

Thrombocytopenia, Liquor Use and Marijuana are Associated with Non-invasive Markers of Liver Fibrosis in People Living with HIV

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Abstract

Objective and aims: Liver disease is currently one of the leading causes of death among people living with HIV. Although platelets alterations are well recognized in the course of liver disease, the impact of thrombocytopenia (TCP: platelet counts <150,000 per microliter), which is highly prevalent among Hazardous Alcohol Users (HAU), is unclear. This lack of information limits the possibility to identify those at risk and to create targeted interventions.

Methods: In this study, a total of 400 people living with HIV underwent laboratory assessments to determine if in addition to alcohol, TCP or its related factors (i.e., serotonin) were associated with non-invasive markers of liver fibrosis. The FIB-4 and the APRI scores were calculated using the Sterling's and the Wai's formulas, respectively.

Results: Liver fibrosis was present in almost half of the study population (47%). As expected, APRI values of Hepatitis C co-infected subjects were higher than in HIV mono-infected patients (0.96 ± 0.18 vs. 0.5 ± 0.45 , $p=0.01$). Notably, the risk was higher among marijuana users (OR= 9 95% CI 1.3-9.5, $p=0.02$). The risk of moderate fibrosis was also higher in the HAU (OR = 1.3; 95% CI, 1-2.5, $p=0.04$) when compared to non-HAU. Additional analyses indicated that consumption of liquor even 1-2 drinks per day, and high daily intakes of beer or wine (> 3 drinks per day) increases the risk of liver fibrosis. Thrombocytopenia, which is still prevalent (HAU=15% versus Hep C=22%) significantly increased the odds of moderate fibrosis (OR = 9; 95% CI, 3.6-23; $p=0.0001$). The odds of having severe fibrosis were 52% higher in the TCP group (95% CI, 13-97; $p=0.0000$). Serotonin, which is stored in the platelets, was also reduced among subjects with liver fibrosis levels (55.7 ± 6.7 vs. 87.3 ± 7.8 ng/mL, $p=0.003$). Multivariate analysis identified gender, hepatitis C, liquor use, TCP, marijuana and serotonin alterations as risk factors associated with liver fibrosis.

Conclusion: Liver fibrosis is a condition that was associated with modifiable risk factors such as HAU, marijuana, TCP and viral hepatitis C. These results indicated that health care providers must be attentive for signs of these conditions to avert the risk of this terminal liver disease. From the clinical perspectives, analyses suggest that platelet-based interventions and serotonin agonist could be potential therapeutic targets.

Keywords: Liver; Serotonin; Thrombocytopenia; Alcohol; Marijuana; HIV/AIDS

Introduction

Thrombocytopenia (TCP), a platelet count of less than 150,000 per microliter, is a common hematological complication which affects a sizable proportion of the people living with HIV (PLWH) [1-3]. For many years it was expected that the introduction of Highly Active Antiretroviral Therapy (HAART) would eliminate this problem. However, since its causes are multifactorial, TCP is still present in approximately 15% of the people receiving HAART [4,5]. In addition to direct destruction caused by the virus, alcohol might be an important contributing factor to TCP persistence [6]. Since TCP is often asymptomatic, clinicians' only concern has been the risk of bleeding [7].

Beyond hemostasis, platelets exert a variety of other functions. These multifunction effects derive from various inflammatory mediators, growth factors, and proteases stored in the platelets, that either enhance or limit tissue injury [8,9]. For example, after produced by the enterochromaffin cells, serotonin is taken up and stored in the platelets. Then when needed, serotonin is transported and released at sites of injury [10]. Although initially related to liver fibrosis, other studies have implicated the release of serotonin by platelets as a mediator of liver regeneration and repair after partial hepatectomy [9,11,12]. These opposite observations let us with a plethora of unanswered questions that we aim to address in this article.

