OPEN

Prevalence and risk factors of nonalcoholic steatohepatitis with significant fibrosis in people with HIV

Maurice Michel[a,b], Christian Labenz[a,b], Alisha Wahl[a,b], Malena Anders[a,b], Angelo Armandi[a,b], Yvonne Huber[a,b], Peter R. Galle[a,b], Martin Sprinzl[a,b] and Jörn M. Schattenberg[a,b]

Objective: Metabolic risk factors and nonalcoholic fatty liver disease (NAFLD) in people with HIV (PWH) have been increasing. Patients exhibiting the inflammatory subtype nonalcoholic steatohepatitis (NASH) are at increased risk of liver-related complications. Therefore, the aim was to investigate the prevalence of NASH with significant fibrosis in PWH using noninvasive tests (NITs).

Design: In this prospectively enrolling cohort study, 282 PWH were explored for hepatic steatosis, fibrosis and steatohepatitis using vibration-controlled transient elastography (VCTE) and the Fibroscan-AST (FAST) score.

Methods: On the basis of controlled attenuation parameter (CAP; dB/m) and liver stiffness measurement (LSM; kPa), patients were categorized according to the presence of steatosis (≥275 dB/m) and significant fibrosis (≥8.2 kPa). The FAST score was calculated according to established cut-offs.

Results: The prevalence of hepatic steatosis in this cohort was 35.5% ($n = 100$) with 75 (75%) of these patients fulfilling the criteria of NAFLD. The prevalence of significant fibrosis (≥ F2) was 6.7% ($n = 19$). The FAST score identified a total of 32 (12.3%) patients with a cut-off greater than 0.35, of whom 28 (87.5%) PWH qualified as NASH. On multivariable analysis, waist circumference was a predictor of hepatic steatosis and type 2 diabetes was a predictor of significant fibrosis. Type 2 diabetes and ALT remained independent predictors of a FAST score greater than 0.35.

Conclusion: NASH with significant fibrosis is highly prevalent among PWH. The FAST score may be helpful to identify patients at risk for significant liver disease.

AIDS 2022, **36**:1665–1674

Keywords: Fibroscan-AST score, HIV, metabolic syndrome, nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, vibration-controlled transient elastography

Introduction

HIV is one the most common infectious diseases worldwide. Although associated with a poor prognosis during earlier decades, significant improvements have been achieved with the introduction of long-term antiretroviral therapy (ART) [1]. However, despite major accomplishments in the life expectancy of people with HIV (PWH), liver-related comorbidities are the second leading cause of mortality [2]. Although the impact of

[a]Metabolic Liver Research Program, and [b]I. Department of Medicine, University Medical Centre Mainz, Germany.

Correspondence to Prof. Dr. Jörn M. Schattenberg, Metabolic Liver Research Program, I. Department of Medicine, University Medical Centre Mainz, Germany.

Tel: +49 6131 17 6074; fax: +49 6131 17 477282; e-mail: joern.schattenberg@unimedizin-mainz.de

Received: 26 January 2022; revised: 3 June 2022; accepted: 12 June 2022.

DOI:10.1097/QAD.0000000000003312

chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) coinfection as the underlying cause of chronic liver diseases is well established, the prevalence of metabolic risk factors and the number of PWH developing nonalcoholic fatty liver disease (NAFLD) has been increasing in recent years [3–5]. In the general population, NAFLD has become one of the most common chronic liver diseases, with a prevalence of approximately 25% globally [6] bearing a high economic impact [7]. NAFLD can progress to a more inflammatory stage, termed nonalcoholic steatohepatitis (NASH), with a significant risk of liver fibrosis, cirrhosis, and even liver cancer [8]. In this context, liver fibrosis has become the most important predictor of mortality [9].

Overall, the metabolic syndrome, a major risk factor for NAFLD, has a higher prevalence in PWH compared with the general population [10]. Both HIV infection itself and ART have been implicated as steatogenic factors [11]. In this context, a recent study suggested that tenofovir alafenamide (TAF) was associated with more weight gain and less favourable metabolic outcomes compared with tenofovir disoproxil fumarate (TDF), independent of age [12]. A meta-analysis, however, could not detect a higher risk of NAFLD on ART [3]. Overall, only few studies have investigated the burden of hepatic steatosis and fibrosis in PWH and data on NASH with significant fibrosis is limited [13,14]. One recent analysis exploring hepatic steatosis in HIV-monoinfected and HCV-coinfected patients in Germany did not report data on hepatic fibrosis [15].

Liver biopsy is not feasible as a screening tool and will be replaced by noninvasive tests (NITs) and biomarkers [16]. Importantly, the histological disease stage is considered as an indication for antisteatotic, antifibrotic, and anti-inflammatory drugs in NASH that are currently being developed. Vibration-controlled transient elastography (VCTE) has been established and validated as an ultrasound-based test to screen for hepatic steatosis and fibrosis [17,18]. More recently, the Fibroscan-AST (FAST) score has been introduced to screen patients at risk of nonalcoholic steatohepatitis with significant fibrosis [19]. For the utility of a diagnostic test, the prevalence of the condition is of importance for the positive-predictive value (PPV). Therefore, the aim of this study was first to analyse the prevalence, risk factors, and independent predictors of NAFLD and significant fibrosis and secondly investigate the prevalence of steatohepatitis by means of VCTE and the FAST score in PWH.

Methods

Study design and population

A total of 282 individuals with an HIV infection were enrolled between 2018 and 2021 in this noninterventional, cross-sectional and prospectively enrolling monocentric cohort study (FLASH, Prevalence of Advanced Fibrosis in Patients Living With HIV, NCT04066608) after informed consent was obtained at the outpatient clinic of the Metabolic Liver Research Program at the University Medical Centre Mainz in Germany. Participants with HIV 18 years of age or older were included into this study. HBV or HCV coinfection status was assessed. Patients with an active malignancy were excluded. Clinical assessment was used to categorize patients according to the amount of alcohol consumption. NAFLD (alcohol intake: male patient <20 g/day, female patient <10 g/day) or alcohol-related liver disease (ALD) were defined according to current practice guidelines [20]. The metabolic syndrome and its associated risk factors including waist circumference (men ≥ 94 cm, women ≥ 80 cm), diabetes mellitus (previously diagnosed type 2 diabetes), raised triglycerides (≥ 150 mg/dl, or treatment of this condition), low HDL cholesterol (men <40 mg/dl; women <50 mg/dl) and arterial hypertension (systolic ≥ 130 mmHg or diastolic ≥ 85 mmHg) were defined according to the criteria of the international diabetes federation (IDF) [21]. In this context, the metabolic syndrome was evident if central obesity according to a waist circumference or a BMI greater than 30 kg/m^2 and any two of the previously mentioned factors were present. BMI [kg/m^2; weight (kg)/height^2 (m^2)] and waist circumference (cm) were assessed as well as laboratory values were obtained at study inclusion. Assessment of medical and treatment history were retrieved from the electronic healthcare records.

