multivariable

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model of demographic and clinical variables, transformed (natural logarithm) of alpha-fetoprotein ($p=0.0003$), age ($p=0.006$), and HCV treatment ($p=0.027$) were significantly associated with fibrosis.

Conclusion—Nearly one-fourth of hemophilic men have Metavir ≥ 3 fibrosis. The odds for developing fibrosis are increased in those with elevated alpha-fetoprotein, increasing age, and past HCV treatment.

Keywords

Hepatitis C; Fibrosis; Hemophilia; Transfusion; Metavir; Receiver operating curve

Introduction

Hepatitis C (HCV) is the most common cause of chronic liver disease and the leading cause of death in individuals with hemophilia. Of the 90% of hemophilic men exposed to HCV through clotting factor concentrates prior to AIDS [1], it has been estimated that 20% have cirrhosis [2], similar to other risk groups [3,4]. However, the extent of fibrosis among hemophilic men with long-term HIV infection is not known. This is an important consideration, given the competing effects of the HIV virus itself, which accelerates liver disease progression [5] and upregulates cytokines promoting liver fibrosis [6–8]; and the effects of highly active antiretroviral therapy (HAART), which improves HIV survival [9], slows HCV progression [10–12] and improves post-transplant survival in co-infected individuals [13]. Although liver biopsy is the gold standard for assessment of liver disease [14], few hemophilic men undergo this procedure, even by the less invasive transjugular route [5]. Thus, noninvasive markers of hepatic fibrosis [16–22], e.g. AST, ALT, platelet count, and combinations, have been increasingly used to predict fibrosis [19]. Few studies, however, are powered to assess markers in HCV(+) hemophilic men with and without HIV infection. [23]. Moreover, little is known about the role of cytokine upregulation by HIV [6–8] or cytokine genotype [24–26] in predicting fibrosis in co-infected individuals. We, therefore, designed a cross-sectional study to determine prevalence and risk factors for fibrosis.

Patients and methods

Patients

A total of 781 men with hemophilia A or B, ≥ 18 years of age, with a positive HCV antibody test, from 34 U.S. hemophilia treatment centers were enrolled on the HIV Impact on Hepatitis C in Hemophilia (HHH) Study. Study subjects were enrolled on this NHLBI-funded cross-sectional, multi-center study between April 2002 and December 2007, during routine comprehensive hemophilia center clinic visits. All subjects provided signed informed consent in accordance with the Declaration of Helsinki. The protocol and informed consent were approved by the Clinical Translational Research Center (CTRC) Advisory Committee and Institutional Review Board (IRB), at the University of Pittsburgh (headquarters), and all participating institutions. Approved consent forms from participating institutions were reviewed by the Coordinating Center (M.V.R.) to assure inclusion of health information protection (HIPAA) and safety procedures regarding liver biopsy. Duration of HCV infection was based on data documenting HCV infection occurs in those with hemophilia with the first blood product exposure [33], which occurs within the first year of life for most such patients. Dates of HIV seroconversion were based on previously published studies establishing 1982 as median year of seroconversion for hemophilia A and 1983 for hemophilia B [1].

Liver histology and quantification of fibrosis

Each patient was asked to undergo an optional liver biopsy at baseline, or to provide permission to review recent liver biopsy slides and blocks. Only 40 subjects underwent liver biopsy during study; 180 already had undergone liver biopsy and refused to undergo a repeat biopsy due to bleeding risk. The 220 biopsies, including 40 fresh tissue and 180 slides and blocks, were coded at each site, shipped to the University of Pittsburgh coordinating center, and independently reviewed by the study pathologist (M.A.N.) and scored by Knodell [27], Ishak [28], and Metavir [29] classification systems, with Ishak fibrosis 0 to 6, Metavir fibrosis F0 to F4, http://www.xcellpath.com/healthcare/other/other_staging_liver.htm. Of the biopsies, 66 (30.0%) were performed within 12 months; 24 (10.9%) within 24 months; 35 (15.9%) within 36 months; 25 (11.4%) within 48 months; 14 (6.4%) within 60 months; and the remainder, 56 (25.4%) between 60 and 180 months of enrollment. By approach, 119 (54.1%) of the biopsies were performed by the percutaneous route, 84 (38.2%) by the transjugular route, 16 (7.3%) intraoperatively, and one (0.4%) was obtained at autopsy.