In addition to serotonin, platelets transport several growth factors (i.e., platelet-derived growth factor, vascular endothelial growth factor, HGF, IGF, EGF and TGF β), that can promote organ regeneration [8,13]. Of relevance for both PLWH and hazardous alcohol users, evidence is accumulating regarding platelets role in hepatic regeneration. The liver volume is mostly composed (80%) of hepatocytes, which are cells that are not terminally differentiated, and thus when needed can rapidly enter a cell division cycle [8]. Hepatocytes respond to various growth factors and cytokines that are present in the platelets, such as, HGF, IGF-1, IL-6, TNF α , EGF, TGF β , and PDGF, that may help to repair and /or rebuild the lost and damaged hepatic tissue [8,9,14-16]. Noteworthy, if liver damage is viral or immune-mediated, platelets seems to exacerbate liver injury by generating an inflammatory

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response and microcirculation alterations [14]. These findings are of particular salience, given the frequent overlap of HIV, hazardous alcohol use, and hepatitis B and C infections.

Liver fibrosis a life-threatening condition, which develops when these insults are chronic, offers another opportunity to illustrate another example of the dual role of platelets. During this phase, platelets accumulate in the sites of damage and release pro-fibrogenic mediators, such as type $\beta 1$ Transforming Growth Factor (TGF $\beta 1$), CXC Chemokine Ligand 4, growth factor BB (PDGF-BB) and C [17-19]. One can imagine that thrombocytopenia may be beneficial under this condition; yet, animal models have shown quite the opposite, TCP aggravates liver fibrosis [20]. These effects have been linked to the down regulation of hepatic stellate cell collagen production by platelet derived hepatocyte growth factor. Furthermore, thrombocytosis reduces liver fibrosis in the chronic CCl₄ mouse model [21]. In summary, how platelets interact during the liver disease process is a fascinating issue, for which many interrogates remain.

While liver biopsy is considered the gold standard to diagnose liver fibrosis, its invasiveness, costs and associated major complications, render liver biopsies as an unpractical diagnostic procedure for large clinical cohorts [3]. Given these limitations, researchers have developed and validated a number of algorithms. These algorithms have been incorporated to both clinical practice and research [22-24]. The aspartate aminotransferase (AST)-to-Platelet Ratio Index (APRI) is a validated marker of hepatic fibrosis [22]. APRI has been widely used among the general population, and has been validated with PLWH [22-24]. Furthermore, in clinical setting APRI has been shown to be a useful predictor of liver-related mortality [24].

Despite these important functions, little is known about the plausible effects of TCP on liver disease among PLWH. These gaps in knowledge highlighting the need for further research. Thus the aim of this study was widening our knowledge of platelets in liver disease among PLWH.

Methods

Sampling

This study utilized baseline data from Platelets Mediating Alcohol and HIV Damage Study (PADS), a large, single-site multi-ethnic cohort consisting of 400 PLWH, who are at least 18 years old and under regular care at Miami's primary open-access public health system. The study was conducted from 2009 to 2012, and examined associations among platelet status, alcohol use, and clinical outcomes in PLWH who were receiving Antiretroviral Therapy (ART). Our choice of PLWH in an open-access public health system with standard treatment protocols was purposefully designed to minimize social, medical, and treatment inequalities. Since the main focus of this cohort study was to assess the potential effects of alcohol, the DSM-IV-TR questionnaire was applied [25], and those participants who were dependent on drugs or injecting illicit psychoactive substances were excluded. Subjects presenting medical co-morbidities such as central nervous system opportunistic infection, major psychiatric disease, severe malnutrition, chronic conditions such cirrhosis, hepatomegaly, splenomegaly, renal failure, thyroid problems, malignancies, autoimmune diseases, or arthritis, were also excluded.

Alcohol use

At each visit, participants reported alcohol intake in the past six months, using two standardized and validated brief screening

questionnaires: the Alcohol Use Disorders Identification Test (AUDIT) and the Alcohol Dependence Scale (ADS) [26,27]. The AUDIT includes three questions on alcohol consumption, three on drinking behavior and dependence, and four on the consequences or problems related to drinking. The ADS assesses alcohol withdrawal symptoms, impaired control over drinking, awareness of a compulsion to drink, increased tolerance to alcohol, and salience of drink-seeking behavior.