Assessment of hepatic steatosis, fibrosis and steatohepatitis

Hepatic steatosis (CAP, dB/m) and fibrosis (LSM, kPa) were noninvasively assessed using VCTE (FibroScan 430 mini; SMART Exam was introduced in 2020; Echosens, Paris, France) [22]. In the majority of patients, the M probe (91.1%; $n = 257$) was used. In cases of severe obesity, the XL probe (8.9%; $n = 25$) was used. The success rate was 93.4%. A total of 20 participants had to be excluded from the study because of invalid measurements using VCTE. The recently suggested cut-off above 275 dB/m in the EASL guidelines on noninvasive tests was used to diagnose hepatic steatosis [23]. For the assessment of liver fibrosis, a cut-off value of at least 8.2 kPa was considered a significant fibrosis ($\geq$F2) [17]. Measurement of LSM was considered reliable if the interquartile range (IQR) was less than 30% and the success rate greater than 70% [24]. To identify patients at risk of more progressive and inflammatory fatty liver disease, that is, NASH, the FAST score with cut-off values of greater than 0.35 and at least 0.67, respectively, were applied [19]. Briefly, the FAST score combines LSM, CAP and AST blood values into a specific equation to rule in NASH and has been validated in non-HIV-infected individuals [19]. Due to missing AST blood values, the FAST score was available in 93.3% ($n = 263$). Additional surrogate scores of advanced fibrosis included

the following: NAFLD fibrosis score (NFS) and Fibrosis-4 (FIB-4) score with published cut-off values to rule in or rule out advanced fibrosis [25–27].

Ethics

All patients provided written informed consent. The study was conducted according to the ethical guidelines of the 1975 Declaration of Helsinki (sixth revision, 2008). The study protocol was approved by the ethics committee of the Landesärztekammer Rhineland-Palatinate [Nr. 873.199.10 (7208)].

Statistical analysis

Descriptive analysis of data is expressed as median values with interquartile ranges (IQR 25th–75th). The Mann–Whitney *U* rank test was used to compare groups and to calculate differences between two groups with continuous variables. Categorical variables are presented as frequencies and percentages. For the comparison of two or more patient-groups, a chi-squared test was applied. All tests were two-tailed, statistically significant values were defined as *P* less than 0.05. All variables with *P* less than 0.05 and the clinical parameters age, sex and alcohol intake were then included into a multivariable logistic regression model to examine associations with NAFLD, fibrosis (LSM $\geq$8.2 kPa) and a FAST score greater than 0.35. Due to the large number of tests, *P* values should be interpreted with caution and in connection with effect estimates. For all data analysis and statistical tests, IBM SPSS Statistic Version 23.0 (IBM Corp., Armonk, New York, USA) was used. For all figures, Microsoft Excel 2016 (Microsoft Corp., Redmond, Washington, USA) was used.

Results

Demographic and clinical characteristics

A total of 282 PWH fulfilling the inclusion and exclusion criteria were analysed. The majority of patients were men ($n = 198$, 70.2%). The median age was 51 years (IQR 42–58) and the median duration of established HIV infection was 12 years (IQR 6–20). The median BMI (kg/m^2) was 25 (IQR 22.3–28.1), and 15.6% ($n = 44$) were obese according to a BMI greater than 30 kg/m^2. The median waist circumference (cm) was 96 (IQR 86.8–104), and a total of 40.8% of male and 25.2% of female participants showed a waist circumference above 94 and 80 cm, respectively. The criteria of the metabolic syndrome were fulfilled in 72 (25.5%) individuals at study inclusion. Alcohol consumption in most individuals was less than 20 g/day (male) and less than 10 g/day (female). Most PWH received NRTI as part of their ART with a majority receiving TAF-containing combinations. The majority showed a controlled HIV disease as indicated by HIV RNA below the threshold and CD4$^+$ cells above the threshold of 500 cells/μl. Baseline characteristics and laboratory results are summarized in Table 1.

Prevalence of hepatic steatosis and fibrosis in people with HIV

The median CAP (dB/m) was 248 (IQR 214.8–300). According to a cut-off value of at least 275 dB/m, a total of 100 PWH (35.5%) presented with hepatic steatosis (Supplementary Figure 1a, http://links.lww.com/QAD/C567). On the basis of the presence of hepatic steatosis according to a CAP greater than 275 dB/m and alcohol intake (g/day), 76 (27%) PWH were considered to have NAFLD, whereas 13 (4.6%) qualified for alcohol-related liver disease (ALD). Because of missing data on alcohol intake, a total of 11 PWH remained undefined.

The median LSM (kPa) in the study cohort was 4.6 (IQR 3.8–5.7). A total of 263 (93.3%) PWH had a LSM below 8.2 kPa excluding relevant fibrosis, whereas 19 (6.7%) participants presented with significant fibrosis (F2; cut-off $\geq$ 8.2 kPa) (Supplementary Fig. 1b, http://links.lww.com/QAD/C567).

Comparison of people with HIV characteristics with nonalcoholic fatty liver disease and fibrosis

A high prevalence ($n = 38$, 38.2%) of the metabolic syndrome and its associated risk factors was seen in PWH with NAFLD. The median BMI (kg/m^2) was significantly higher if NAFLD was present [27.7 (IQR 25.4–31.6)]. Obesity (BMI >30) was more prevalent in PWH with NAFLD. Median waist circumference (cm) was higher in NAFLD [102 (IQR 97–113)], and a higher prevalence of a waist circumference (cm) greater than 94 (men; $n = 49$) and greater than 80 (women; $n = 16$), respectively, was seen. The blood levels of ALT, triglycerides and uric acid showed higher median values in NAFLD. PWH with significant fibrosis (LSM $\geq$8.2 kPa) were older [55 (IQR 50–63)] and showed a longer disease duration (time since diagnosis). Furthermore, a higher median BMI (kg/m^2) [27.1 (IQR 24.5–34.7)] and waist circumference (cm) [104 (95–117.3)] were observed. Liver enzymes were significantly higher in PWH presenting with fibrosis [ALT: 36 (IQR 24–59); AST: 35 (IQR 28–40.5)]. In contrast, HIV-related parameters were not significantly different between these groups. Numerically, a higher proportion of PWH and NAFLD were treated with TAF compared with TDF. A comparison of patients based on the presence of NAFLD and significant fibrosis is shown in Table 2. Furthermore, anti-HBc and anti-HCV were positive in $n = 5$ and $n = 2$ in the subgroup with a LSM at least 8.2 kPa, respectively. The NFS identified a higher number of PWH at risk of fibrosis than the FIB-4. The surrogate scores of advanced fibrosis are shown in Supplementary Table 1, http://links.lww.com/QAD/C567.