Serum fibrosis markers

On the day of study enrollment, demographic, clinical information, and a one-time blood sample was obtained for ALT, AST, alpha-fetoprotein, ferritin, HCV antibody, HCV viral load, HCV genotype, HIV antibody, HIV viral load, CD4 count, platelet count, hemoglobin, and cytokines TGF- β , IL-6, IL-10, TNF- α , and IFN- γ . APRI score was calculated as AST/ULN x 100/platelet count $10^9/L$.

Cytokine immunoassay, mRNA, and promoter genotype

Plasma cytokine immunoassay (TGF- β , IL-6, IL-10, TNF- α , and IFN- γ) was measured by enzyme-linked immunosorbent assay (Quantikine R&D Systems, Minneapolis, MN) [30,31]. Cytokine mRNA (TGF- β , IL-6, IL-10, TNF- α , and IFN- γ) was measured on liver tissue by ribonuclease protection assay (Riboquant, Pharmingen, San Diego CA) with radiolabeled bands determined by densitometry [26]. Genomic DNA was extracted from peripheral venous blood samples using the QIAamp DNA Blood Midi Kit^R, followed by whole genome amplification using the REPLI-g^R Mini/Midi Kit (Qiagen Inc, Valencia CA). The allelic polymorphisms of IL-6 (174 G/C) and IL-10 (1082G/A, 819C/T, 592C/A), and TGF- β (C/T codon 10, C/G codon 25) were identified by PCR with sequence specific primers (PCR-SSP) using a five-cytokine polymorphism kit (One Lambda Inc, Canoga Park CA) [32].

Statistical analysis

Subjects with and without a liver biopsy were compared on demographic, behavioral, and clinical variables to assess the generalizability of the findings. Wilcoxon rank sum test was used for continuous variables and chi-square, or Fisher's exact, test was used for categorical variables. Using the same statistical tests, subjects with and without HIV infection were compared on the same variables to establish differences between the two groups that might be important to consider in multivariable analyses. Spearman rank correlation was used to determine the association between the biochemical markers (AST, ALT, platelet count, AST/ALT, APRI ferritin, alpha fetoprotein) and fibrosis score [29] for each classification system for all subjects and stratified by HIV status. The same analysis was used in a subset of subjects with cytokine measurements to determine the association between cytokine mRNA (TGF- β , IL-6, IL-10, TNF- α , and IFN- γ). Logistic regression and the area under the receiver operating curves (AUROC) were used to determine the diagnostic accuracy of the biochemical markers for Metavir F3-F4 fibrosis [29]. Although time between enrollment and biopsy was not significantly different between those with Metavir F3-F4 fibrosis and those without, two types of sensitivity analyses were conducted to assess its impact on

biochemical marker association with Metavir F3-F4 fibrosis. In the first sensitivity analysis, we stratified by time between enrollment and biopsy date (<3 , ≥ 3 years) to compare the regression coefficients and AUROCs. In the second sensitivity analysis, regression models were conducted in all subjects, controlling for time between enrollment and biopsy. No impact was noted (data not shown) in terms of inference for the associations of biochemical markers with Metavir F3-F4 fibrosis: therefore, analyses are presented from all subjects. Multiple logistic regression was used to determine demographic, clinical, behavioral, and laboratory variables significantly associated with fibrosis. Variables were considered for inclusion in the final model if they were significant as univariates at the 0.2 level and significant at the 0.05 level in multivariable models within each type of variable. Among subjects with HIV, the association between HAART and presence of fibrosis was tested by Fisher's exact test for categorical variables. The median fibrosis score by HAART therapy was assessed by Wilcoxon rank sum test, comparing those treated vs. untreated. The same procedures were used to test the impact of HCV treatment on fibrosis among all biopsied subjects, irrespective of HIV status. All data analyses were performed using SAS 9.1.3 software package (SAS Institute, Cary NC), and receiver operating curves (ROC) were analyzed with Stata software (StataCorp LP, College Station TX).