Alcohol consumption scores were computed by averaging cross products of quantity and frequency of beer/wine and hard liquor reported on the AUDIT and ADS responses. This information is highly relevant, given that we previously observed significant differences in immune and biochemical profiles, depending on the type of alcoholic beverage consumed. Then, based on the National Institute of Alcohol Abuse and Alcoholism guidelines criteria [28], men who reported consuming >14 drinks/week or >4 drinks in one day, and women >7 drinks/week or >3 drinks in one day, were classified as HAU, while those who reported fewer drinks were categorized as non-HAU. Participants who drank more than five standard drinks in a given day were considered binge drinkers.

Platelet assessments

Blood was drawn in fasting subjects in order to best evaluate immunological, hematological, and platelet associated factors profiles. Blood cell counts were obtained using the cell-Dyn 4000, a multi-parameter automated hematology analyzer system, recommended for specimens with low or high platelet concentrations. Thrombocytopenia (absolute thrombocytopenia) was defined as platelets counts below 150×10^3 cells/mm³.

Marker of liver fibrosis

The aspartate transaminase (AST) and the alanine aminotransferase (ALT) were abstracted from medical records if results were available within a three month window period from the baseline visit. For subjects without an AST/ALT results, testing was performed in the UM/JMH clinical laboratory. These values were used to calculate liver fibrosis using two non-invasive markers. The Fibrosis 4 (FIB-4) score was calculated using Sterling's formula [29], as follows:

$$FIB-4 = Age \times AST [IU/L]$$

$$Platelet\ count \times ALT$$

The APRI score was calculated using Wai's formula [22]:

$$APRI = 100 \times [(AST\ level) / (upper\ limit\ of\ normal)]$$

$$Platelet\ count$$

The upper limit normal was defined as an AST level of 35 U/mL. An APRI < 0.5 is considered within normal range; 0.5-1.5 represents moderate fibrosis; while an APRI > 1.5 has a positive predictive value of 91% for significant liver fibrosis.

Serotonin

Serotonin concentrations were determined in plasma by using a competitive ELISA kit (DRG international, Inc. USA). Samples were prepared (derivatization of serotonin to N-acetylserotonin) by incubation of respective plasma samples with the acylation reagent. Standards, acylated controls and acylated sample (50 μ L each) were incubated overnight at 2-8°C with serotonin biotin and serotonin antiserum followed by incubation with enzyme conjugate. Micro plates were washed and incubation was continued with PNPP substrate solution at room temp with shaking. The reaction was stopped by adding PNPP

Stop Solution. Optical density was measured with a photometer at 405 nm within 60 min after addition of stop solution. The serotonin concentrations were expressed in ng/mL.

Cytokines

Tumor necrosis factor alpha (TNF) was quantified using a commercially available kit (R&D Systems, Minneapolis, MN, USA). This immunoassay is a 3.5-hour solid phase ELISA that recognizes natural human TNF in serum and plasma. This ELISA system is characterized by its high sensitivity detecting low amounts of TNF- α (0.1 pg/ml).

Levels of IL-6 were measured by commercial Enzyme-Linked Immunosorbent Assays (ELISAs) (Beckman/Coulter Corporation, Miami, FL), in which standard curves using recombinant cytokines were generated. Both assays were appropriately quality-controlled with different known sources of recombinant and natural cytokines (including National Institute for Biological Standards and Control/World Health Organization reference standards).

Covariates

Upon entry into the study, sociodemographic, medications, and medical history information (e.g., ART details) were obtained via standardized questionnaires. Hepatitis B and C antibodies were obtained in all subjects. Viral hepatitis was defined by either hepatitis B or hepatitis C positive antibodies. For those that tested positive at enrollment, medical records were reviewed searching for a positive HBsAg, HCV RNA positive. Please note that subjects under treatment for viral hepatitis or with previous diagnosis of cirrhosis were excluded from the study. The following parameters were collected at baseline and scheduled every 6 months: CD4⁺ T cell count, HIV RNA level (Amplicor HIV monitor test Roche Diagnostic System), biochemical profile

Statistical analyses

The data were analyzed using SAS version8, and SPSS version 11, and *p* values <0.05 were considered to be statistically significant. Following descriptive statistical analyses, mean variables were compared using Student's *t* test, and one-way analysis of variance (ANOVA) procedures. Correlations among the main variables of interest were examined with Pearson's coefficients.

