Prevalence of nonalcoholic fatty liver disease using noninvasive techniques among children, adolescents, and youths living with HIV

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Objective: The prevalence of subclinical liver abnormalities is high among people with HIV, but data regarding perinatally HIV-infected children and adolescents (PHIV) are scarce. Noninvasive image techniques offer an opportunity to address nonalcoholic fatty liver disease (NAFLD) in a population in which the scores validated for adults have not been tested.

Design: Prospective cross-sectional study including PHIV and uninfected controls.

Methods: Noninvasive imaging techniques for the diagnosis of NAFLD and/or fibrosis were performed, and four scores to predict NAFLD were evaluated.

Results: Seventy-six participants (59.2% women) with a median of 19 years old (interquartile range: 15.5–25.6) were included, 38 were PHIV and 38 were age and sex-matched controls. All HIV participants were on ART at the moment of inclusion, and 86.8% were virologically suppressed. A total of 11 PHIV and three controls were diagnosed with NAFLD (28.9% vs. 7.9%; $P = 0.02$) by noninvasive imaging techniques. The performance of scores based on clinical and analytical parameters was very poor. Although nonsignificant, overweight was more common among participants with NAFLD, who had a significantly higher BMI. Differences in HIV-related parameters between the groups were nonsignificant, except for the $CD4^+/CD8^+$ T-cells ratio, decreased among PHIV diagnosed with NAFLD ($P = 0.04$).

Conclusions: The prevalence of NAFLD was high (28.9%) among PHIV, and only partially explained by overweight and metabolic syndrome defining factors. The scores based on clinical and analytical parameters did not accurately identify participants at risk. Therefore, liver ultrasound assessment should be considered for the screening of NAFLD among PHIV in routine clinical practice.

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Introduction

Since the introduction of antiretroviral therapy (ART), HIV infection has turned into a chronic condition [1]. As a result of this increase in life expectancy, the quality of life of people with HIV is threatened by comorbidities, including liver, kidney, cardiovascular disease, or cancer [2,3]. Worldwide, the liver disease remains one of the major causes of morbidity and mortality among people with HIV [4,5]. However, thanks to the efficacy of direct-acting antivirals (DAA) to treat the hepatitis C virus (HCV), the burden of diseases associated with this virus is decreasing dramatically in areas with access to treatment. Instead, the weight of nonalcoholic fatty liver disease (NAFLD) and steatohepatitis on chronic liver disease is increasing.

NAFLD is defined as a fat accumulation higher than 5% in the liver, encompassing different stages of abnormal liver ranging from simple steatosis to nonalcoholic steatohepatitis (NASH) and cirrhosis [3,6]. The increase in NAFLD keeps pace with the increasing prevalence of obesity, insulin resistance [7,8] and other components of the metabolic syndrome [9,10], and is linked to mitochondrial dysfunction [11] and the cytopathic effect of HCV infection [12]. Some reports suggest that NAFLD/NASH will turn into the first cause of liver disease in the general population, both for children and adults, and the first cause of liver transplant in western countries in years to come [3,10]. However, the diagnosis of NAFLD/NASH is challenging, and although we have very sensitive and specific noninvasive methods for steatosis and fibrosis diagnosis in our daily clinical practice, to date, liver biopsy is considered the gold standard [13]. Scores combining clinical and laboratory parameters, such as the hepatic steatosis index (HSI) [14] or triglycerides and glucose (TyG) [15], have been validated in adults for screening and are able to predict NAFLD in the adult population. The AST to platelet ratio index [16] and the fibrosis-4 index and NAFLD fibrosis score [17] are recommended to detect fibrosis. None of these scores has been validated for children [18,19].

New, noninvasive imaging techniques as transient elastography (TE) or point shear wave elastography (p-SWE) offer the possibility to stratify the degrees of steatosis [13,20], assessing hepatic rigidity in a quantitative way and can be combined with the mentioned scores to improve accuracy. Their main limitation is a low sensitivity for the diagnosis of early stages of NAFLD/NASH, according to the European Association for the Study of the Liver [21]. However, up today, the use of noninvasive imaging techniques is recommended for the diagnosis and follow-up both in adults and children [16,21,22].

