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Increased Prevalence of Liver Fibrosis in People Living With Human Immunodeficiency Virus Without Viral Hepatitis Compared to Population Controls

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Background. Liver fibrosis is associated with poor liver-related outcomes and mortality. People with human immunodeficiency virus (PWH) may be at increased risk. We aimed to estimate the prevalence and factors associated with liver fibrosis in PWH compared to population controls.

Methods. This was a cross-sectional cohort study comparing 342 PWH with 2190 population controls aged 50–70 years.

Transient elastography was performed and elevated liver stiffness measurement (LSM) defined as 7.6 kPa as a proxy for significant liver fibrosis. Adjusted odds ratios (aORs) and 95% confidence intervals (95% CIs) were computed by logistic regression.

Results. The prevalence of elevated LSM was higher in PWH than in uninfected controls (12% vs 7%; $P < .01$). Human immunodeficiency virus (HIV) infection was independently associated with elevated LSM. In multivariate analysis, elevated LSM was associated with HIV (aOR, 1.84 [95% CI, 1.17–2.88]; $P < .01$); higher age (per decade: aOR, 3.34 [95% CI, 1.81–6.18]; $P < .01$); alanine aminotransferase (ALT) (per 10 IU/L: aOR, 1.25 [95% CI, 1.05–1.49]; $P < .01$); body mass index (BMI) (per 1 kg/m^2: aOR, 1.17 [95% CI, 1.05–1.29]; $P < .01$), and previous exposure to didanosine (per year: aOR, 2.26 [95% CI, 1.01–5.06]; $P = .04$).

Conclusions. The prevalence of elevated LSM was higher in PWH compared to population controls. Higher age, BMI, ALT, previous exposure to didanosine, and positive HIV status were independently associated with higher odds of elevated LSM.

Keywords. human immunodeficiency virus; liver disease; transient elastography; hepatotoxicity; NAFLD.

After the introduction of combination antiretroviral therapy (ART), the human immunodeficiency virus (HIV) epidemic in high- and middle-income countries has changed markedly with a substantial reduction in mortality among people with HIV (PWH) [1]. Today, HIV infection is a chronic disease and the population of PWH is aging. Consequently, the number of age-related comorbidities has increased, and by 2030 >80% of PWH are predicted to have at least 1 age-related comorbidity [2]. Liver disease is the second leading cause of death among PWH with high mortality rates among PWH coinfected with hepatitis B virus (HBV) or hepatitis C virus (HCV) [3]. However, the spectrum of liver disease among PWH likely will change due to the ability to suppress HBV replication with ART, direct-acting antiviral therapy for HCV infection, and the World Health Organization's 2030 HCV elimination plan [4, 5]. In PWH, the prevalence of liver fibrosis has been reported to be 15% in unselected PWH [6]. This is higher than in the general population where the prevalence has been reported to be 2%–9% [7, 8], and PWH seem to be at higher risk of liver fibrosis. Possible explanations may be adverse lifestyle behavior, microbial translocation [9, 10], immune activation or immunosenescence [11, 12], ART-induced liver toxicities [13–16], and nonalcoholic fatty liver disease (NAFLD) [17]. However, studies that assess the prevalence and risk of liver fibrosis among unselected PWH without viral hepatitis and with an uninfected comparator group are few [18, 19]. In this study, we aimed to estimate the prevalence of and factors associated with liver fibrosis in PWH without viral hepatitis compared to HIV-uninfected controls, and to estimate if positive HIV status was independently associated with liver fibrosis. We hypothesized that PWH had a higher prevalence of liver fibrosis compared to the general population and that a positive HIV status was independently associated with liver fibrosis.

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METHODS

Study Populations

The Copenhagen Co-Morbidity in HIV Infection (COCOMO) Study is an observational, prospective cohort study of PWH aged 20 years and above in the area of Copenhagen, Denmark [20]. From March 2015 through November 2016, 1099 PWH were consecutively recruited from the outpatient clinics of the Department of Infectious Diseases, at Rigshospitalet and Amager-Hvidovre Hospital, both in Copenhagen, Denmark. For this study, PWH aged 50–70 years were included for further analysis. The Rotterdam Study is a prospective, population-based study of adult people living in the area of Ommoord, Rotterdam, the Netherlands [21]. The study was initiated in 1989 and comprises 2 cohorts with inhabitants aged 55 years and older (RS-I, RS-II), and 1 cohort with inhabitants aged 45 years and older (RS-III). For this study, participants aged 50–70 years enrolled from March 2011 to November 2014 from RS-II and RS-III were used as a comparator group. The comparator group was assumed to be HIV uninfected, as the prevalence of PWH in the Netherlands in 2017 was 0.1% [22]. Individuals with HBV infection and/or HCV infection were excluded from the analyses.