Clinical predictors of nonalcoholic fatty liver disease and fibrosis in people with HIV

A multivariable logistic regression model was built including all clinical variables with a *P* less than 0.05 in the univariable analysis as well as the clinical parameters

Table 1. Baseline characteristics and metabolic profile of people with HIV.

Variable	Total cohort ($n = 282$)
Age in years	51 (42; 58)
Time since diagnosis (years) ($n = 268$)	12 (6; 20)
Male	198 (70.2)
Female	84 (29.8)
VCTE	
CAP (dB/m)	248 (214.8; 300)
LSM (kPa)	4.6 (3.8; 5.7)
Metabolic comorbidities	
BMI (kg/m^2) ($n = 272$)	25 (22.3; 28.1)
Underweight (<18.5 kg/m^2)	9 (3.2)
Normal weight (18.5 to <25 kg/m^2)	126 (44.7)
Overweight (25 to <30 kg/m^2)	93 (33)
Obese (>30 kg/m^2)	44 (15.6)
Waist circumference (cm) ($n = 270$)	96 (86.8; 104)
Male >94 cm	115 (40.8)
Female >80 cm	71 (25.2)
Type 2 diabetes ($n = 261$)	30 (10.6)
Total cholesterol >200 mg/dl ($n = 181$)	91 (32.3)
Triglycerides >150 mg/dl ($n = 175$)	71 (25.2)
HDL-cholesterol: male <40 mg/dl	39 (13.8)
female <50 mg/dl ($n = 149$)	13 (4.6)
Arterial hypertension ($n = 268$)	85 (30.1)
Metabolic syndrome	72 (25.5)
Alcohol consumption ($n = 282$)	
Male >20 (g/day)	No: 165 (90.2); yes: 22 (9.8)
Female >10 (g/day)	No: 68 (90.7); yes: 7 (9.3)
Laboratory values	
ALT (U/l) ($n = 263$)	24 (18; 32)
AST (U/l) ($n = 263$)	26 (23; 32)
Triglycerides (mg/dl) ($n = 175$)	131 (91; 190)
Cholesterol (mg/dl) ($n = 181$)	200 (174; 226)
HDL (mg/dl) ($n = 133$)	48 (39; 57.5)
LDL (mg/dl) ($n = 133$)	120 (102; 144)
HbA1c (%) ($n = 133$)	5.4 (5.1; 5.7)
Uric acid (mg/dl) ($n = 146$)	5.5 (4.8; 6.4)
Hepatitis serology	
Anti-HCV positive ($n = 134$)	8 (2.8)
HBsAg positive ($n = 129$)	4 (1.4)
HIV-related parameters and medication (ART)	
CDC stage ($n = 185$)	A: 80 (43.2); B: 47 (16.7); C: 58 (20.6)
HIV RNA ($n = 274$)	
Above threshold	102 (36.2)
Below threshold	172 (61)
CD4$^+$ (cells/μl) ($n = 266$)	723.5 (515.8; 910.8)
>500 cells/μl	207 (73.4)
NRTI	253 (89.7)
TAF as part of ART	179 (63.5)
TDF as part of ART	31 (11)
NNRTI	62 (22)
PI	41 (14.5)
INSTI	188 (66.7)
DTG	68 (36.2)
TAF and INSTI	124 (44)

Data are expressed as numbers, median, percentage (%) or interquartile ranges (IQR 25th–75th). ALT, alanine-aminotransaminase; ART, antiretroviral therapy; AST, aspartate-aminotransaminase; CDC, Centres for Disease Control and Prevention; DTG, dolutegravir; HDL, high-density lipoprotein; INSTI, integrase inhibitors; LDL, low-density lipoprotein; TAF, tenofovir alafenamide; TDF, tenofovir disoproxil fumarate; NRTI, nucleoside reverse-transcriptase inhibitors; NNRTI, nonnucleotide reverse transcriptase inhibitors; PI, protease inhibitors.

age and sex to assess predictive factors of NAFLD and fibrosis in this cohort. Waist circumference (OR = 1.1, 95% CI 1.067–1.135, $P < 0.001$) remained the only independent predictor of NAFLD. The variable type 2 diabetes (OR = 5.056, 95% CI 1.386–18.44, $P = 0.014$) remained the only independent predictor of significant fibrosis (Table 3). In turn, HIV-related parameters showed no association with NAFLD and fibrosis in the univariable analysis (data not shown).

Prevalence of steatohepatitis with significant fibrosis in people with HIV

To identify PWH with steatohepatitis and at least significant fibrosis, the FAST score was explored ($n = 263$). In total, the FAST score was above the cut-off more than 0.35 in 32 (12.1%) of the patients. Of these, 9.1% ($n = 24$) of the cohort were in the intermediate range between greater than 0.35 and less than 0.67, whereas 3% ($n = 9$) ruled-in applying the cut-off of at least 0.67 (Supplementary Figure 2, http://links.lww.com/QAD/C567). In turn, 231 (87.8%) of PWH were FAST score negative. In the 32 individuals with a FAST score greater than 0.35, $n = 28$ were classified as NASH, whereas $n = 2$ qualified for alcoholic steatohepatitis (ASH). Due to missing data on alcohol intake, a total of two PWH remained undefined.

Comparison of people with HIV characteristics with a FAST score greater than 0.35 and at least 0.67

The majority of PWH with a FAST score greater than 0.35 were of male sex (84.4%). Metabolic comorbidities were generally more prevalent in PWH with a FAST score greater than 0.35. The BMI (kg/m^2) was significantly higher in both groups (>0.35 and ≥0.67) in comparison to less than 0.35, and more patients were obese (>30 kg/m^2). Median waist circumference was also higher in both groups, and particularly more men showed a waist circumference of more than 94 cm. Likewise, a diagnosis of type 2 diabetes at study inclusion was more prevalent in both groups. Liver-related blood values (ALT, AST) as well as triglycerides and HbA1c (%) were significantly higher in PWH and a FAST score greater than 0.35. No major differences were seen in the analysis of HIV-related parameters within these groups. A comparison of these characteristics is shown in Table 4. Additionally, in the group with a FAST score greater than 0.35, $n = 7$ were anti-HBc-positive and $n = 2$ anti-HCV-positive.