Among people with HIV, data suggest an increased prevalence of NAFLD [23–29] ranging from 28.8% [30] to 48.0% [31] when the diagnosis is based on imaging techniques to 57.1% [32] to 72.6% [25] when based on liver biopsy. Although the pathogenesis of NAFLD/NASH is unclear and most probably multifactorial, in the context of HIV infection, authors have suggested a potential deleterious effect of the chronic inflammation and activation of the immune system secondary to the virus [28,33–35] and its treatment [36,37]. In the unique population of perinatally HIV-infected children who face lifelong exposure to antiretroviral treatment and its metabolic consequences, and the deleterious effects of the virus itself [18] these phenomena have been described since birth [38–40]. However, data addressing the prevalence of NAFLD in this population are scarce [41]. The reliability of noninvasive assessment of NAFLD, including clinical scores in the population of adults living with HIV, has been the focus of intense research, but their use among children and youths has not been established [17]. Although hypertransaminasemia is quite common during follow-up in people with HIV its significance remains unknown [40].

The aim of this study was to determine the prevalence of subclinical liver abnormalities based on the use of noninvasive image techniques (TE/p-SWE) in perinatally HIV-infected children and youths (PHIV). Secondary objectives included the description of clinical, epidemiological, virological, and immunological determinants and evaluation of clinical scores for the diagnosis of NAFLD among children and youths.

Methods

Study design and participants

We carried out a prospective longitudinal study in two tertiary hospitals in Madrid, Spain. PHIV followed up in the Spanish National Network of Children and Adolescents Living with HIV (CoRISpe) were included. Uninfected siblings, partners, and adolescents who attended for postexposure prophylaxis were included as controls, matched by sex and age (±2 years). HIV participants and uninfected controls were recruited at Hospital Universitario La Paz and Hospital General Universitario Gregorio Maran on in Madrid (Spain) from June 2018 to December 2020. All patients included in the

CoRISpe cohort undergo serology at inclusion and during follow-up according to clinical criteria, and all patients included in the study were screened for a full panel of diseases with liver involvement, including viral infections. Additional exclusion criteria for participants and controls were history of HBV or HCV infection or co-infection, acute infections or opportunistic diseases, or chronic inflammatory diseases at the moment of inclusion, and reporting >30 g in men and >20 g in females of alcohol intake. The study was approved by the Ethics Committee of participating hospitals and conducted according to the Declaration of Helsinki. All participants signed informed consent before inclusion in the study.

Demographic, clinical, analytical, and virological data

Sociodemographic and clinical data were collected from medical records. Virological and immunological data, including HIV-specific variables and ART history, were collected for PHIV from the CoRISpe database. CDC 2014 classification was used to categorize HIV disease stages. HIV viral load was considered suppressed when <50 copies/ml. HBV and HCV participants were excluded, and no other hepatic diseases were discarded. Liver enzymes (AST, ALT, and GGT) were considered elevated according to laboratory ranges: ALT > 35 UI/l, AST, and GGT when >40 UI/l. Fasting total cholesterol was considered elevated when >200 mg/dl and triglycerides when > 150 mg/dl. Weight, height, waist, and hip measurements were taken according to a unified protocol. Body mass index (BMI) was automatically calculated according to the formula: BMI = weight (kg)/height^2 (m). All measurements were *z*-score adjusted. The standard deviations for BMI according to age and gender were calculated using the 2010 Spanish Growth charts [42]. Overweight was defined as a BMI *z*-score between + 1 and +2 SD, and obesity was defined as BMI *z*-score ≥2 SD. For adults ≥ 18 years of age, we used the WHO definition for overweight and obesity based on a BMI ≥ 25 and ≥ 30, respectively [43].

Noninvasive techniques for nonalcoholic fatty liver disease diagnosis

All patients included in the study underwent both transient elastography (with CAP) and shear wave elastography, as well as hematology and biochemistry, serological and virological studies. Ultrasound elastography measurements were performed in a supine position with the right arm maximally abducted, after ≥6 h of fasting. Two trained clinicians, blind to HIV status, performed the ultrasound studies according to a unified protocol.

Liver fibrosis was evaluated by means of TE, FibroScan [44], as well as shear wave elastography. Liver elasticity/stiffness was quantified based on the speed of ultrasound waves when passing through the liver. p-SWE uses an internal acoustic radiation force impulse or ARFI, measuring the speed of shear wave propagation through the hepatic parenchyma at a fixed point or region of interest (ROI). It was performed with a 4−9 MHz convex probe in a Samsung Prestige RS80 ultrasound scanner [45]. Ten valid measurements were acquired at 2− 4 cm in-depth in the liver. The measured speed is given in m/s and converted to kPa for tissue stiffness estimation [46]. Then, the median is calculated, as well as the interquartile range. Normal values have been defined for children [47]. The risk for advanced fibrosis was stratified by APRI (<0.5 mild fibrosis), advanced fibrosis p-SWE and/or TE Liver fibrosis was defined according to the Metavir Scale for the TE as the absence of fibrosis F0-F1 (<7.6 kPa), moderate fibrosis F2 (7.6−9.5 kPa), advanced fibrosis F3 (9.5−14 kPa) and F4 (14 kPa); and for p-SWE as the absence of fibrosis F0−F1 (<5.7 kPa), moderate fibrosis F2 (>7.6 kPa), advanced fibrosis F3 (<11.6 kPa), and F4 (>11.6 kPa).