Results

Baseline characteristics and liver fibrosis scores

Of 781 HCV(+) hemophilic men enrolled in the study, 220 (28.2%) underwent liver biopsy (Table 1). Those who underwent biopsy were more likely to be older, Caucasian, HIV(+), to have a detectable HCV viral load, and to have received HCV antiviral therapy than those not biopsied. Of the subjects undergoing biopsy, 43.6% received interferon (IFN) and/or ribavirin (RBV) therapy, and, among HIV(+) biopsied subjects, 80.7% received highly active antiretroviral therapy (HAART). Co-infected subjects also had lower platelet counts ($p<0.001$) and higher AST ($p=0.002$) than mono-infected subjects. Of all biopsied subjects, 82 (38.2%) had Ishak grade > 3 fibrosis, higher among HIV(+), 46.2%, than HIV(-), 32.3%, $p=0.035$. A total of 52 (23.6%) had $\geq$ F3 Metavir fibrosis, and the Metavir score was higher in co-infected than HCV mono-infected subjects, 1.6 ± 1.2 (standard deviation) vs. 1.3 ± 1.2 , $p=0.044$ (Table 2).

Correlation between serum biomarkers and fibrosis scoring systems

Overall, AST, ALT, platelet count, APRI, ferritin, and alpha-fetoprotein were significantly associated with fibrosis by the Knodell, Ishak, and Metavir systems (Table 3). This was also true for the HIV(-) subgroup, while among HIV(+), only AST, ferritin, and alpha-fetoprotein were positively correlated with all three scoring systems. In all groups, alpha-fetoprotein was the variable most strongly associated with Metavir fibrosis (Table 3).

Relationship between HAART therapy and fibrosis

Among HIV(+) subjects, a significantly greater proportion of those treated with HAART, 23 of 75 (30.7%), had Metavir $\geq$ F3 fibrosis than those not receiving HAART, 3 of 18, 16.7%, $p=0.380$. There was also no difference between median Metavir fibrosis score between subjects treated with HAART and untreated (data unshown) $p=0.578$.

Relationship between HCV infection, HCV treatment, and fibrosis

Duration of HCV was greater in those with Metavir F3-F4 fibrosis than F0-F2, 44.5 ± 11.2 vs. 38.8 ± 12.2 years, $p=0.0014$. Significantly more of those receiving HCV treatment (interferon $\pm$ ribavirin), 31 of 96 (32.3%), had Metavir $\geq$ F3 fibrosis than those not receiving HCV treatment, 21 of 123 (17.1%), $p=0.009$. Median Metavir fibrosis score was higher

among HCV-treated subjects, median (25th, 75th percentile), 1.0 (1.0, 3.0) compared to untreated subjects, 1.0 (0.0, 2.0), $p=0.004$.

Logistic regression model and predictors of Metavir $\geq$ F3 fibrosis

In univariate logistic regression, alpha-fetoprotein was most highly associated with Metavir $\geq$ F3, overall, and for HIV(+) and HIV(−) groups, AUROC=0.76, 0.71, and 0.80, respectively, Table 4. In multiple logistic regression models, APRI, ALT, and alpha-fetoprotein were significantly associated with F3-F4 fibrosis, AUROC=0.77 (95% CI 0.69, 0.86, Figure 1a. Among HIV(+), platelets and alpha-fetoprotein were predictors of Metavir $\geq$ F3 fibrosis, AUROC=0.77 (95% CI 0.65, 0.88), Figure 1b. Among HIV(−), APRI, ferritin, and alpha-fetoprotein were significant predictors of Metavir $\geq$ F3 fibrosis, AUROC=0.82 (95% CI 0.72, 0.92), Figure 1c. Since APRI is a function of AST and platelet count, AUROC curves were assessed with just APRI, and considering AST and platelet count as separate variables. Overall, when APRI was excluded from the model, platelet count was significantly associated with fibrosis, but not AST (data not shown).

Cytokine analysis

IL-6 mRNA was negatively associated with all three classification systems, overall and stratified by HIV status. IL-10, TNF- α , and IFN- γ mRNA showed a similar negative association, with all classification systems, in all individuals and in those who were HIV(−), Table 5. Neither cytokine ELISA nor cytokine promoter genotypes was associated with fibrosis in any group (data not shown).

Multivariable model

In multiple logistic regression, age at enrollment, HCV treatment, transformed (natural logarithm, ln) platelet count, and transformed (natural logarithm, ln) alpha-fetoprotein were positively associated with Metavir $\geq$ F3 fibrosis, Table 6. Despite the effect of HIV on severity of fibrosis (above), HIV was not significantly associated with fibrosis, nor did it alter the association among the variables with fibrosis in the final model.