Clinical predictors of a FAST score greater than 0.35 in people with HIV

Next, clinical predictors of a FAST score greater than 0.35 were analysed by means of univariable and multivariable logistic regression models. Blood levels of AST were excluded from the analysis to avoid multicollinearity as it is a part of the FAST score. Type 2 diabetes (OR = 17.31, CI 4.188–71.52, $P < 0.001$) and

Table 2. Clinical characteristics of people with HIV with and without NAFLD and fibrosis.

Variable	No steatosis ($n = 182$; 64.5%) n (% or IQR)	NAFLD ($n = 76$; 27%) n (% or IQR)	P value	No fibrosis ($n = 263$; 93.3%) n (% or IQR)	Fibrosis ($n = 19$; 6.7%) n (% or IQR)	P value
Age (years)	50 (41–57)	54 (49–60)	**0.011**	51 (41–58)	55 (50–63)	0.061
Time since diagnosis (years)	12 (6–19)	14 (6–23)	0.475	12 (6–19)	21 (7–27)	0.061
Sex			**0.025**			0.389
Male	118 (64.8)	60 (78.9)		183 (69.6)	15 (78.9)	
Female	64 (35.2)	16 (21.1)		80 (30.4)	4 (21.1)	
Metabolic comorbidities						
BMI (kg/m^2)	23.4 (21.5; 26.4)	27.7 (25.4–31.6)	**<0.001**	24.9 (22.3–27.9)	27.1 (24.5–34.7)	**0.039**
Obese (>30 kg/m^2)	12 (6.8)	26 (35.1)	**<0.001**	37 (14.6)	7 (38.8)	**0.007**
Waist circumference (cm)	91 (84–100)	102 (97–113)	**<0.001**	96 (86–103)	104 (95–117.3)	**0.001**
Male >94	52 (44.1)	49 (81.6)	**<0.001**	102 (55.7)	13 (86.6)	**0.020**
Female >80	51 (79.7)	16 (100)	**0.049**	68 (85)	3 (75)	0.589
Type 2 diabetes	15 (8.7)	11 (16.9)	0.071	24 (9.8)	6 (40)	**<0.001**
High triglycerides	31 (29.2)	33 (61.1)	**<0.001**	66 (40)	5 (50)	0.532
High cholesterol	51 (45.9)	40 (61.1)	0.067	87 (50.6)	4 (44.4)	0.720
Arterial hypertension	47 (26.5)	38 (39.1)	0.053	77 (30.5)	8 (55)	0.105
Metabolic syndrome	34 (18.6)	38 (38.2)	**0.001**	64 (24.3)	8 (42.1)	0.086
Laboratory values						
ALT (U/l)	22 (17–30)	28 (18.3–38)	**0.001**	23 (17.8–31)	36 (24–59)	**0.001**
AST (U/l)	26 (22–30)	26 (23–32.8)	0.507	26 (22–31)	35 (28–40.5)	**0.001**
Triglycerides (mg/dl)	108.5 (82.8–157.3)	183 (123.8–246.3)	**<0.001**	131 (86.5–188)	154.5 (106–242.5)	0.337
Cholesterol (mg/dl)	196 (173–222)	207 (182.8–228)	0.147	200 (173.3–226)	186 (178–217)	0.669
HDL (mg/dl)	48 (41–59.5)	43.5 (38–52)	0.071	48 (39–58.3)	45 (40–53)	0.614
LDL (mg/dl)	120 (102.5–144.5)	122.5 (103.5–143.5)	0.589	120 (102–144)	127 (114–148)	0.789
HbA1c (%)	5.4 (5.1–5.6)	5.5 (5.2–6.1)	0.101	5.4 (5.1–5.7)	5.7 (5.5–7.0)	**0.024**
Uric acid (mg/dl)	5.3 (4.6–6.3)	6.0 (5.1–6.9)	**0.013**	5.5 (4.8–6.3)	6.9 (4.7–8.3)	0.230
HIV-related parameter						
HIV RNA at inclusion			0.684			0.297
Above threshold	67 (37.9)	26 (35.1)		98 (37.9)	4 (25)	
Below threshold	110 (62.1)	48 (64.8)		160 (62.1)	12 (75)	
CD4$^+$ (cells/μl)	695 (515.8–881.5)	802.5 (533.5–1019)	0.114	723 (515.8–897)	769.5 (436.8–1105.5)	0.685
>500 CD4$^+$ cells/μl	135 (74.2)	72 (72.4)	0.764	195 (74.1)	12 (63.2)	0.295
CDC C	36 (31)	22 (25)	0.426	53 (31.3)	5 (31.3)	0.993
NRTI (TAF vs. TDF)			0.369			0.303
TAF	112 (83.6)	52 (88.1)		170 (85.9)	9 (75)	
TDF	22 (16.4)	7 (11.9)		28 (14.1)	3 (25)	
INSTI	120 (66.6)	52 (70.3)	0.577	175 (67.6)	13 (72.2)	0.683
TAF and INSTI	73 (76)	39 (78)	0.790	119 (76.3)	5 (71.4)	0.768

Data are expressed as numbers, median, percentage (%) or interquartile ranges (IQR 25th–75th). ALT, alanine-aminotransaminase; AST, aspartate-aminotransaminase; CAP, controlled attenuation parameter; HDL, high-density lipoprotein; INSTI, integrase inhibitors; LDL, low-density lipoprotein; LSM, liver stiffness measurement; NAFLD, nonalcoholic fatty liver disease; NRTI, nucleoside reverse transcriptase inhibitors; TAF, tenofovir alafenamide; TDF, tenofovir disoproxil fumarate. Mann–Whitney U test and chi-square test were used to compare continuous and categorical values, respectively. Boldface indicates statistical significance. A P value less than 0.05 was considered statistically significant.

Table 3. Multivariable analyses of predictors for NAFLD and fibrosis in people with HIV.

	NAFLD			Fibrosis		
	Multivariable logistic regression[a]			Multivariable logistic regression[b]		
Variable	OR	CI	*P* value	OR	CI	*P* value
Waist circumference (cm)	1.1	1.067–1.135	**<0.001**			
Type 2 diabetes				5.056	1.386–18.44	**0.014**

Multivariable logistic regression analysis of data is shown. With all factors showing a *P* value less than 0.05 in the univariable analysis and the variables, age and sex, a multivariable regression model was built. Age, sex, BMI and ALT were not predictive of NAFLD. Likewise age, sex, BMI, ALT, AST and alcohol intake were not predictive of fibrosis. Metabolic syndrome was excluded from the multivariable analysis to avoid multicollinearity. The variable triglycerides was excluded because of limited numbers available. Odds ratio (OR) and 95% confidence interval (CI) are shown. Boldface indicates statistical significance. A *P* value less than 0.05 was considered statistically significant. NAFLD, nonalcoholic fatty liver disease.