Both p-SWE and the controlled attenuation parameter (CAP) by TE were used to establish NAFLD diagnosis. CAP evaluates the ultrasonic attenuation in the liver at 3.5 MHz at depth 25−65 mm using FibroScan and represents a noninvasive assessment of liver steatosis. CAP values in dB/m were reported as the median of 10 acquisitions, and the cut-off point of 248 dB/m defined by Karlas *et al.* [48] was used to define the presence of steatosis. The criteria defined by Dasaranthy et al. [49] and Bril et al. [50] were used for the diagnosis of steatosis by p-SWE: increased parenchymal echogenicity of the liver, hepatic vein blurring, portal vein blurring, and visualization of the diaphragm.

Clinical and analytical scores

Four scores validated to predict liver abnormalities in the adult population were calculated for study participants, and their accuracy to predict fibrosis/NAFLD was compared to noninvasive imaging techniques. As there are no specific pediatric cut-off points, the ones described for adults were used. Triglyceride glucose index (TyG), hepatic steatosis index (HSI), AST to platelet ratio index (APRI), and fibrosis 4 (FIB-4) were calculated automatically. TyG, an index to determine insulin resistance and able to identify individuals at risk for NAFLD, was calculated according to the equation: TyG = ln[fasting triglyceride (mg/dl) × fasting glucose (mg/dl)]/2. An index of 4.49 or larger indicates a patient is likely to suffer from insulin resistance and an index of 8.38 or larger indicates steatosis. HSI, an index used to identify candidates for liver study, was calculated using the equation: HSI = 8 × ALT/AST + BMI (+2 if type 2 diabetes, +2 if female). For interpretation, NAFLD was diagnosed >36, ruled out with a value <30, whereas values between 30 and 36 are considered inconclusive. Two scores for fibrosis evaluation were performed: APRI = AST (IU/L)/AST upper normal limit (IU/l)/platelet count (10^9/l) × 100. When APRI <0.5, fibrosis

can be ruled out. FIB-4 was also used to evaluate liver fibrosis: FIB-4 = (age × AST)/(platelet count × $\sqrt{}$ALT). A value >2.67 suggests fatty liver, and >3.25 suggests cirrhosis by HCV; a value <1.3 is relevant to exclude significant fibrosis. All index and interpretations were performed using MDCalc (https://www.mdcalc.com/).

Statistical analysis

Categorical variables were presented as total counts and percentages (%), and continuous variables appeared as median and interquartile ranges (IQR). Chi-square test or Fisher test, as appropriate, were performed to compare categorical variables and Mann—Whitney *U*-test to compare continuous variables. Due to sample size restrictions, we did not perform multivariate analysis to assess determinants for NAFLD. A P-value <0.05 was considered statistically significant. All analyses were conducted in IBM-SPSS Statistics Version 25.0 (IBM Corp., Armonk, New York, USA).

Results

General characteristics of the study population

Globally, 76 participants (59.2% women) with a median age of19.0 (IQR: 15.5–25.6) years old were included in our study. Of them, 38 were PHIV, and 38 were age and sex-matched HIV-negative controls. The sociodemographic and clinical characteristics of both groups are shown in Table 1. Participants living with HIV had been diagnosed at a median of19.2 (IQR: 3.6–56.4) months of age. All PHIV were on ART at the moment of inclusion in the study, and 86.8% of them were virologically suppressed. Overall, the most used recent combination (44.7%) was two nucleoside reverse transcriptase inhibitors (NRTI) + one integrase strand transfer inhibitors (INSTI), followed by an 18.4% using two NRTI + one protease inhibitor (PI) and a 15.8% using two NRTI + one non-nucleoside reverse transcriptase inhibitors (NNRTI). The median number of ART regimens received since diagnosis was 4.5 (IQR: 3.0–8.8). Half of PHIV had been exposed to zidovudine, 45% to stavudine (d4T) and over 36% to didanosine (ddI). Main HIV-related variables are shown in Table 2, according to NAFLD diagnosis.