Discussion

Few studies have documented the histologic impact of HIV infection on HCV infection in those with hemophilia, a unique population that differs from other risk groups in their early acquisition of HCV, now the leading cause of mortality in this group [1,5,10]. Knowledge of stage of fibrosis is critical to appropriate HCV management and treatment. Because of the potential bleeding risks associated with liver biopsy, there has been interest in identifying noninvasive biomarkers that predict fibrosis. Yet fibrosis models derived in non-hemophilic HCV-infected groups may not accurately predict fibrosis in those with hemophilia, due to differences in age at acquisition of HCV and HIV, duration of HCV [33] and HIV infection [1], CD4 count, and HAART therapy [10]. To address these issues, the impact of HIV infection on HCV was evaluated by comparing prevalence and factors associated with fibrosis in HIV/HCV co-infected and HCV mono-infected men. The findings of this observational study, which represents the largest multi-center study of histologic HCV infection in hemophilia, demonstrate that 41 years after the introduction of HCV into this population, 23.6% have biopsy-confirmed fibrosis, and, among HIV/HCV co-infected, the prevalence of fibrosis, 28.0%, is 1.4 fold higher than in HCV-mono-infected men, 20.5%, or pre-HIV [2] and post-HIV rates in hemophilic men [16,34].

All variables except AST/ALT were strongly associated with fibrosis by all three systems. Alpha-fetoprotein was the strongest univariate marker associated with Metavir $\geq$ F3 fibrosis. Platelet count and alpha-fetoprotein were significantly associated with fibrosis in the co-

infected group, similar to previous findings [35]; and APRI, ferritin, and alpha-fetoprotein were significantly associated with fibrosis in the HIV(−) group, similar to published studies [22]. These biomarkers, however, were not as strongly associated with fibrosis in co-infected as mono-infected subjects, in contrast to previous studies [20,21,36]. Although the reason for this discrepancy is not known, it may reflect the effects of long duration HIV infection which increases HCV progression [5,10], resulting in liver dysfunction and lower platelets in co-infected, but not mono-infected subjects. This may also account for differences between our study and past studies of shorter duration HIV infection.

The likelihood of having fibrosis (Metavir F3 fibrosis) was increased in those with elevated α -fetoprotein, increasing age, and past HCV treatment. The latter is a somewhat surprising finding: HCV treatment, if successfully completed, is associated with reduction in HCV viral load and *reduced* liver disease progression. Although there is no obvious explanation, this may be a limitation of the variables we assessed, “interferon ever” or “ribavirin ever”, but not the dates of treatment, dose, tolerance, compliance, or virologic outcome. This could also be a survivor effect, as those not treated for HCV could have died earlier, so those living treated longer may be more likely to live long enough to develop fibrosis. Alternatively, those receiving treatment did so late in the course of their HCV infection, as long duration HCV infection distinguishes hemophilic from non-hemophilic subjects, with potentially poorer outcomes, as noted in hemophilic transplant candidates [37,38].

It would seem reasonable, based on these findings, to monitor HCV(+) hemophilia patients routinely with α -fetoprotein levels, and, if elevated or increasing, consider liver biopsy, antiviral treatment, careful assessment for end-stage liver disease, and early referral for transplant evaluation. These findings underscore the need for prospective studies to confirm these findings and determine whether early identification of these abnormalities is associated with clinical benefit.

The findings of this study also establish that fewer than one-third of HCV (+) hemophilic men overall receive HCV antiviral therapy; and, despite beneficial effects of HAART in slowing HCV liver disease [10], one-fifth of co-infected men may not receive HAART therapy. Emerging rates of pre-transplant mortality in co-infected transplant candidates [39], among whom 10% have hemophilia, further suggest not only the need for optimal treatment, but also early recognition of end-stage liver disease, including routine monitoring of all co-infected individuals with model for end-stage liver disease (MELD), a predictor of post-transplant mortality, based on bilirubin, creatinine, INR [13,39].

The mechanism of fibrosis progression among those with co-infection is not well understood. It has been proposed that HIV upregulates cytokines that cause fibrosis [6–8], yet, in contrast to previous studies [24,40], we found that cytokine mRNA was negatively associated with fibrosis.