Data Collection

Data collection for the COCOMO Study and the Rotterdam Study has been described in detail elsewhere [20, 21]. For the COCOMO Study, data were collected through blood sampling and comprehensive questionnaires on health and lifestyle. Information on HIV-specific parameters (eg, CD4 T-cell count, HIV RNA, exposure and duration of ART) and hepatitis serology (hepatitis B surface antigen [HBsAg] and hepatitis *C* virus antibodies [anti-HCV]) were retrieved from medical records. Hepatic steatosis was assessed by unenhanced computed tomographic (CT) scan of the upper abdomen in the COCOMO Study as described previously [20]. In short, CT scans were analyzed by a trained physician and the average liver attenuation in the Couinaud liver segments 5 and 6 was estimated. Moderate-to-severe hepatic steatosis was defined as an average liver attenuation ≤48 Hounsfield units according to Pickhardt et al [23].

For the Rotterdam Study, data were collected through blood sampling including hepatitis serology (HBsAg and anti-HCV antibody), and comprehensive questionnaires. Hepatic steatosis was assessed by abdominal ultrasonography [24]. In short, abdominal ultrasonography images were reevaluated by an experienced hepatologist, and hepatic steatosis was defined as presence of hyperechogenic liver parenchyma according to Hamaguchi et al [25].

Transient Elastography

Transient elastography was performed by trained personnel using Fibroscan (Echosens, Paris, France) in both cohorts. With the nonfasting participant in supine position, the transducer was placed on the skin in an intercostal space in the right midaxillary line at the level of the right liver lobe. In the COCOMO Study, the liver stiffness was measured with the standard M probe and in the Rotterdam study an M or XL probe was used according to instructions by the manufacturer. The liver stiffness measurement (LSM) was expressed as kilopascals (kPa). The transient elastography was considered valid if at least 10 valid measurements were obtained; the interquartile range (IQR) was <30% of the median LSM; and the success rate was at least 60% [26]. The transient elastography was considered failed if no valid measurements were obtained after at least 10 attempts.

Definitions

The physiologic stiffness of the liver parenchyma is 5.5 ± 1.6 kPa by transient elastography [7]. Liver stiffness is positively correlated with liver fibrosis, yielding higher LSM with higher amounts of liver fibrosis. In this study we defined elevated LSM as a LSM ≥7.6 kPa as a proxy for significant liver fibrosis with an area under the receiver operating characteristic curve of 87% (95% confidence interval [CI], 82%–91%) for discriminating F2–F4 fibrosis from F0–F1 fibrosis and with a specificity of 80%, a sensitivity of 75%, a positive predictive value of 72%, and a negative predictive value of 83% [27].

We defined metabolic syndrome as a minimum of 3 of the following 5 items: (1) waist circumference of ≥94 cm for men and ≥80 cm for women; (2) systolic blood pressure ≥130 mm Hg and/or antihypertensive treatment; (3) plasma high density lipoprotein (HDL) ≤1.036 mmol/L for men, and plasma HDL ≤1.295 mmol/L for women; (4) plasma triglycerides ≥1.693 mmol/L; (5) self-reported diabetes mellitus and/or antidiabetic treatment and/or nonfasting plasma glucose ≥11.1 mmol/L [28]. We defined HBV infection as the presence of HBsAg; HCV infection as presence of hepatitis C antibodies (anti-HCV); elevated alanine aminotransferase (ALT) as plasma ALT ≥70 IU/L for males and ALT ≥45 IU/L for females. Individuals with missing information on viral hepatitis serology (n = 42) were included in the study population as not having viral hepatitis. None of these individuals had elevated ALT or a history of antiviral therapy for HCV at time of enrollment.

Ethical Considerations

The COCOMO Study has been approved by the regional ethics committee of the Capital Region of Denmark (protocol number H-8-2014-004). The Rotterdam Study has been approved by the Netherlands Ministry of Health, Welfare and Sports and by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam, the Netherlands. The studies were conducted in accordance with the Helsinki declaration. All study participants provided informed consent.

Statistical Analyses

Clinical and demographic characteristics were summarized as median with IQR for continuous variables and as number with

percentage for categorical variables. Comparison between the study cohorts were performed by Fisher exact test and χ^2 test for categorical variables and by Mann–Whitney *U* test or Kruskal–Wallis for continuous variables. Univariate and multivariate logistic regression analyses were performed to assess factors associated with elevated LSM as a proxy for significant liver fibrosis (dependent variable). Covariates included in the adjusted model were age (continuous), sex (binary), plasma ALT (continuous), body mass index (BMI) (continuous), plasma triglycerides (continuous), and plasma cholesterol (continuous). Sensitivity analyses were performed excluding individuals with elevated ALT, as this may be induced by liver inflammation and lead to falsely higher liver stiffness measurements. Associations between elevated LSM and previous (yes vs no) or cumulative exposure (per 5 years) to antiretroviral (ARV) drug classes were assessed by crude logistic regression analysis. If statistically significant additional logistic regression analysis was conducted for individual ARVs within the given drug class. Didanosine (ddI), stavudine, and zidovudine were selected a priori for individual testing in logistic regression analysis based on previous literature, where these ARVs have been associated with significant liver fibrosis [29]. Results are presented as crude and adjusted odds ratios (aORs) with 95% CIs. *P* values <.05 are considered statistically significant. Missing values of ALT, aspartate aminotransferase (AST), albumin, and platelets were imputed by predictive mean matching for the COCOMO study cohort. All statistical analyses were conducted in R version 3.4.1 software.