Univariate analyses were used to calculate Odds Ratios (OR), and 95% Confidence Intervals (CI). The primary analysis was APRI as continuous values, and liver fibrosis using following pre-established cutoffs: APRI \leq 0.5 (mild/no fibrosis), APRI 0.5-1.5 (intermediate fibrosis), and APRI >1.5 (significant fibrosis). Binary logistic regression analyses were used to evaluate the effects of alcohol (hazardous vs. non-hazardous), thrombocytopenia (thrombocytopenia vs. normal platelet counts) viral hepatitis (yes/no). Potential predictors (i.e., gender, race/ethnicity, CDC status, serotonin, cytokines and body mass index) were selected on the basis of the HIV medical literature, and were added to the model. Non-significant variables (*p* \geq 0.05) were removed, beginning with the least significant variable, until the final full model was achieved.

Ethics

PADS were approved by the central governing Institutional Review Boards at Florida International University and University of Miami. The study was conducted according to the principles expressed in the Declaration of Helsinki. Upon signing the written informed consent

and medical release forms, subjects were consecutively enrolled and followed over a period of six months.

Results

Study population characteristics

Most (80%) of the individuals in the study had normal platelets, hemoglobin, and hematocrit measurements. No significant differences in mean hemoglobin, hematocrit, or proportion of anemia were observed between patients with TCP (13.2 \pm 2.0 g/dl) and without TCP (13.0 \pm 1.9 g/dl). A total of 179 subjects reported past history of cannabis and/or crack use. However; occasional cannabis smoking was reported by 40 of 400 participants, who reported smoking marijuana occasionally and mostly during the weekends. Fifty subjects reported recreational use of crack, 30 of whom were also occasional marijuana users. Rates of drug use were similar between TCP and non-TCP.

Liver fibrosis

Though, the vast majority of the sample had normal liver enzymes (Table 1), the mean APRI for the cohort was 0.66 \pm 0.5, suggestive of intermediate fibrosis. According to the APRI values, 38% had intermediate fibrosis, 7% had significant fibrosis, and the remaining 55% of the PLWH had no fibrosis. As per the FIB-4, 31% of the sample had liver fibrosis. The concordance analyses showed moderate agreement between FIB-4 and APRI (kappa=0.7). In particular, twenty patients who were in class 1 according to FIB-4 were in class 2 according to APRI. Therefore, APRI seemed to be stricter than FIB-4, and was used for the remaining analyses.

Males were twice more likely to have fibrosis, compared to their female counterparts (OR, 1.6; 95% CI, 1.1-2.15; *p*=0.007). Males had 12 times an increased odd of having severe fibrosis (95% CI, 1.6-

Variable	Non-TCP	TCP	P value
Age	43.1 \pm 6.7	42.6 \pm 7	0.80
Gender			
Men	74%	56%	0.01
Women	26%	44%	
Race/ethnicity			
African American	63%	73%	0.10
Black Caribbean	3%	2%	
Hispanic	27%	20%	
White	7%	5%	
Education			
Years of school	11.6 \pm 2.3	11.3 \pm 2.3	0.20
Albumin mg/dl	4.0 \pm 0.6	4.2 \pm 0.5	0.90
Metabolism			
Glucose mg/dL	96.9 \pm 50.5	93.7 \pm 28.9	0.70
Diabetes	4.5%	5%	0.80
Liver Enzymes			
AST IU/L	35.0 \pm 20.5	41.2 \pm 23.9	0.06
ALT IU/L	33.0 \pm 30.0	39.9 \pm 27.0	0.30
Total Number of Drinks per week	15 \pm 1.3	19 \pm 3.5	0.04
HIV Disease			
CD4 Cell Counts	450.1 \pm 284.8	307.6 \pm 237.8	0.00
Viral Log	2.6 \pm 1.3	3.3 \pm 1.5	0.02

BMI=Body Mass Index, ALT= Alanine Transaminase, AST= Aspartate Transaminase

*Data are *n* (%) or group means + Standard Deviation.