We recognize several limitations of this study. First, only a small proportion of the group underwent liver biopsy; however, it is among the largest hemophilic groups to undergo biopsy. Secondly, many subjects had undergone biopsies prior to study and were unwilling to undergo another. Despite this, the results of the study remain unchanged even after adjusting for or stratifying by time between serum measurement and biopsy. Thirdly, the true rate of fibrosis may be underestimated, as those who died of AIDS or liver disease before the 2002 start of study may have had fibrosis [5], were not able to be included, while most of those co-infected included in the study received HAART, which slows HCV progression [10]. Fourthly, although the duration of HCV infection was based on data documenting that HCV infection in hemophilia occurs with the first blood product [33], typical for those with severe and moderately severe hemophilia, it may not be true for those

with mild disease, who are treated for traumatic bleeds, typically after first year of life. Finally, a one-time cytokine level may be insufficient to evaluate HIV impact on liver fibrosis, and moreover, the negative association between cytokine mRNA and fibrosis suggests other mechanisms by which HIV increases HCV progression, which will require further study

The strengths of this study are its size, i.e. the largest histologic assessment of HCV infection in hemophilia, liver histopathology assessment by a single central pathologist expert in HCV fibrosis, and comparisons between HIV(+) and HIV(−). However, until the accuracy of biomarkers approaches that of liver biopsy, the latter will remain the gold standard for fibrosis detection.

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M.V.R. and K.S. designed the research; M.A.N. and A.J.Z. performed study procedures; A.C.H., T.L.W., S.H., and A. Z. performed laboratory assays; M.V.R., K.S., C.G.M., and J.L. analyzed the results and made figures; and M.V.R. wrote the paper with contributions from K.S., C.G.M., and O.S.S.

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Figure 1. Metavir $\geq$ F3 Fibrosis in Hemophilic Men with Hepatitis C

Receiver Operating Characteristic (ROC) Curves of the APRI score for the detection of fibrosis, defined as Metavir $\geq$ F3, are shown for all HCV(+) hemophilic men (Figure 1a), for HIV/HCV co-infected hemophilic men (Figure 1b); and for HCV mono-infected hemophilic men (Figure 1c). The AUROC for the whole group (Figure 1a), based on a model including variables significantly associated with fibrosis, i.e. APRI, ALT, and alfa-fetoprotein, was 0.77 (95%CI 0.69, 0.86). The AUROC for the HIV(+) group (Figure 1b), based on a model including platelet count and alpha-fetoprotein, was 0.77 (95%CI 0.65, 0.88). The AUROC for the HIV(−) group (Figure 1c), based on a model including APRI, ferritin, and alpha-fetoprotein, was 0.82 (95%CI 0.72, 0.92).