RESULTS

Clinical and Demographic Characteristics

A total of 342 PWH from the COCOMO Study, and 2190 controls from the Rotterdam Study were included for this study (Figure 1). Clinical and demographic characteristics are shown in Table 1 and HIV-specific characteristics in Table 2.

Elevated LSM in PWH and Population Controls

Forty-one (12%) of PWH without viral hepatitis had elevated LSM assessed by transient elastography compared to 154 (7%) population controls ($P < .01$). The proportion of PWH with mild, moderate, and severe fibrosis was higher compared to population controls ($P < .01$) (Figure 2). The proportion of PWH with CT-defined moderate-to-severe hepatic steatosis was 24 (8%), while the proportion of population controls with ultrasound-defined steatosis was 776 (35%). In sensitivity analyses, individuals with elevated ALT were excluded, and the proportion of individuals with elevated LSM remained higher in PWH with normal ALT compared to population controls (11% vs 7%; $P < .01$). Compared to PWH without elevated LSM, PWH with elevated LSM were older (62 vs 56 years; $P < .01$),

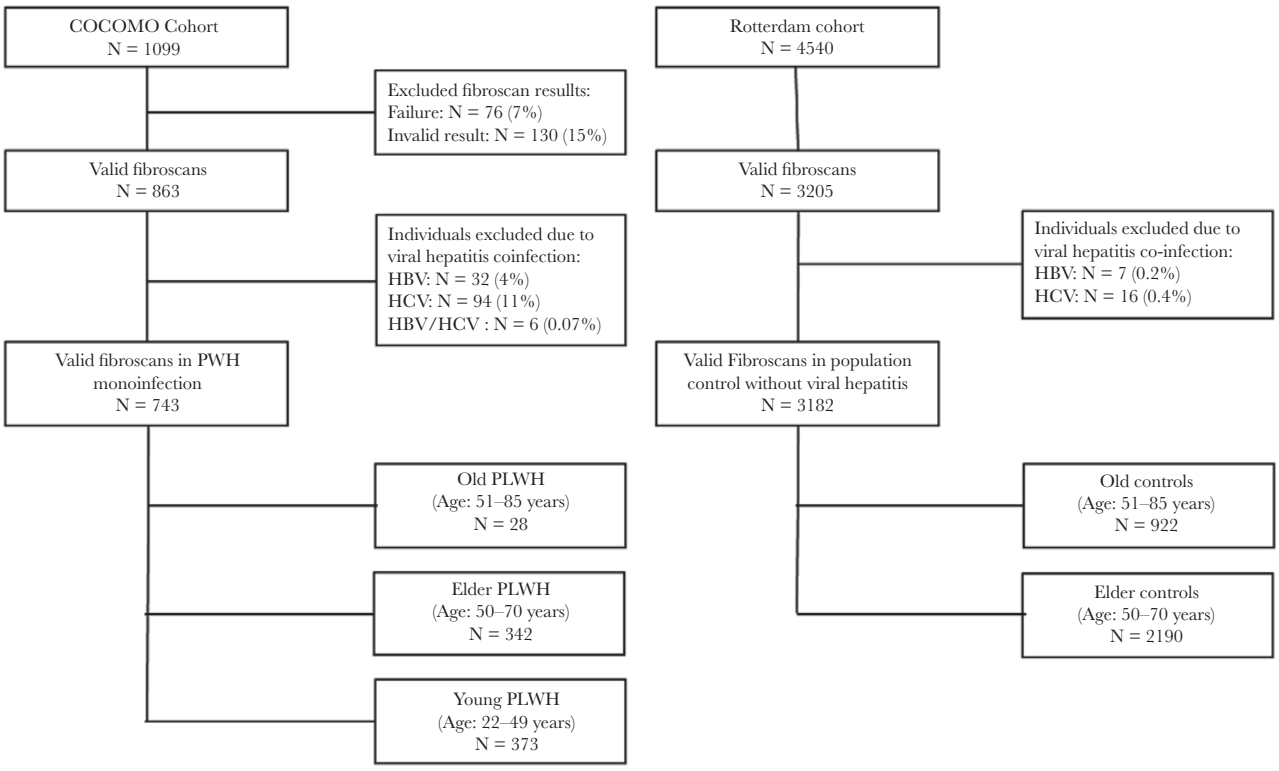

Figure 1. Flowchart of study participants. Selection of study participants from the Copenhagen Co-Morbidity in HIV Infection Study and Rotterdam Study cohorts. Hepatitis B virus coinfection was defined as the presence of hepatitis B surface antigens. Hepatitis C virus (HCV) infection was defined as the presence of anti-HCV antibodies. Abbreviations: COCOMO, Copenhagen Co-Morbidity in HIV Infection Study; HBV, hepatitis B virus; HCV, hepatitis C virus; PWH, people with human immunodeficiency virus.