Table 1: Sample Characteristics by TCP Group

86; $p=0.0006$). While previously a significant relationship between age and liver fibrosis had been observed, in our sample age did not exert any significant effect. Albumin levels were similar among the groups and within normal limits suggesting that these individuals were compensated according to the Child-Turcotte-Pugh score that clinicians use to assess decompensated liver cirrhosis.

In our sample all subjects were prescribed ART, yet 4% were non-compliant. Analyses between these two groups indicated a tendency for lower AST values among those on therapy (ART = 35.9 ± 21 vs. 46.5 ± 21 U/L; $p=0.08$). Though in previous studies [3,30] risks of liver fibrosis were reduced with ART (yes/no), we did not identify any significant associations between receiving ART and APRI. However, APRI values were significantly lower in those who have undetectable viral loads, compared with those who had not fully responded to the therapy (0.39 ± 0.25 vs. 0.66 ± 0.59 ; $p=0.001$). Subjects with undetectable viral loads exhibited significantly lower AST (27.9 ± 21 vs. 37 ± 21 U/L; $p=0.02$), as well as ALT enzyme values (26.9 ± 14 vs. 35.9 ± 31 U/L; $p=0.02$). However, subjects with and without ART did not differ in their platelet counts (234.6 ± 106 vs. 233.4 ± 69 counts; $p=0.9$).

APRI by hepatitis status

The prevalence of Hep B and Hep C infection was low (12% and 11%), and only 3% of the study population had dual co-infection. AST and ALT levels were significantly higher in HIV/HCV co-infected than in HIV mono-infected patients (AST = 46.8 ± 33.6 vs. 35 ± 20 U/L; $p=0.003$; ALT = 43.9 ± 27.2 vs. 33 ± 30 U/L; $p=0.04$). A similar trend was observed for HepB co-infected patients than in HIV mono-infected patients ($p<0.01$). Of interest, Hep C co-infected subjects exhibited significantly lower platelet counts when compared to those with mono-infection (195.9 ± 73 vs 237.6 ± 75 ; $p=0.003$)

APRI values of Hepatitis C co-infected subjects were higher than in HIV mono-infected patients (0.96 ± 0.18 vs. 0.5 ± 0.45 ; $p=0.01$). APRI values of HepB and Hep C subjects were nearly identical (0.92 ± 0.65) and were significantly higher compared with the HIV-mono-infected uninfected group ($p=0.02$).

APRI values by drugs of abuse

Cannabis use was significantly associated with higher APRI values when compared to non-marijuana users (0.58 ± 0.48 vs. 0.45 ± 0.32 ; $p=0.02$). Crack users tended to have higher APRI values compared to non-crack users (0.60 ± 0.5 vs. 0.47 ± 0.36 ; $p=0.07$). Noteworthy, univariate analyses indicated that the risk of having fibrosis was highest among marijuana users (OR= 2.2; 95% CI: 1.1-4.1; $p=0.02$).

Since a prior study suggests that marijuana risk was significant among individuals with viral hepatitis, we compared the risk between subjects with and without viral hepatitis. Analyses indicated that among individuals with viral hepatitis the risk for having liver fibrosis was higher among marijuana users (OR = 9 95% CI 1.3-9.5, $p=0.02$). Among those without viral hepatitis the risk of marijuana users to develop liver fibrosis was present, yet, the risk was lower (OR=1.16; 95% CI, 1.0-1.4; $p=0.045$).

APRI values by alcohol status

The non-HAU group had the lowest median APRI at 0.56 ± 0.4 , compared with the HAU (0.72 ± 0.69 ; $p=0.004$). As expected, the risk of moderate fibrosis was higher in the HAU (OR=1.3; 95% CI, 1-2.5; $p=0.04$) compared with the non-HAU. However, the prevalence of severe fibrosis was similar between HAU and non-HAU (8% and 5.8%, respectively). As depicted in Table 2, additional analyses indicated

that both high daily consumption as well as frequent alcohol intakes was associated with liver fibrosis. More important, liquor use, but not beer or wine increases the risk of liver fibrosis at low doses. Analyses indicated that more than one drink of liquor per day was associated with liver fibrosis. On the other hand, those with liver fibrosis (APRI > 0.5) take over 4 drinks of beer or wine versus only 2 per day in those with normal APRI values ($p=0.02$).