Table 1

Characteristics of HCV (+) Hemophilic Men Enrolled on Study

		Biopsy N = 220	No Biopsy N = 561	p value
Age at Enrollment*	Years	40 ± 12	38 ± 13	0.007 <i>a</i>
Race	Caucasian	193 (87.7%)	435 (77.5 %)	0.001 <i>b</i>
	Non-Caucasian	27 (12.3%)	126 (22.5%)	
Type Hemophilia	A	179 (81.7%)	452 (80.6%)	0.710 <i>b</i>
	B	40 (18.3%)	109 (19.4%)	
ETOH Use	< 2–3/week	47 (21.5%)	126 (22.5%)	0.754 <i>b</i>
	≥ 2–3/week	172 (78.5%)	434 (77.5%)	
Smoking	Current	74 (33.6%)	213 (38.0%)	0.081 <i>b</i>
	Never	94 (42.7%)	254 (45.3%)	
	Quit	52 (23.6%)	94 (16.8%)	
Analgesic Use	≥ weekly	99 (45.8%)	261 (47.2%)	0.733 <i>b</i>
	< weekly	117 (54.2%)	292 (52.8%)	
Alternative Drugs	Ever	65 (29.6%)	131 (23.4%)	0.073 <i>b</i>
	Never	155 (70.5%)	430 (76.6%)	
Duration HCV	Years	40 ± 12	38 ± 13	0.007 <i>a</i>
HCV Genotype	Type 1	128 (81.0%)	262 (77.5%)	0.376 <i>b</i>
	Types 2–6	30 (19.0%)	76 (22.5%)	
IFN ± RBV Rx	Ever	96 (43.6%)	140 (25.0%)	<0.001 <i>b</i>
	Never	123 (56.2%)	419 (75.0%)	
HCV Viral Load	≥ 600 U/ml	157 (77.7%)	334 (65.8%)	0.002 <i>b</i>
	< 600 U/ml	45 (22.3%)	174 (34.3%)	
HIV Infection	HIV(+)	93 (42.3%)	196 (34.9%)	0.056 <i>b</i>
	HIV(–)	127 (57.7%)	365 (65.1%)	
Median CD4	No./ul	673 (n=206)	738 (n=520)	0.658 <i>a</i>
HIV Viral load	≥ 400 copy/ml	18 (32.1%)	38 (36.9)	0.549 <i>b</i>
	< 400 copy/ml	38 (67.9%)	65 (63.1%)	
HAART Therapy	Yes	75 (80.7%)	135 (71.1%)	0.083 <i>b</i>
	No	18 (19.4%)	55 (29.0%)	
Hemoglobin	gm%	15.1 ± 1.6 (n=192)	14.8 ± 1.9 (n=481)	0.037 <i>a</i>
Platelets	No.×10 ³ /uL	204 ± 66 (n=184)	215 ± 79 (n=460)	0.043 <i>a</i>
AST	U/L	48 ± 48 (n=205)	44 ± 36 (n=514)	0.158 <i>a</i>
ALT	U/L	58 ± 59 (n=204)	51 ± 46 (n=515)	0.063 <i>a</i>
Ferritin	ng/ml	212 ± 220 (n=201)	198 ± 211 (n=500)	0.737 <i>a</i>
Alpha-fetoprotein	ng/ml	7.8 ± 25.5 (n=197)	7.0 ± 31.8 (n=502)	0.484 <i>a</i>

* Mean ± standard deviation.

^aWilcoxon rank sum test for continuous variables.

^bPearson chi-square test for categorical variables.

Table 2

Characteristics of HCV (+) Hemophilic Men Undergoing Liver Biopsy

		HIV(+) N = 93	HIV(-) N = 127	p value
Age at Enrollment*	Years	41.4 ± 9.6	39.3 ± 13.8	0.104 <i>a</i>
Race	Caucasian	80 (86.0%)	113 (89.0%)	0.509 <i>b</i>
	Non-Caucasian	13 (14.0%)	14 (11.0%)	
Type Hemophilia	A	83 (89.3%)	96 (76.2%)	0.013 <i>b</i>
	B	10 (10.8%)	30 (23.8%)	
ETOH Use	< 2–3/week	24 (26.1%)	23 (18.1%)	0.156 <i>b</i>
	≥ 2–3/week	68 (73.9%)	104 (81.9%)	
Smoking	Current	22 (23.7%)	52 (40.9%)	0.007 <i>b</i>
	Never	41 (44.1%)	53 (41.7%)	
	Quit	30 (32.3%)	22 (17.3%)	
Analgesic Use	≥ Weekly	48 (52.2%)	51 (41.1%)	0.107 <i>b</i>
	< Weekly	44 (47.8%)	73 (58.9%)	
Alternative Drug	Ever	31 (33.3%)	34 (26.8%)	0.292 <i>b</i>
	Never	62 (66.7%)	93 (73.2%)	
Duration HCV	Years	41.4 ± 9.6	39.3 ± 13.8	0.067 <i>a</i>
HCV Genotype	1	56 (81.2%)	72 (80.9%)	0.967 <i>b</i>
	2–6	13 (18.8%)	17 (19.1%)	
IFN ± RBV Rx	Ever	44 (47.8%)	52 (40.9%)	0.311 <i>b</i>
	Never	48 (52.2%)	75 (59.1%)	
HCV Viral Load	≥ 600 U/ml	68 (78.2%)	89 (77.4%)	0.896 <i>b</i>
	< 600 U/ml	19 (21.8%)	26 (22.6%)	
Duration HIV	Years	23.6 ± 1.9	-	-
Median CD4	No./ul	494	799	<0.001 <i>a</i>
HIV Viral load	≥ 400 Copy/ml	18 (32.1%)	-	
	< 400 Copy/ml	38 (67.9%)	-	
HAART Therapy	Yes	75 (80.7%)	-	
	No	18 (19.4%)	-	
Hemoglobin	Gm%	14.9 ± 1.9	15.3 ± 1.4	0.076 <i>a</i>
Platelets	No.x10 ³ /uL	184 ± 59	220 ± 67	<0.001 <i>a</i>
AST	U/L	57 ± 56	42 ± 40	0.002 <i>a</i>
ALT	U/L	63 ± 70	55 ± 49	0.420 <i>a</i>
Ferritin	Ng/ml	216 ± 264	210 ± 182	0.195 <i>a</i>
Alpha-fetoprotein	Ng/ml	10.0 ± 32.7	6.3 ± 18.7	0.074 <i>a</i>
Age at Biopsy	Years	38.9 ± 9.7	36.0 ± 14.1	0.024 <i>a</i>
Duration HIV at Biopsy	Years	21.1 ± 2.5	-	
Duration HCV at Biopsy Years	38.9 ± 9.7	36.0 ± 14.0	0.024 <i>a</i>	