Table 1. Clinical and Demographic Characteristics of People With Human Immunodeficiency Virus and Population Controls

Characteristics	PWH (n = 342)	Controls (n = 2190)	*P* Value
Age, y, median (IQR)	57.0 (52.0–63.0)	63.0 (58.1–66.4)	<.01
Sex, male	296 (86.5)	1059 (48.4)	<.01
White race	264 (78.3)	1905 (96.7)	<.01
Smoking			.20
Current smoker	86 (25.1)	558 (27.1)	
Previous smoker	151 (44.2)	805 (39.1)	
Never smoker	105 (30.7)	698 (33.9)	
Alcohol			.03
None	63 (20.3)	304 (17.7)	
Within recommendations	159 (51.3)	787 (45.9)	
Above recommendations	88 (28.4)	623 (36.3)	
Abdominal obesity	252 (75.9)	1327 (60.6)	<.01
Waist circumference, cm, median (IQR)	94.0 (87.0–101.0)	93.0 (84.0–101.0)	.02
BMI, kg/m^2, median (IQR)	24.3 (22.1–26.5)	26.6 (24.3–29.3)	<.01
BMI WHO category			<.01
Underweight (<18.4 kg/m^2)	7 (2.1)	11 (0.5)	
Normal weight (18.5–24.9 kg/m^2)	192 (56.5)	659 (30.1)	
Overweight (25–29.9 kg/m^2)	119 (35.0)	1062 (48.5)	
Obese (≥30 kg/m^2)	22 (6.5)	458 (20.9)	
Diabetes	23 (6.9)	226 (10.3)	.06
Metabolic syndrome	132 (43.0)	689 (31.5)	<.01
Plasma ALT, IU/L, median (IQR)	25.5 (20.0–34.8)	20.0 (15.0–26.0)	<.01
Plasma AST, IU/L, median (IQR)	29.0 (25.0–35.0)	24.0 (21.0–28.0)	<.01
Plasma total cholesterol, mM, median (IQR)	5.0 (4.3–5.7)	5.5 (4.8–6.3)	<.01
Plasma triglycerides, mM, median (IQR)	1.8 (1.3–2.8)	1.3 (0.9–1.7)	<.01
Route of HIV transmission		NA	
MSM	240 (70.8)		
Heterosexual	77 (22.7)		
Injection drug use	3 (0.9)		
Other	19 (5.6)		
Blood CD4 T-cell count, cells/μL		NA	
<200	3 (0.9)		
200–349	17 (5.0)		
350–500	57 (16.8)		
>500	263 (77.4)		
Blood CD4 nadir, cells/μL, median (IQR)	200.0 (84.2–290.0)	NA	
Plasma HIV RNA ≥50 copies/mL	10 (2.9)	NA	
Duration of HIV infection, y, median (IQR)	19.3 (11.5–25.8)	NA	
ART (yes)	334 (97.7)	NA	

Data are presented as No. (%) unless otherwise indicated. Missing variables for Copenhagen Co-Morbidity in HIV Infection Study and Rotterdam Study, respectively: white race, 5 and 220; smoking, 0 and 129; alcohol, 32 and 476; education, 15 and 10; abdominal obesity, 10 and 1; waist circumference, 10 and 1; BMI, 2 and 0; diabetes, 8 and 0; metabolic syndrome, 35 and 4; total cholesterol, 19 and 0; triglycerides, 19 and 0.

Abbreviations: ALT, alanine aminotransferase; ART, antiretroviral therapy; AST, aspartate aminotransferase; BMI, body mass index; HIV, human immunodeficiency virus; IQR, interquartile range; MSM, men who have sex with men; NA, not applicable; PWH, people with human immunodeficiency virus; WHO, World Health Organization.

with higher waist circumference (102 vs 93 cm; $P < .01$), BMI (26 vs 24 kg/m^2; $P = .02$), and AST (31 vs 28 IU/L; $P = .02$). They were more frequently diabetic (23% vs 5%; $P < .01$), overweight and obese (56% vs 39%; $P = .02$), and more frequently had metabolic syndrome (62% vs 40%; $P = .02$) and hepatic steatosis (27% vs 6%; $P < .01$).

HIV Infection and Elevated LSM

HIV infection was associated with higher odds of elevated LSM (aOR, 1.84 [95% CI, 1.17–2.88]; $P < .001$). The association between HIV infection and elevated LSM increased with age (Figure 3); individuals aged 57–63 years had higher odds of elevated LSM (aOR, 4.35 [95% CI, 1.27–14.88]; $P = .02$) when compared to individuals aged 50–52 years, and the odds were even higher in individuals aged 63–79 years (aOR, 8.67 [95% CI, 2.56–29.35]; $P < .01$).