To characterize the differential effect of hepatitis C viral infection on HAU, we compared the APRI means of HCV-infected individuals with HIV mono-infected HAU. HC co-infected individuals exhibited higher APRI values when compared to the HIV mono-infected group (1.2 ± 0.4 vs. 0.5 ± 0.4 ; $p=0.007$)

Although a significant relationship between excessive alcohol uses was evident, not all HAU exhibited abnormal APRI scores, suggesting that additional factors have to be explored. Based on prior literature, we proceeded to analyze the effect of platelet status in our results.

APRI and platelet status

As depicted in Table 1, thrombocytopenic subjects were more likely to be males (22% vs. 7%; $p=0.0001$), had significantly lower CD4 counts and higher viral loads. They also tended to have higher AST ($p=0.09$), but did not differ in ALT values. Additional analyses indicated that thrombocytopenic patients significantly differed in their patterns of alcohol consumption, when compared to patients with normal platelet counts. Subjects with thrombocytopenia consumed alcohol more days per week (4.3 ± 1 vs. 3.2 ± 2.6 ; $p=0.045$) and for more years (17 ± 11 vs. 10 ± 9.5 ; $p=0.01$).

In PLWH, platelets strongly correlated with both APRI values ($r=-.398$; $p=0.0001$) and liver fibrosis groups ($r=0.44$; $p=0.0001$). A main effect of platelet group was observed, with subjects with normal platelet counts having significantly lower APRI, compared with TCP (0.49 ± 0.32 vs. 1.26 ± 0.92 ; $p=0.003$). Group differences were substantial, with the TCP group mean APRI being 2.6 higher than the reference group.

Variable	Normal APRI < 0.5 N=236	Risk of Liver Fibrosis APRI>0.5 N=164	P value
Age	42.6 ± 6	43.4 ± 6	0.84
Men	53%	74%	0.003
Women	47%	26%	
African American	67%	70%	0.81
Hispanic	27%	23%	
White	6%	7%	
Education (years of school)	11.3 ± 2.3	11.2 ± 2.5	0.62
Albumin mg/dl	4.1 ± 0.6	4.2 ± 0.5	0.61
AST IU/L	27.0 ± 7.8	52.7 ± 26	0.00
ALT IU/L	25.9 ± 11.7	49.2 ± 44.0	0.00
BMI	29.5 ± 8.3	26.7 ± 5.6	0.06
CD4	442.7 ± 275.0	359.6 ± 246.8	0.001
Viral Log	2.6 ± 1.3	3.2 ± 1.5	0.02
Total Number of Drinks/week	12.0 ± 1.5	20.7 ± 3.4	0.04
Number of days Drinking	2.0 ± 0.2	3.0 ± 0.3	0.01
Number of Liquor Drinks/day	0.6 ± 0.1	1.1 ± 0.2	0.05
Number of non-Liquor drinks/day	2.0 ± 0.3	4.0 ± 0.6	0.02

BMI=Body Mass Index, ALT= Alanine Transaminase, AST= Aspartate Transaminase, Data are n (%) or means + Standard Deviation.

Table 2: Sample Characteristics by APRI

Bivariate analyses indicated that the risk of having moderate fibrosis was highest in the TCP group (OR= 9; 95% CI, 3.6-23; $p=0.0001$) and even higher of having severe fibrosis (OR= 12; 95% CI, 13-97; $p=0.0000$).

Given that serotonin is stored in platelets and has been associated with liver status, we proceeded to analyze its levels. Analyses indicated the patients with fibrosis (APRI > 0.5) had significantly lower serotonin levels (55.7 ± 6.7 vs. 87.3 ± 7.8 ng/mL; $p=0.003$). However, levels did not differ between those with moderate and severe liver fibrosis (59 ± 21 vs. 58 ± 8 ng/mL, $p=0.7$) suggesting a bell-shaped relationship. Based on prior studies indicating that HIV directly can impact serotonin we pursued such analyses. In accord with prior studies, subjects with detectable viral loads exhibited significantly lower serotonin levels 79.5448 ± 4.7 vs. 112.2 ± 14.9 ng/mL, ($p=0.02$).