[a]Multivariable logistic regression: age, sex, BMI, waist circumference, ALT ($n = 230$).

[b]Multivariable logistic regression: age, sex, BMI, waist circumference, ALT, AST, type 2 diabetes, alcohol intake ($n = 212$).

ALT (OR = 1.118, CI 1.070–1.167, $P < 0.001$) were the only independent predictors of a FAST score greater than 0.35 in this cohort (Table 5). HIV-related parameters showed no impact on a FAST score greater than 0.35 in the univariable analysis (data not shown).

Discussion

In this analysis, we used a novel NIT for the detection of NASH with significant fibrosis in PWH. The FAST score was developed and validated in patients with NASH and showed a sensitivity of 90% to rule-out NASH with significant fibrosis using a cut-off of less than 0.35 [19]. In the current study, the FAST score exceeded the lower threshold of 0.35 in 32 (11.3%) individuals, of whom the majority were related to NASH. We observed hepatic steatosis in 35.5% of PWH as assessed by CAP, and most patients presented with a NAFLD. Significant fibrosis (≥8.2 kPa) was detected in 6.7% of PWH.

A number of emerging medical therapies for NASH are under way. The FAST score has been developed to determine those patients that are potential candidates for pharmacotherapy. To date, little data are available on the prevalence of NASH with significant fibrosis in PWH. In this cohort study, we define NASH based on the FAST score in PWH, which identified a similar prevalence of NASH reported in monoinfected HIV patients when using liver histology as a reference [28]. In the same study, ALT levels of at least 36 U/l were identified to have the highest predictive value for NASH, which is in line with our observations. Type 2 diabetes and other metabolic risk factors (hypertension and obesity) are highly associated with NAFLD/NASH and are well known risk factors for liver cirrhosis and hepatocellular carcinoma (HCC) in these patients [29]. Interestingly, type 2 diabetes and ALT remained independent predictors of a FAST score greater than 0.35 in this cohort. The FAST score may additionally aid in the identification of patients at risk for NASH, although future studies are needed to verify these findings.

Cut-off values were chosen according to the recently published guidelines on noninvasive tests to rule-in hepatic steatosis and from a UK-based study for ruling-in significant fibrosis [17,23]. A meta-analysis of HIV and HCV-coinfected patients that analysed hepatic steatosis by liver biopsy reported a prevalence of roughly 50% [30]. Other studies have reported a similar prevalence ranging from 40 to 48% in HCV-coinfected and monoinfected PWH using a lower cut-off of 238 dB/m in cohorts from Spain and Canada, respectively [13,31]. In a study from Brazil, excluding patients with excessive alcohol use, the prevalence of NAFLD remained at 38% applying a lower cut-off of 238 dB/m [32]. According to the ANRS-ECHAM study, which used a liver biopsy as a reference, the optimal cut-off was considered to be greater than 280 dB/m to detect hepatic steatosis in PWH [28]. The estimated overall prevalence of NAFLD in the general population is around 25% globally and 23% in Germany [6,33]. The differences in prevalence of NAFLD in PWH differs across previous studies and may need to be considered with respect to the applied cut-off value. Yet, the prevalence of NAFLD is higher in PWH as this and other studies confirm.

In our analysis, waist circumference remained an independent predictor of NAFLD. Other studies have identified BMI to be the strongest predictor of steatosis [31]. Although BMI showed a strong association, it did not remain an independent predictor of steatosis in comparison to waist circumference in this study. Furthermore, the risk of developing NAFLD increases with both higher BMI and waist circumference [34]. Waist circumference is considered a surrogate of visceral fat and central obesity, and is an integral part of the definition of the metabolic syndrome [21]. Moreover, previous studies have highlighted the close relationship of waist circumference and visceral adiposity with the prevalence of NAFLD [35]. In PWH, central obesity may also be a result of lipodystrophy, which, however, has

Table 4. Comparison of people with HIV characteristics with a FAST-score greater than 0.35 and at least 0.67.

Variable	FAST score <0.35 ($n=231$; 87.8%) *n* (% or IQR)	FAST score >0.35 ($n=32$; 12.2) *n* (% or IQR)	*P* value	FAST score <0.67 ($n=255$; 97%) *n* (% or IQR)	FAST score ≥0.67 ($n=8$; 3%) *n* (% or IQR)	*P* value
Age in years	52 (42–58)	51.5 (44.3–59.5)	0.512	52 (42–58)	52 (46.3–59.3)	0.808
Time since diagnosis (years)	12 (6–20)	11 (5–22.5)	0.790	12 (6–20)	8 (2.5–24)	0.588
Sex			0.052			0.735
Male	156 (67.5)	27 (84.4)		177 (69.4)	6 (75)	
Female	75 (32.5)	5 (15.6)		78 (30.6)	2 (25)	
Metabolic comorbidities						
BMI (kg/m^2)	24.7 (22.2–27.7)	27.8 (25.4–33.7)	**<0.001**	25 (22.3–27.9)	27.5 (24.5–37.7)	**0.049**
Obese (>30 kg/m^2)	31 (13.7)	10 (33.3)	**0.006**	38 (15.3)	3 (50)	**0.050**
Waist circumference (cm)	95 (86; 102)	104.5 (95.8; 116.5)	**<0.001**	96 (86.8; 103.3)	104 (100; 133)	**0.009**
Male >94	86 (55.1)	22 (81.5)	**0.010**	102 (57.6)	6 (100)	**0.038**
Female >80	64 (85.3)	4 (80)	0.746	67 (85.9)	1 (50)	0.160
Type 2 diabetes	16 (7.4)	10 (38.5)	**<0.001**	22 (9.3)	4 (66.6)	**<0.001**
High triglycerides	58 (38.2)	13 (56.5)	0.095	67 (39.8)	5 (57.4)	0.362
High cholesterol	81 (51.3)	10 (45.5)	0.610	91 (52.6)	0	**0.006**
Arterial hypertension	67 (29.9)	11 (39.3)	0.312	74 (30.1)	4 (66.6)	0.055
Metabolic syndrome	56 (24.2)	14 (33.8)	**0.019**	69 (25.5)	5 (62.5)	**0.020**
Alcohol intake (g/day)						
Male			0.259			0.575
Male >20 (g/day)	16 (11.1)	1 (3.8)		16 (9.7)	1 (16.6)	
Male <20 (g/day)	129 (89.9)	25 (96.2)		149 (89.3)	5 (83.3)	
Female			0.296			0.739
Female >10 (g/day)	6 (8.9)	1 (25)		7 (10)	1 (100)	
Female <10 (g/day)	61 (91.1)	3 (75)		63 (90)	0	
Laboratory values						
ALT (U/l)	22 (17–29.3)	50 (36–76.3)	**<0.001**	23 (18–31)	83.5 (44–127)	**<0.001**
AST (U/l)	25 (22–29)	40 (35–52.3)	**<0.001**	26 (22–31)	56 (39.5–125.5)	**<0.001**
Triglycerides (mg/dl)	129 (86–184.5)	157 (109–239)	**0.038**	130.5 (88–189.5)	177 (100–239)	0.431
Cholesterol (mg/dl)	201 (174–226)	186.5 (172.8–223.3)	0.735	201 (174–226.5)	177 (173–183)	**0.021**
HDL (mg/dl)	48 (38.5–58.5)	45.5 (40–51.5)	0.538	48 (39–58)	44.5 (37.3–45.8)	0.251
LDL (mg/dl)	120 (102.5–143)	124 (92.8–168)	0.592	124 (102.5–144.5)	102 (90–125.3)	0.201
HbA1c (%)	5.4 (5.1–5.7)	5.9 (5.5–7.2)	**0.004**	5.4 (5.1–5.7)	6.9 (5.7–7.8)	**0.024**
Uric acid (mg/dl)	5.5 (4.8–6.3)	6.1 (5.1–7.2)	0.123	5.5 (4.8–6.4)	5.2 (4.4–5.5)	0.294
HIV-related parameters						
HIV RNA at inclusion			0.547			0.633
Above threshold	87 (37.8)	10 (32.3)		95 (37.4)	2 (28.6)	
Below threshold	143 (62.2)	21 (67.7)		159 (62.6)	5 (71.4)	
CD4 (cells/μl)	724 (516–905)	764 (549.3–991.5)	0.439	724.5 (516.8–910.8)	816 (669–1193)	0.265
>500 CD4$^+$ cells/μl	174 (75.3)	24 (75)	0.968	192 (75.3)	6 (75)	0.985
CDC C	46 (31.3)	6 (23.1)	0.400	51 (30.7)	1 (14.3)	0.353
NRTI (TAF vs. TDF)			0.299			**0.016**
TAF	150 (85.7)	17 (77.3)		164 (85.9)	3 (50)	
TDF	25 (14.3)	5 (22.7)		27 (14.1)	3 (50)	
INSTI	157 (68.5)	17 (58.6)	0.282	169 (67.3)	5 (71.4)	0.819
TAF and INSTI	106 (77.9)	9 (56.3)	0.056	113 (76.4)	2 (50)	0.226