Factors Associated With Elevated LSM in PWH

In univariate regression analysis, higher age, BMI, waist circumference, ALT, and triglycerides and presence of diabetes and steatosis were all associated with higher odds of elevated LSM in PWH, whereas higher total cholesterol was associated

Table 2. Factors Associated With Liver Fibrosis in People With Human Immunodeficiency Virus and Population Controls

	PWH		Controls	
Factors	Crude OR (95% CI)	*P* Value	Crude OR (95% CI)	*P* Value
Age, y (per decade)	2.39 (1.41–4.06)	<.01	1.67 (1.18–2.37)	<.01
Age groups (quartiles)				
50–52 y	Ref		Ref	
53–57 y	0.68 (.21–2.23)	.53	1.44 (.84–2.47)	.18
58–63 y	2.14 (.81–5.64)	.13	1.27 (.73–2.18)	.4
64–70 y	3.43 (1.33–8.87)	.01	2.08 (1.25–3.45)	<.01
Sex (male vs female)	1.14 (.42–3.06)	.80	2.51 (1.77–3.57)	<.01
White race (no vs yes)	1.06 (.48–2.33)	.89	0.60 (.18–1.92)	.39
Smoking				
Never smoker	Ref		Ref	
Current smoker	1.25 (.49–3.16)	.64	1.24 (.81–1.91)	.32
Previous smoker	1.53 (.69–3.41)	.29	0.90 (.59–1.36)	.62
Alcohol				
None	Ref		Ref	
Within recommendations	0.77 (.31–1.81)	.54	0.74 (.44–1.23)	.25
Above recommendations	0.77 (.29–2.02)	.59	0.89 (.53–1.49)	.65
BMI (per 1 kg/m^2)	1.13 (1.03–1.23)	<.01	1.08 (1.04–1.12)	<.01
BMI category (yes vs no)				
Normal weight (18.5–24.9 kg/m^2)	Ref		Ref	
Overweight (25–29.9 kg/m^2)	0.93 (.11–8.26)	.95	1.13 (.75–1.71)	.56
Obese (≥30 kg/m^2)	2.80 (.28–27.91)	.38	2.01 (1.29–3.14)	<.01
Diabetes (yes vs no)	5.81 (2.32–14.51)	<.01	3.92 (2.67–5.76)	<.01
Waist circumference (per 1 cm)	1.06 (103–1.10)	<.01	1.04 (1.02–1.05)	<.01
Abdominal obesity (yes vs no)	1.36 (.60–3.07)	.46	2.16 (1.47–3.15)	<.01
Plasma ALT (per 10 IU/L)	1.26 (1.07–1.47)	<.01	1.21 (1.11–1.33)	<.01
Plasma triglyceride (per 1 mM)	1.21 (1.03–1.42)	.02	1.05 (.90–1.23)	.54
Plasma total cholesterol (per 1 mM)	0.63 (.45–.88)	<.01	0.74 (.63–.86)	<.01
Hepatic steatosis[a]	6.00 (2.37–15.16)	<.01	2.69 (1.93–3.75)	<.01
Duration of HIV infection (per 5 y)	1.19 (.98–1.46)	.08	NA	
MSM HIV transmission (no vs yes)	1.30 (.65–2.60)	.46	NA	
Plasma HIV RNA ≥50 (yes vs no)	0.82 (.10– 6.67)	.86	NA	
Blood CD4 T-cell count (cells/μL)			NA	
<200	Ref			
200–349	0.15 (.01–2.18)	.17		
350–500	0.04 (.00–.51)	.01		
>500	0.07 (.01–.76)	.03		
Previous ART exposure (yes vs no)			NA	
NRTI	1.38 (.47–4.07)	.56		
NNRTI	0.79 (.41–1.51)	.47		
Protease inhibitor	1.22 (.64–2.35)	.54		
Integrase inhibitor	0.37 (.08–1.58)	.18		
Thymidine analogue	1.74 (.80–3.79)	.16		
Stavudine	1.53 (.74–3.17)	.25		
Zidovudine	1.69 (.80–3.57)	.17		
Didanosine	2.57 (1.29–5.12)	<.01		
Cumulative ART exposure (per 5 y)			NA	
NRTI	1.00 (.98–1.02)	.98		
NNRTI	0.94 (.66–1.34)	.74		
Protease inhibitor	1.08 (.86–1.35)	.5		
Integrase inhibitor	2.88 (.95–8.70)	.06		
Thymidine analogue	1.20 (.78–1.84)	.4		
Stavudine	1.08 (.85–1.38)	.54		
Zidovudine	1.02 (.93–1.11)	.72		
Didanosine	1.08 (.44–2.70)	.86		

Abbreviations: ALT, alanine aminotransferase; ART, antiretroviral therapy; BMI, body mass index; CI, confidence interval; HIV, human immunodeficiency virus; MSM, men who have sex with men; NA, not applicable; NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; OR, odds ratio; PWH, people with human immunodeficiency virus.

[a]Moderate to severe hepatic steatosis assessed by computed tomographic scan (Copenhagen Co-Morbidity in HIV Infection Study) or ultrasound (Rotterdam Study).