Given that inflammatory cytokines can modulate hepatic fibrogenesis and in our prior studies IL-6 and TNF were associated with TCP and HAU, we included these cytokines in the study. Analyses indicated that subjects with liver fibrosis (APRI > 0.5) exhibited significantly different TNF values (log TNF 0.29 ± 0.04 vs. 0.11 ± 0.05 ; $p=0.02$). Similar to serotonin levels, TN only differed between the control group and those with moderate fibrosis. IL-6 levels were similar between the groups.

Longitudinal analyses

Factors considered in adjusted models were time with HIV, CD4 cell counts viral load, gender, race, alcohol use, age, serotonin and TNF. Likelihood Ratio (LR) tests and Wald tests for association were used with a significance level of 0.05. When final analysis was performed platelet status, liquor, serotonin and marijuana use were the only predictors of liver fibrosis (Table 3). Male sex was confirmed as being a risk factor for liver fibrosis. It needs to be noted that once we introduced serotonin and TNF in the model neither viral load nor ART remained significant.

Discussion

Our data suggest a high burden of liver fibrosis in almost half (41%) of our urban sample of PLWH in South Florida. Given that liver fibrosis represent one of the leading causes of mortality among PLWH in the United States and in Europe [31], the elucidation of factors associated with liver fibrosis should be prioritized. Consistent with platelets broad impact on physiology and health, our analyses revealed that in the setting of immunosuppression, TCP is strongly associated with liver fibrosis. Even though the study design and the

lack of liver biopsies do not allow us to make definitive conclusions, several facts reassured our tenants. 1) Our results were consistent with previous studies and in the final model were highly significant ($p=0.000$). 2) There is biological evidence supporting these findings. 3) Though the reversibility of effects, were not established here, there is evidence emerging from animal models indicating that correction of TCP, with platelet transfusion, reduced liver fibrosis [32]. These results provide a sound basis for additional studies to explore the development of interventional strategies aimed at correcting platelet deficits to modulate this important aspect of the pathophysiological process. Additional studies are also needed to further determine if TCP could be an early phenomenon in the pathological process, given that we have excluded those with any indication of advanced disease (i.e., cirrhosis, hepatomegaly, splenomegaly and those with liver enzymes > 1 SD normal limits). Such analyses will be highly relevant considering that TCP can be an early HIV phenomenon; and the high rates of HAU among PLWH. Equally relevant for clinical practices are our findings indicating that liquor and marijuana use maybe further increasing the risks of liver fibrosis.

Although the multivariate analyses confirm typical fibrosis risk factors, such as male sex, and HCV infection. In our cohort, viral hepatitis was only present in 12% of the sample and thus does not appear to account for a large portion of liver fibrosis. On the other hand, HAU is an important contributor. One of our remarkable findings is the effect of liquor, but not of wine or beer alcohol on liver fibrosis. It needs to be highlighted that oxidative stress and inflammatory cytokines are important contributors to liver damage and therefore the anti-oxidants present in wine and beer but not in hard liquor may be exert a protective effect. Further supporting these findings are our prior publications demonstrating that IL-6 and TNF are increased in HAU particularly liquor users [5,6]. Analyses also emphasize the value of alcohol abstinence in PLWH as the main effective preventive measure against fibrosis. Nonetheless, not all HAU exhibited abnormally high APRI values suggesting that additional factors were increasing the risks; in this case we focused on platelets.