	HIV(+) N = 93	HIV(-) N = 127	p value
Liver Histopathology			
Knodel Score (0–22)	5.5 ± 3.1	5.1 ± 2.9	0.393 ^a
Ishak Fibrosis Score (0–6)	2.3 ± 1.9	1.9 ± 1.9	0.064 ^a
No. Ishak Score ≥ 3	43 (46.2%)	41 (32.3%)	0.035 ^b
Metavir Fibrosis Score (F0–F4)	1.6 ± 1.2	1.3 ± 1.2	0.044 ^a
No. Metavir Score ≥ F3	26 (28.0%)	26 (20.5%)	0.197 ^a

* Mean ± standard deviation.

^a Wilcoxon rank sum test for continuous variables.

^b Pearson chi-square test for categorical variables.

^c Fisher's exact test.

Table 3
Fibrosis in HCV (+) Hemophilic Men: Spearman Correlation Coefficients Between Biomarkers and Histologic Scoring Systems

	HIV(+) N = 93				HIV(-) N = 127				All N = 220			
	N	ρ	p value	N	ρ	p value	N	ρ	p value	N	ρ	p value
AST												
Knodell	89	0.26	0.0147	114	0.29	0.0019	203	0.28	<0.0001			
Ishak	89	0.24	0.0209	116	0.34	0.0004	205	0.31	<0.0001			
Metavir	89	0.23	0.0303	116	0.38	<0.0001	205	0.34	<0.0001			
ALT												
Knodell	89	0.20	0.0620	113	0.26	0.0060	202	0.24	0.0007			
Ishak	89	0.18	0.0887	115	0.27	0.0031	204	0.24	0.0004			
Metavir	89	0.17	0.1112	115	0.33	0.0004	204	0.27	<0.0001			
Platelet Count												
Knodell	80	-0.02	0.8579	102	-0.24	0.0165	182	-0.16	0.0327			
Ishak	80	-0.24	0.0300	104	-0.31	0.0012	184	-0.29	<0.0001			
Metavir	80	-0.23	0.0440	104	-0.26	0.0080	184	-0.26	0.0003			
AST/ALT												
Knodell	89	0.06	0.6043	113	0.03	0.7508	202	0.06	0.4287			
Ishak	89	0.09	0.4172	115	0.02	0.8022	204	0.07	0.3106			
Metavir	89	0.09	0.3815	115	-0.01	0.8834	204	0.06	0.4198			
APRI*												
Knodell	79	0.13	0.2397	102	0.32	0.0010	181	0.26	0.0003			
Ishak	79	0.27	0.0145	104	0.38	<0.0001	183	0.36	<0.0001			
Metavir	79	0.26	0.0188	104	0.40	<0.0001	183	0.38	<0.0001			
Ferritin												
Knodell	86	0.28	0.0095	113	0.21	0.0241	199	0.23	0.0009			
Ishak	86	0.28	0.0100	115	0.22	0.0159	201	0.25	0.0004			
Metavir	86	0.25	0.0191	115	0.22	0.0188	201	0.23	0.0008			

HIV(+) N = 93			HIV(-) N = 127			All N = 220		
N	ρ	p value	N	ρ	p value	N	ρ	p value
Alpha-fetoprotein								
Knodel	83	0.38	0.0004	112	0.25	0.0082	195	0.32
Ishak	83	0.40	0.0002	114	0.40	<0.0001	197	0.42
Metavir	83	0.40	0.0002	114	0.42	<0.0001	197	0.44

* APRI is AST/ULN×100/platelets (10⁹/L).