Data are expressed as numbers, median, percentage (%) or interquartile ranges (IQR 25th–75th). ALT, alanine-aminotransaminase; AST, aspartate-aminotransaminase; HDL, high-density lipoprotein; INSTI, integrase inhibitors; LDL, low-density lipoprotein; NRTI, nucleoside reverse transcriptase inhibitors; TAF, tenofovir alafenamide; TDF, tenofovir disoproxil fumarate. Mann–Whitney *U* test and chi-square test were used to compare continuous and categorical values, respectively. Boldface indicates statistical significance. A *P* value less than 0.05 was considered statistically significant.

Table 5. Multivariable analysis of predictors for a FAST score greater than 0.35 in people with HIV.

	FAST score >0.35		
	Multivariable logistic regression[a]		
Variable	OR	CI	*P* value
Type 2 diabetes	17.31	4.188–71.52	**<0.001**
ALT (U/l)	1.118	1.070–1.167	**<0.001**

Multivariable logistic regression analysis of data is shown. With all factors showing a *P* value less than 0.05 in the univariable analysis and the clinical variables age, sex and alcohol intake, a multivariable regression model was built. Age, sex, BMI, waist circumference and alcohol intake were not predictive of a FAST score greater than 0.35. Metabolic syndrome and AST were excluded to avoid multicollinearity. Odds ratio (OR) and 95% confidence interval (CI) are shown. Boldface indicates statistical significance. A *P* value less than 0.05 was considered statistically significant.
[a]Multivariable logistic regression: age, sex, BMI, waist circumference, ALT, type 2 diabetes, alcohol intake ($n = 212$).

become less common because of advances in modern treatments. In the clinical setting, despite its low costs, the variability of waist measurement across different centres limits its applicability.

Hepatic steatosis can give rise to inflammation and subsequent hepatic fibrosis, which are constituting NASH. In the current cohort, significant fibrosis was seen in 6.7% of PWH as assessed by VCTE (LSM) at a cut-off at least 8.2 kPa (≥F2). Similar results were previously reported in studies from Denmark (12%, >7.6 kPa) [36] and Brazil (10%, >8.7 kPa M probe and >7.2 kPa XL probe) [32]. A study from Germany showed a higher prevalence of 16% using a lower cut-off greater than 7.1 in HCV-coinfected PWH [37]. However, a recent study has shown a poor performance of LSM to detect significant fibrosis (≥F2) at a cut-off of 7.1 kPa in PWH [28]. Importantly, the PPV of LSM to rule-in for significant fibrosis (≥F2) greatly depends on the disease prevalence in the population [17]. In our cohort, type 2 diabetes was an independent predictor of significant fibrosis. Type 2 diabetes is generally known as a major driver of disease progression in patients with NAFLD and especially if fibrosis is present [38].

Despite the strong association of metabolic factors with NAFLD, significant fibrosis and NASH in this cohort, other studies have reported the impact of the HIV infection itself and components of the ART regimen as steatogenic factors [12,15]. These have largely focused on the assessment of weight gain and body composition in the context of ART [12,39], and not hepatic steatosis specifically. In the current analysis, treatment with TAF was not associated with a higher prevalence of hepatic steatosis. In this context, larger and longitudinal conducted studies are required to investigate the impact of ART on hepatic steatosis. The numerically larger number of TAF in patients with NAFLD might potentially be related to the selective prescription of TAF over TDF in patients with metabolic risk factors, which could be detrimental for kidney or bone health.

This study has several limitations. Liver biopsy remains the reference standard for the assessment of hepatic steatosis, fibrosis and steatohepatitis. However, it is an invasive procedure with a relevant risk and costs [40]. MRI may also provide a noninvasive assessment of steatosis with a high accuracy [28] but it is associated with high costs and not routinely available for this purpose. Furthermore, in separating NAFLD from ALD, borderline cases can introduce bias. In PWH, the history of chronic viral infection with HBV and HCV can add to the fibrosis burden in a specific patient. Although we applied the FAST score regardless of alcohol intake, the number of participants with high alcohol consumption was low and did not affect our results. On the contrary, based on the prospective recruitment at a single centre with a high experience of performing VCTE, this data set represents a large and well characterized cohort that carries a high validity.