Even though most studies have seen platelets as a promoter of liver injury and a marker of late stage of liver disease, emerging literature and clinical data have revealed that platelets play a critical role in the liver repair processes [32]. Differences with prior studies are probably the result of the type of populations studied, but particularly the underlying causes associated with liver damage (i.e. alcohol, viral infection, and trauma). In the case of viral infection, studies consistently demonstrated the beneficial role of platelets. Thrombocytopenia may

Model		Coefficients ^a						
		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95.0% Confidence Interval for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
1	(Constant)	2.466	.217		9.694	.000	2.080	3.144
	Thrombocytopenia	-.710	.103	-.470	-6.913	.000	-.913	-.507
	Gender	-.214	.090	-.160	-2.382	.018	-.391	-.037
	Total Drinks	.004	.002	.148	1.896	.060	.000	.007
	Hepatitis C	-.252	.103	-.158	-2.461	.015	-.455	-.050
	Liquor	-.061	.024	-.200	-2.556	.011	-.108	-.014
	Serotonin	.001	.001	.148	2.273	.024	.000	.003
Marijuana	-.126	.063	.080	-3.371	.005	-.425	-.122	

a. Dependent Variable: liver index

Table 3: Multivariate Analyses Predictors of Liver Fibrosis (Liver Index)

exacerbate liver fibrosis; because platelets exert an antifibrotic role in suppressing type I collagen expression, [20]. Another plausible mechanism is by regulating hepatic stellate cell, which induced fibrosis, via the platelet-derived growth factors [17,19]. Therefore, the presence of TCP in a sizable proportion of our urban HIV population in South Florida is of concern and indicates that we should direct our efforts to assure PLWH are maintaining normal platelet counts. In light of emerging studies suggesting that liver fibrosis is reversible, the need of corrective measures to normalize platelet counts that ultimately leads to liver repair should be prioritized.

Our study also translated findings from animal models, by confirming that serotonin and TNF are also important predictors of liver fibrosis among PLWH [33,34]. In this regard, analyses uncovered serotonin depletions among subjects with liver fibrosis. It is possible that HAU among this group could compromise the supply of tryptophan and reduce liver capacity to regenerate. Ethanol deleterious effects over tryptophan levels have been linked to its effects over liver Trp pyrrolase (TP) activity [35]. HIV can also directly affect serotonin levels [36,37]. The breakdown of tryptophan could also be increased due to the chronic inflammation, which can lead to reductions in serotonin synthesis. Notably, knockout mice lacking peripheral serotonin or tryptophan exhibited limited liver regeneration after partial hepatectomy, while the injection of the serotonin or its precursor restored the impeded liver regeneration [38].

Despite to exclude daily drug abusers, occasional cannabis smoking emerged as an independent predictor of liver fibrosis. The literature regarding marijuana effects on the liver is conflicting [39-41]. The relevance of marijuana use on liver fibrosis has been confirmed by laboratory experiments as well as a prior study among the general population [39-41]. Those studies have demonstrated a relationship between the cannabinoid system and the pathogenesis of portal hypertension [39]. The increased risks have also been attributed to the profibrogenic role of CB1 receptors [39]. On the other hand, similar analyses among PLWH failed to identify a significant relationship [41]. Yet the study populations were different as Brunet's study was limited to subjects with both HIV, hepatitis C co-infection while our population includes both hepatitis C positive and negative individuals. Considering the expanding use of marijuana, additional research is needed. Meanwhile, physicians should caution patients with dual co-infection to avoid the use of marijuana until further studies confirmed if it is deleterious or not.

However, our results have some limitations: the lack of liver biopsies or elastography. Results can only be applicable to PLWH. Another limitation is that given the study design we cannot establish causality. Therefore, additional studies are needed to determine the long-term clinical significance and the validity of our results.

Nonetheless, these findings may have important research, clinical, and therapeutic implications. First, our data highlights the importance of being attentive to drops in platelet counts on PLWH- a practice that many times is overlooked by physicians dealing with HIV patients. Since, TCP is an early event during HIV infection, thus treatment of TCP in PLWH may prevent long term complications such as liver fibrosis. Secondly, findings may also guide the development of future therapies based on anti-inflammatories and or serotonin agonists. These results, together with recent animal studies showing that platelets contribute to liver regeneration [21,32], suggest that platelet increment therapy may provide new clinical approaches for the treatment of liver diseases. Lastly, given the expanding use of marijuana, particularly in some states physician should be recommending to avoid marijuana until additional information is available.

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