Fibrosis in HCV (+) Hemophilic Men: Spearman Correlation Coefficients Between Cytokines and Histologic Scoring Systems

Table 4

HIV(+) N = 16						HIV(-) N = 25						All N = 41					
N	p	p value	N	p	p value	N	p	p value	N	p	p value	N	p	p value	N	p	p value
TGF-β mRNA																	
Knodell	16	0.09	0.7398	25	-0.49	0.0130	41	0.22	0.1597								
Ishak	16	-0.07	0.7993	25	-0.33	0.1034	41	-0.20	0.2185								
Metavir	16	-0.06	0.8110	25	-0.32	0.1137	41	-0.20	0.2190								
IL-6 mRNA																	
Knodell	16	-0.51	0.0415	25	-0.65	0.0004	41	-0.57	<0.0001								
Ishak	16	-0.65	0.0065	25	-0.70	0.0001	41	-0.65	<0.0001								
Metavir	16	-0.57	0.0208	25	-0.70	0.0001	41	-0.62	<0.0001								
IL-10 mRNA																	
Knodell	16	-0.19	0.4758	25	-0.39	0.0551	41	0.31	0.0454								
Ishak	16	-0.15	0.5903	25	-0.48	0.0164	41	-0.33	0.0376								
Metavir	16	-0.08	0.7553	25	-0.49	0.0120	41	-0.32	0.0389								
TNF-α mRNA																	
Knodell	16	-0.40	0.1211	25	-0.56	0.0034	41	-0.50	0.0009								
Ishak	16	-0.34	0.1921	25	-0.58	0.0025	41	-0.48	0.0015								
Metavir	16	-0.28	0.2916	25	-0.56	0.0039	41	-0.46	0.0028								
IFN-γ mRNA																	
Knodell	16	-0.41	0.1110	25	-0.60	0.0014	41	-0.56	0.0002								
Ishak	16	-0.19	0.4827	25	-0.37	0.0649	41	-0.33	0.0336								
Metavir	16	-0.24	0.3794	25	-0.40	0.0480	41	-0.36	0.0218								

Table 5
Logistic Regression Coefficients for Metavir $\geq$ F3 Fibrosis in Hemophilic Men with HCV Infection *

	HIV(+)N=93				HIV(-) N=127				All N=220			
	β (SE)	AUROC	p value		β (SE)	AUROC	p value		β (SE)	AUROC	p value	
AST	0.631 (0.365)	0.557	0.0834		1.720 (0.434)	0.718	<0.0001		1.155 (0.269)	0.653	<0.0001	
ALT	0.363 (0.310)	0.552	0.2415		0.917 (0.323)	0.669	0.0045		0.649 (0.220)	0.615	0.0031	
Platelets	-1.915 (0.775)	0.708	0.0134		-2.393 (0.851)	0.722	0.0049		-2.129 (0.558)	0.721	0.0001	
AST/ALT	0.384 (0.612)	0.574	0.5310		0.732 (0.539)	0.543	0.1744		0.640 (0.395)	0.567	0.1053	
APRI**	0.655 (0.324)	0.629	0.0432		1.546 (0.410)	0.739	0.0002		1.040 (0.243)	0.699	0.0001	
Ferritin	0.047 (0.229)	0.508	0.8387		0.928 (0.308)	0.709	0.0026		0.384 (0.180)	0.608	0.0324	
Alpha-fetoprotein	0.918 (0.321)	0.707	0.0043		1.580 (0.382)	0.798	<0.0001		1.248 (0.252)	0.763	<0.0001	

* All independent variables were natural log transformed for modeling purposes.

** APRI is AST/ULN $\times$ 100/platelets ($10^9/L$).

Table 6Multivariable Model for Metavir $\geq$ F3 Fibrosis in Hemophilic Men with HCV Infection *

Predictors	Odds Ratio (OR)	95% CI	p value
Age	1.05	1.01, 1.08	0.0083
HCV Treatment	2.86	1.18, 6.97	0.0206
Alcohol Use	0.50	0.15, 1.67	0.2600
ln Platelet Count *	0.32	0.10, 1.00	0.0512
ln Alpha-fetoprotein	3.29	1.69, 6.38	0.0004

* Adjusted for time between serum measurement and biopsy

** ln is natural logarithm; CI is confidence intervals