This study identified metabolic risk factors that may also be addressed by lifestyle interventions in PWH. More importantly, this study highlights the applicability of a NIT to detect NASH with significant fibrosis, and thus, helps to identify patients that are metabolically unhealthy. Addressing these factors may improve the metabolic health and prevent the progression to more severe chronic liver diseases in PWH.

Acknowledgements

M.M. is supported by the Clinician Scientist Fellowship 'Else Kröner Research College: 2018_Kolleg.05'. This study contains parts of the medical thesis of A.W. and M. A. We thank all the patients that participated in this study.

Funding: this research was funded in part by Gilead Sciences.

Authorship: performed research: M.M., A.W., M.A. Contributed to acquisition of data: M.M., C.L., A.W., M. A., A.A., Y.H., M.S., J.M.S.; designed the experiments and analysed the data: M.M., J.M.S.; contributed reagents/materials/analysis tools: P.R.G., J.M.S. Wrote the manuscript: M.M., J.M.S. Revised and edited the

manuscript: M.M., C.L., J.M.S. Statistical analysis: M.M., J.M.S. All authors approved the final version of the manuscript and the authorship list. Guarantor of the article: J.M.S.

Institutional Review Board Statement: the study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of Landesärztekammer Rhineland-Palatine Nr. 873.199.10 (7208).

Informed Consent Statement: informed consent was obtained from all patients involved in the study.

Data availability statement: the data presented in this study are available on request from the corresponding author.

Conflicts of interest

J.M.S. has acted as consultant to Boehringer Ingelheim, BMS, Genfit, Gilead Sciences, Intercept Pharmaceuticals, Madrigal, Novartis, Novo Nordisk, Nordic Bioscience, Pfizer, Roche, Sanofi, Siemens Healthcare GmbH. Research Funding: Gilead Sciences, Boehringer Ingelheim. Speakers Bureau: Falk Foundation MSD Sharp & Dohme GmbH. The other authors declare that they have no competing interests.

References

1. Mocroft A, Vella S, Benfield TL, Chiesi A, Miller V, Gargalianos P, *et al*. **Changing patterns of mortality across Europe in patients infected with HIV-1.** *Lancet* 1998; **352**:1725–1730.
2. Data Collection on Adverse Events of Anti-HIV drugs (D:A:D) Study Group. Smith C, Sabin CA, Lundgren JD, Thiebaut R, Weber R, *et al*. **Factors associated with specific causes of death amongst HIV-positive individuals in the D:A:D Study.** *AIDS (London, England)* 2010; **24**:1537–1548.
3. Maurice JB, Patel A, Scott AJ, Patel K, Thursz M, Lemoine M. **Prevalence and risk factors of nonalcoholic fatty liver disease in HIV-monoinfection.** *AIDS* 2017; **31**:1621–1632.
4. Hernando V, Perez-Cachafeiro S, Lewden C, Gonzalez J, Segura F, Oteo JA, *et al*. **All-cause and liver-related mortality in HIV positive subjects compared to the general population: differences by HCV co-infection.** *J Hepatol* 2012; **57**:743–751.
5. Konopnicki D, Mocroft A, Wit Sde, Antunes F, Ledergerber B, Katlama C, *et al*. **Hepatitis B and HIV: prevalence, AIDS progression, response to highly active antiretroviral therapy and increased mortality in the EuroSIDA cohort.** *AIDS* 2005; **19**:593–601.
6. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. **Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes.** *Hepatology* 2016; **64**:73–84.
7. Schattenberg JM, Lazarus JV, Newsome PN, Serfaty L, Aghemo A, Augustin S, *et al*. **Disease burden and economic impact of diagnosed nonalcoholic steatohepatitis in five European countries in 2018: a cost-of-illness analysis.** *Liver Int* 2021; **41**:1227–1242.
8. Diehl AM, Day C. **Cause, pathogenesis, and treatment of nonalcoholic steatohepatitis.** *N Engl J Med* 2017; **377**:2063–2072.
9. Dulai PS, Singh S, Patel J, Soni M, Prokop LJ, Younossi Z, *et al*. **Increased risk of mortality by fibrosis stage in nonalcoholic fatty liver disease: systematic review and meta-analysis.** *Hepatology* 2017; **65**:1557–1565.
10. Bonfanti P, Giannattasio C, Ricci E, Facchetti R, Rosella E, Franzetti M, *et al*. **HIV and metabolic syndrome: a comparison with the general population.** *J Acquir Immune Defic Syndr* 2007; **45**:426–431.
11. Squillace N, Soria A, Bozzi G, Gori A, Bandera A. **Nonalcoholic fatty liver disease and steatohepatitis in people living with HIV.** *Expert Rev Gastroenterol Hepatol* 2019; **13**:643–650.
12. Surial B, Mugglin C, Calmy A, Cavassini M, Günthard HF, Stöckle M, *et al*. **Weight and metabolic changes after switching from tenofovir disoproxil fumarate to tenofovir alafenamide in people living with HIV: a cohort study.** *Ann Intern Med* 2021; **174**:758–767.
13. Vuille-Lessard É, Lebouché B, Lennox L, Routy J-P, Costiniuk CT, Pexos C, *et al*. **Nonalcoholic fatty liver disease diagnosed by transient elastography with controlled attenuation parameter in unselected HIV monoinfected patients.** *AIDS* 2016; **30**:2635–2643.
14. Bischoff J, Gu W, Schwarze-Zander C, Boesecke C, Wasmuth J-C, van Bremen K, *et al*. **Stratifying the risk of NAFLD in patients with HIV under combination antiretroviral therapy (cART).** *EClinicalMedicine* 2021; **40**:101116.
15. Mohr R, Boesecke C, Dold L, Schierwagen R, Schwarze-Zander C, Wasmuth J-C, *et al*. **Return-to-health effect of modern combined antiretroviral therapy potentially predisposes HIV patients to hepatic steatosis.** *Medicine* 2018; **97**:e0462.
16. Alqahtani SA, Schattenberg JM. **Nonalcoholic fatty liver disease: use of diagnostic biomarkers and modalities in clinical practice.** *Expert Rev Mol Diagn* 2021; **21**:1065–1078.
17. Eddowes PJ, Sasso M, Allison M, Tsochatzis E, Anstee QM, Sheridan D, *et al*. **Accuracy of FibroScan controlled attenuation parameter and liver stiffness measurement in assessing steatosis and fibrosis in patients with nonalcoholic fatty liver disease.** *Gastroenterology* 2019; **156**:1717–1730.
18. Michel M, Schattenberg JM. **Leberspezifische Diagnostik bei der nichtalkoholischen Fettlebererkrankung (NAFLD) - wann kann die Leberbiopsie ersetzt werden? [Liver-specific diagnostic for nonalcoholic fatty liver disease (NAFLD) - time to replace liver biopsy?].** *Zeitschrift fur Gastroenterologie* 2020; **58**:1233–1240.
19. Newsome PN, Sasso M, Deeks JJ, Paredes A, Boursier J, Chan W-K, *et al*. **FibroScan-AST (FAST) score for the noninvasive identification of patients with nonalcoholic steatohepatitis with significant activity and fibrosis: a prospective derivation and global validation study.** *Lancet Gastroenterol Hepatol* 2020; **5**:362–373.
20. **EASL-EASD-EASO Clinical Practice Guidelines for the management of nonalcoholic fatty liver disease.** *J Hepatol* 2016; **64**:1388–1402.
21. International Diabetes Federation (IDF). The IDF consensus worldwide definition of the metabolic syndrome. Available at: https://www.idf.org/e-library/consensus-statements/60-idf-consensus-worldwide-definitionof-the-metabolic-syndrome.html. [Accessed 12 December 2021]
22. Wong VW-S, Vergniol J, Wong GL-H, Foucher J, Chan HL-Y, Le Bail B, *et al*. **Diagnosis of fibrosis and cirrhosis using liver stiffness measurement in nonalcoholic fatty liver disease.** *Hepatology* 2010; **51**:454–462.
23. Berzigotti A, Boursier J, Castera L, Cazzagon N, Friedrich-Rust M, Petta S, *et al*. **EASL Clinical Practice Guidelines (CPGS) on non-invasive tests for evaluation of liver disease severity and prognosis- 2020 update.** *J Hepatol* 2021; **75**:659–689.
24. Pezzini MF, Cheinquer H, Araujo Ade, Schmidt-Cerski CT, Sprinz E, Herz-Wolff F, Poeta J. **Hepatic steatosis among people living with HIV in Southern Brazil: prevalence and risk factors.** *Sci Rep* 2020; **10**:8282.
25. McPherson S, Stewart SF, Henderson E, Burt AD, Day CP. **Simple noninvasive fibrosis scoring systems can reliably exclude advanced fibrosis in patients with nonalcoholic fatty liver disease.** *Gut* 2010; **59**:1265–1269.
26. Angulo P, Hui JM, Marchesini G, Bugianesi E, George J, Farrell GC, *et al*. **The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD.** *Hepatology* 2007; **45**:846–854.
27. Sun W, Cui H, Li N, Wei Y, Lai S, Yang Y, *et al*. **Comparison of FIB-4 index, NAFLD fibrosis score and BARD score for prediction of advanced fibrosis in adult patients with nonalcoholic fatty liver disease: a meta-analysis study.** *Hepatol Res* 2016; **46**:862–870.