Nonalcoholic fatty liver disease in people with HIV

By means of noninvasive imaging techniques, a total of11 PHIV participants were diagnosed with steatosis, vs. only three of the HIV-negative controls (28.9% vs. 7.9%; $P = 0.02$). Comparisons between participants with and without NAFLD, including data regarding HIV-related parameters, are shown in Table 2. Overall, 11 PHIV were diagnosed with NAFLD by imaging techniques, at a median age of17.9 years (IQR: 11.3–26.6), ranging from 9 to 33 years old. Characteristics of PHIV diagnosed with NAFLD are shown in Table 3. Seven were women and only two presented overweight (BMI > 25). Their immunological situation was good, with only one participant having a CD4+ nadir below 200 cells/μl. The median number of previous ART regimens was 5 (IQR: 3–9), with five participants with previous exposure to ddI and d4T and eight to zidovudine. Fibrosis (stage F2 or higher) was detected in 3 (7.9%) PHIVand none of the HIV-negative controls. The three HIV-infected patients with fibrosis were male, aged 18.1, 19.2, and 27.8 years, respectively. All were F2 according to the Metavir scale (9.7, 8.2 and

Table 1. Characteristics of participants living with perinatally acquired HIV and HIV-negative controls.

Parameters	PHIV ($n = 38$)	HIV-negative controls ($n = 38$)	*P*-value
Age			0.16
Median (IQR), years	18.0 (14.9–24.2)	21.93 (14.9–27.0)	
Range	6.9–33.5	6.1–33.7	
Women (%)	22 (57.9)	23 (60.5)	0.82
Origin (%)			**0.02**
Spain	24 (63.2)	32 (84.2)	
Latin America	5 (13.2)	4 (10.5)	
Sub-Saharan Africa	8 (21.1)	0 (0.0)	
Asia	1 (2.6)	2 (5.3)	
Weight (kg)	54.0 (44.6–60.3)	60.7 (48.9–72.3)	**0.04**
Height (cm)	158.0 (152.0–168.0)	169.3 (152.9–180.0)	**0.02**
BMI	20.3 (18.4–23.6)	22.0 (19.4–23.5)	0.27
Overweight (1 < z-score < 2 SD or BMI > 25)	6 (15.8)	7 (18.4)	0.76
Obesity (≥2SD z-score or BMI > 30)	1 (2.6)	0 (0.0)	0.31
Waist (cm)	71.0 (65.0–77.0)	75.3 (68.1–81.0)	0.25
Hip (cm)	87.5 (79.0–90.8)	90.0 (77.1–94.0)	0.44
Waist—hip ratio	0.86 (0.79–0.91)	0.87 (0.83–0.92)	0.57
NAFLD diagnosis by imaging techniques (%)	11 (28.9)	3 (7.9)	**0.02**
Fibrosis (%)	3 (7.9)	0 (0)	0.21
FibroScan result (kPa)	5.0 (4.2–5.6)	4.1 (3.6–5.1)	**0.01**

Continuous variables are expressed as medians and interquartile rates. Frequencies are expressed as percentages (%). BMI, body mass index; cm, centimeters; IQR, interquartile range; kg, kilograms; kPa, kilopascals; NAFLD, nonalcoholic fatty liver disease; PHIV, people with HIV.

Table 2. Characteristics of participants living with perinatally acquired HIV with and without NAFLD.