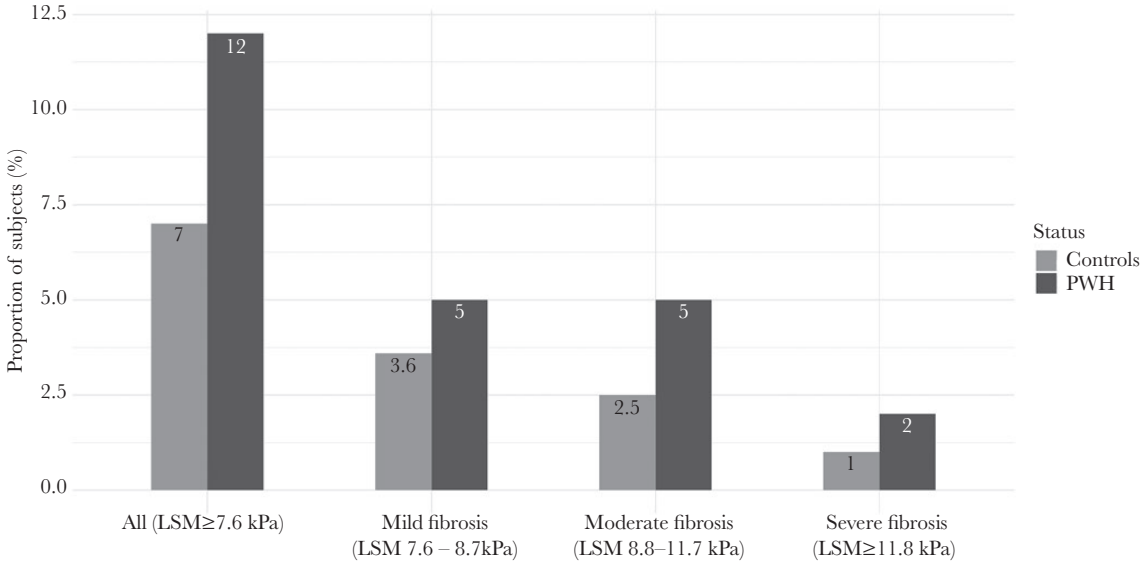

Figure 2. Proportion of subjects with fibrosis in people living with human immunodeficiency virus (dark gray) compared to population controls (light gray). Abbreviations: LSM, liver stiffness measurement; PWH, people with human immunodeficiency virus.

with lower odds of elevated LSM (Table 2). CD4 T-cell count >350 cells/μL was associated with lower odds of elevated LSM, while previous exposure (but not cumulative exposure time) to ddI was associated with higher odds of elevated LSM (OR, 2.57 [95% CI, 1.29–5.12]; $P < .01$) in univariate analyses. Neither duration of HIV infection, route of HIV transmission, plasma HIV RNA, nor previous or cumulative exposure to nucleoside reverse transcriptase inhibitors, nonnucleoside reverse transcriptase inhibitors, protease inhibitors, integrase inhibitors, thymidine analogues, stavudine, or zidovudine was associated with elevated LSM and thus were not tested in multivariate analysis.

In multivariate analyses, higher age, ALT, and BMI were independently associated with elevated LSM in PWH (Figure 4). The effect of previous exposure to ddI remained statistically significant in multivariate analyses (aOR, 2.26 [95% CI, 1.01–5.06];

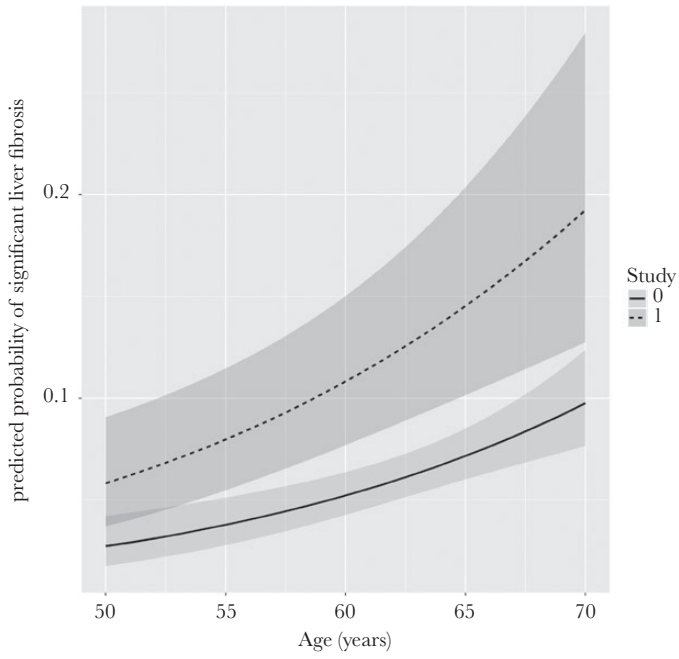

Figure 3. Predicted probability of significant liver fibrosis according to age in people living with human immunodeficiency virus without viral hepatitis (dotted line) compared to population controls (solid line). The predicted probabilities are adjusted by age and plasma alanine aminotransferase, triglycerides, and cholesterol.