28. Lemoine M, Assoumou L, Wit Sde, Girard P-M, Valantin MA, Katlama C, *et al.*, ANRS-ECHAM Group. **Diagnostic accuracy of noninvasive markers of steatosis, NASH, and liver fibrosis in HIV-monoinfected individuals at risk of nonalcoholic fatty liver disease (NAFLD): results from the ECHAM Study.** *J Acquir Immune Defic Syndr* 2019; **80**:e86–e94.
29. Alexander M, Loomis AK, van der Lei J, Duarte-Salles T, Prieto-Alhambra D, Ansell D, *et al*. **Risks and clinical predictors of cirrhosis and hepatocellular carcinoma diagnoses in adults with diagnosed NAFLD: real-world study of 18 million patients in four European cohorts.** *BMC Med* 2019; **17**:95.
30. Machado MV, Oliveira AG, Cortez-Pinto H. **Hepatic steatosis in patients coinfected with human immunodeficiency virus/hepatitis C virus: a meta-analysis of the risk factors.** *Hepatology* 2010; **52**:71–78.
31. Macías J, González J, Tural C, Ortega-González E, Pulido F, Rubio R, *et al*. **Prevalence and factors associated with liver steatosis as measured by transient elastography with controlled attenuation parameter in HIV-infected patients.** *AIDS* 2014; **28**:1279–1287.
32. Yanavich C, Pacheco AG, Cardoso SW, Nunes EP, Chaves U, Freitas G, *et al*. **Diagnostic value of serological biomarkers for detection of nonalcoholic fatty liver disease (NAFLD) and/or advanced liver fibrosis in people living with HIV.** *HIV Med* 2021; **22**:445–456.
33. Estes C, Anstee QM, Arias-Loste MT, Bantel H, Bellentani S, Caballeria J, *et al*. **Modeling NAFLD disease burden in China, France, Germany, Italy, Japan, Spain, United Kingdom, and United States for the period 2016–2030.** *J Hepatol* 2018; **69**:896–904.
34. Guaraldi G, Squillace N, Stentarelli C, Orlando G, D'Amico R, Ligabue G, *et al*. **Nonalcoholic fatty liver disease in HIV-infected patients referred to a metabolic clinic: prevalence, characteristics, and predictors.** *Clin Infect Dis* 2008; **47**:250–257.
35. Bedogni G, Miglioli L, Masutti F, Tiribelli C, Marchesini G, Bellentani S. **Prevalence of and risk factors for nonalcoholic fatty liver disease: the Dionysos nutrition and liver study.** *Hepatology* 2005; **42**:44–52.
36. Kirkegaard-Klitbo DM, Bendtsen F, Lundgren J, de Knegt RJ, Kofoed KF, Nielsen SD, Benfield T. **Increased prevalence of liver fibrosis in people living with HIV without viral hepatitis compared to population controls.** *J Infect Dis* 2020; **224**:443–452.
37. Vermehren J, Vermehren A, Mueller A, Carlebach A, Lutz T, Gute P, *et al*. **Assessment of liver fibrosis and associated risk factors in HIV-infected individuals using transient elastography and serum biomarkers.** *BMC Gastroenterol* 2012; **12**:27.
38. Labenz C, Huber Y, Kalliga E, Nagel M, Ruckes C, Straub BK, *et al*. **Predictors of advanced fibrosis in noncirrhotic nonalcoholic fatty liver disease in Germany.** *Aliment Pharmacol Ther* 2018; **48**:1109–1116.
39. Grant PM, Kitch D, McComsey GA, Collier AC, Bartali B, Koletar SL, *et al*. **Long-term body composition changes in antiretroviral-treated HIV-infected individuals.** *AIDS* 2016; **30**:2805–2813.
40. Bravo AA, Sheth SG, Chopra S. **Liver biopsy.** *New Engl J Med* 2001; **344**:495–500.