P = .04) in the total population of PWH. To test whether this was an independent effect of ddI exposure or an effect of longer duration of HIV infection, we tested the effect of ddI in a subgroup of PWH with comparable duration of HIV infection (≥20 years). In this population, 62 PWH were previously exposed to ddI and 95 PWH were not exposed to ddI (median duration of HIV infection, 25 vs 27 years; P = .13). Although statistically nonsignificant, the effect of ddI exposure remained associated with higher odds of elevated LSM in univariate analysis and after adjustment for sex and age (OR, 2.26 [95% CI, .92–5.53] and aOR, 2.26 [95% CI, .90–5.63], respectively; both P = .08).

DISCUSSION

In this cross-sectional study of 342 unselected PWH aged 50–70 years without viral hepatitis, we show that elevated LSM, a surrogate marker of liver fibrosis, was more prevalent in PWH compared to population controls. Interestingly, a positive HIV status in individuals without viral hepatitis was independently associated with higher odds of elevated LSM. Moreover, age, higher BMI, and plasma ALT were associated with elevated LSM, as well as previous exposure to ddI.

Our results are comparable with previous studies, where the prevalence of liver fibrosis has been reported to range from 8% to 18% in adult PWH without viral hepatitis when assessed by transient elastography [6, 18, 30–36]. Two studies included an HIV-negative comparator group. Lui et al found a prevalence of significant liver fibrosis in 14% of PWH monoinfected compared to 3% of HIV-uninfected controls in a cohort from Hong Kong [18], and Stabinski et al reported a prevalence of fibrosis of 18% in PWH compared to 11% in uninfected controls in a cohort from Uganda [19]. However, these studies were conducted in Asian and African settings and results may not be comparable to a European setting. Furthermore, Stabinski et al included individuals with HBV, which may contribute to the fibrogenesis, and no information on HCV was provided. Interestingly, an independent association between HIV infection and significant liver fibrosis was identified in these studies as well as in ours, suggesting that HIV itself may play a role in the pathogenesis of liver fibrosis. Several studies have demonstrated a direct effect of HIV on hepatic cells. Hepatic fibrogenesis may be induced by HIV entering the hepatic stellate cells [37], oxidative stress and hepatic apoptosis may be triggered by the HIV gp120 signaling pathway [38], and immune-mediated liver injury may be triggered by HIV through alterations of the functions of the stellate cells and Kupffer cells [39]. However, future studies are needed to directly link HIV-induced alterations in the hepatic cells to liver fibrosis development.

HIV-associated factors may also contribute to the development of liver fibrosis. Although duration of HIV infection, low CD4 T-cell counts, high plasma HIV RNA, and route of HIV transmission were not associated with significant liver fibrosis, previous exposure to ddI was independently associated with higher odds of fibrosis. The association persisted in PWH with comparable duration of HIV infection. This finding supports the existing literature from both HIV-monoinfected and HIV/HCV-coinfected individuals, where ddI has been associated with liver fibrosis, variceal bleeding, noncirrhotic portal hypertension, cirrhosis, and end-stage liver disease [15, 16]. Several mechanisms for this potential hepatotoxic effect of ddI have been proposed and include mitochondrial toxicity, hepatic steatosis, and hepatocellular injury [13]. These findings emphasize that PWH without viral hepatitis who were previously exposed to ddI should be monitored for their liver function including development of liver fibrosis.

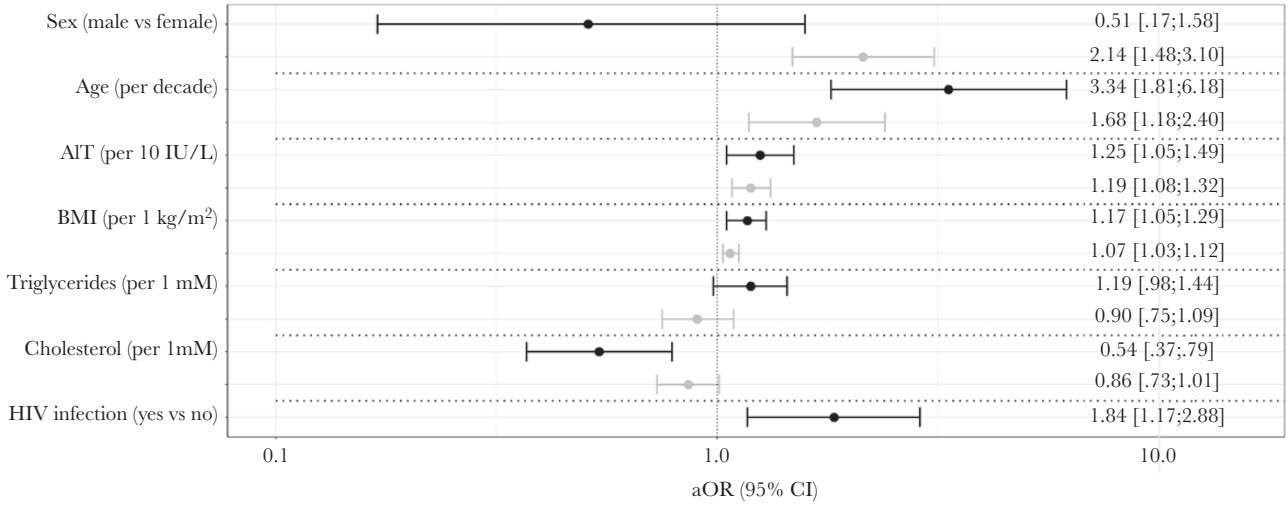

Figure 4. Factors associated with significant liver fibrosis in people living with human immunodeficiency virus (black) and population controls (gray). Adjusted odds ratios with 95% confidence intervals assessed by multivariate logistic regression model adjusted for sex, age, alanine aminotransferase, body mass index, triglycerides, and cholesterol. Abbreviations: ALT, alanine aminotransferase; aOR, adjusted odds ratio; BMI, body mass index; CI, confidence interval; HIV, human immunodeficiency virus.

Age was associated with significant liver fibrosis, and the association between HIV infection and liver fibrosis increased with age, although this may partly be explained by the fact that older PWH tended to have been treated with more hepatotoxic agents no longer used. In the general population, the pathogenesis of nonalcoholic steatohepatitis (NASH) and liver fibrosis is described as a "multiple-parallel hit" model [40]. In short, environmental factors (eg, diet, sedentary lifestyle), metabolic factors (eg, obesity, insulin resistance), and genetic factors (eg, PNAPL3) contribute to lipid accumulation of the hepatocyte [41], production of proinflammatory cytokines (eg, tumor necrosis factor alpha [TNF-α], interleukin 6 [IL-6]), activation of Kupffer cells, and secretion of inflammatory cytokines (eg, TNF-α, interleukin 1β [IL-1β]). Kupffer cells and recruited innate immune cells may induce inflammation in the liver, and IL-1β secretion in particular plays a crucial role in the progression of NAFLD/NASH. Eventually, hepatic stellate cells are activated by, for example, proinflammatory cytokines and IL-1β, resulting in fibrogenesis in the liver parenchyma. In PWH, increased levels of proinflammatory cytokines (including TNF-α, IL-6, and IL-1β) have been demonstrated as well as reduced production of naive CD4 T cells, increased numbers of late differentiated $CD4^+$ and $CD8^+$ T cells, and shortened telomere length [42, 43]. Thus, the immune profile of PWH is similar to the aging and immunosenescent immune profiles as in NASH and may contribute to both increased inflammation and increased fibrogenesis in the liver. Interestingly, ALT and AST levels were higher in PWH compared to uninfected controls and have been linked to liver fibrosis by several study groups including ours [6, 44]. Prospective studies are needed to explore these age-specific differences in more detail and to characterize specific phenotypes of PWH at risk of liver fibrosis.

Excessive alcohol consumption and drug use are known causes of liver disease. In this study, fewer PWH reported excessive alcohol consumption compared to population controls, and no association was found between alcohol consumption and liver fibrosis in either of the 2 populations. Individuals with HBV and HCV coinfection were excluded from the analysis, excluding many individuals with potential drug use. Thus, these adverse lifestyle behaviors do not seem to contribute to the high prevalence of fibrosis observed in PWH.

Higher BMI was independently associated with higher odds of significant liver fibrosis. Given the close correlation between high BMI and hepatic steatosis, it is possible that liver fibrosis is at least partly induced by the NAFLD pathway. However, the number of overweight and obese individuals as well as the prevalence of hepatic steatosis was highest in population controls, a finding that is consistent with previous studies [45, 46]. Thus, one would have expected a higher prevalence of liver fibrosis in population controls. However, the effect of hepatic steatosis on liver fibrosis in PWH seemed stronger than in population controls, and suggests that a positive HIV status may induce a pathway of synergistic effects leading to accelerated fibrogenesis [47]. Importantly, different performance characteristics of CT scans and ultrasonography may also contribute to the differences observed. Interestingly, a higher total cholesterol level was associated with lower odds of liver fibrosis after adjustments. Whether this association is related to residual confounding or if the total cholesterol serves as a proxy for a beneficial effect of lipid-lowering therapy or a healthier lifestyle is unclear and should be explored in future studies.

To our knowledge, this is the largest study to date to evaluate liver fibrosis by transient elastography in an unselected population of monoinfected PWH with a large comparator group of population controls. The study has some limitations. The lack of liver biopsies limits accurate fibrosis diagnostic and staging. PWH and population controls were included from different countries, which may introduce bias due to methodological differences and country-specific differences. Different imaging techniques were used for steatosis assessment in the 2 cohorts, which limits the comparability due to different performance characteristics. An XL probe was available, and a higher failure rate was observed in the Rotterdam study, which may have affected the results. Finally, the cross-sectional study design limits the ability to infer on causality, and residual confounding such as genetic and ethnical variability and microbial translocation, among others, cannot be precluded.

In conclusion, HIV infection was independently associated with higher odds of elevated LSM, and the proportion of individuals with elevated LSM was higher in PWH compared to population controls. Higher age, BMI, ALT, and previous exposure to ddI were independently associated with elevated LSM, suggesting that liver fibrosis may be induced by a combination of hepatotoxic drugs, aging, and steatosis. Future studies on the pathogenesis of liver fibrosis in PWH without viral hepatitis are warranted.

Notes

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