Parameter	NAFLD ($n=11$)	No NAFLD ($n=27$)	Univariate (P)
Age (years)	18.0 (11.3–26.6)	18.1 (15.8–23.3)	0.32
Women, (%)	7 (63.6)	15 (55.6)	0.65
Origin, (%)			0.19
Spain	9 (81.8)	13 (48.2)	
Latin America	2 (18.2)	3 (11.1)	
Sub-Saharan Africa	0 (0.0)	8 (29.6)	
Asia	0 (0.0)	3 (11.1)	
BMI	23.3 (21.2–25.0)	19.1 (18.4–22.5)	**0.04**
Overweight, *z*-score + 1–2 SD or BMI > 25 (%)	3 (27.3)	3 (11.1)	0.22
Obese, *z*-score +2 SD or BMI > 30 (%)	1 (9.1)	0 (0.0)	0.11
Waist–hip ratio	0.91 (0.87–0.94)	0.83 (0.77–0.89)	**0.01**
FibroScan value (kPa)	4.9 (3.9–6.4)	5.0 (4.5–5.6)	0.82
Time since HIV diagnosis (years)	15.3 (10.1–25.7)	15.0 (11.3–22.9)	0.64
CDC HIV stage			0.12
N	1 (9.1)	1 (3.7)	
A	6 (54.5)	5 (18.5)	
B	3 (27.3)	8 (29.6)	
C	1 (9.1)	10 (37.0)	
Unknown	0 (0.0)	3 (11.1)	
HIV VL <50 copies/ml (%)	11 (100.0)	22 (81.5)	0.13
$CD4^+$ T-cells count	839 (680–1420)	845 (678–1182)	0.61
$CD4^+$ T-cells nadir	302 (216–360)	393 (197–493)	0.32
$CD4^+$ T-cells nadir < 200 cells/μl	1 (9.1)	6 (22.2)	0.37
$CD4^+/CD8^+$ T-cells ratio	0.9 (0.6–1.1)	1.3 (1.0–1.7)	**<0.05**
$CD4^+/CD8^+$ ratio < 1	4 (36.4)	5 (18.5)	0.21
ART regimens since diagnosis	5 (3–9)	3 (4–8)	0.76
Current ART (%)			0.09
1 PI (%)	2 (18.2)	1 (9.7)	
1 PI+ 1 INSTI (%)	0 (0.0)	2 (7.4)	
1 NNRTI + 1 INSTI (%)	1 (9.1)	0 (0.0)	
2 NRTI + 1 INSTI (%)	3 (27.3)	14 (51.9)	
2 NRTI + 1 PI (%)	1 (9.1)	6 (22.2)	
2 NRTI + 1 NNRTI (%)	4 (36.4)	2 (7.4)	
2 NNRTI + 1INSTI (%)	0 (0.0)	2 (7.4)	
Glucose (mg/dl)	80 (77–86)	82 (75–84)	0.68
ALT (U/l)	17 (13–26)	22.0 (11.8–29.3)	0.61
AST (U/l)	18 (15–21)	22.5 (15.5–30.5)	0.16
Altered transaminases	0 (0.0)	0 (0.0)	
GGT (U/l)	18.5 (14.0–32.3)	17.5 (13.8–21.5)	0.65
Cholesterol (mg/dl)	173 (138–181)	153 (132–174)	0.41
Cholesterol >200 mg/dl	1 (9.1)	4 (14.8)	0.64
LDL (mg/dl)	101 (75–117)	83 (68–99)	0.16
HDL (mg/dl)	45 (36–55)	50 (44–56)	0.16
Triglycerides (mg/dl)	83 (63–144)	79 (57–122)	0.59
Triglycerides >150 mg/dl	2 (18.2)	4 (14.8)	0.80
Platelets (cells/mm^3)	311 (228–336)	254 (225–316)	0.28
Bilirubin (mg/dl)	0.4 (0.4–57.0)	0.5 (0.4–16.5)	0.81
his	33.2 (28.5–39.3)	27.8 (26.8–34.5)	**0.01**
Altered his	3 (27.3)	2 (7.4)	0.38
APRI	0.2 (0.1–0.2)	0.2 (0.2–0.3)	0.10
Altered APRI	0 (0)	2 (7.4)	0.66
FIB-4	0.3 (0.2–0.4)	0.4 (0.2–0.6)	0.48
Altered FIB-4	0 (0)	0 (0)	
TyG	4.4 (4.3–4.7)	4.4 (4.2–4.6)	0.63
Altered TyG	0 (0)	0 (0)	

Continuous variables are expressed as medians and interquartile rates. Frequencies are expressed as percentages (%). ALT, alanine aminotransferase;APRI, AST to platelet ratio index;ART, antiretroviral treatment; AST, aspartate aminotransferase;BMI, body mass index;d4T, stavudine;ddI, didanosine;FIB-4, fibrosis-4 score;GGT, gamma-glutamyl transferase;HSI, hepatic steatosis index;INSTI, integrase strand transfer inhibitors; *n*, number; NAFLD, nonalcoholic fatty liver disease;NNRTI, non-nucleoside reverse transcriptase inhibitors; NRTI, nucleoside reverse transcriptase inhibitors; PI, protease inhibitor;TyG, triglycerides and fasting glucose index;ULN, upper limit normality;VL, viral load.

8.8 kPa, respectively) and only the last one had steatosis (patient #10, Table 3). The BMI of the other two patients was 22.5 and 22.7, respectively, $CD4^+/CD8^+$ ratio was >1 in both cases with a $CD4^+$ nadir > 200 cells/μl. None of them had an altered APRI or FIB-4 index. No other risk factors for fibrosis were identified.

When compared to PHIV non presenting NAFLD, no differences were found regarding age, gender or ethnicity.

Table 3. Characteristics of PHIV diagnosed with NAFLD.

Patient	Gender	Age (years)	Tanner	BMI	Waist—hip ratio	Fibrosis	Nadir $CD4^+$	$CD4^+/CD8^+$ ratio	CDC stage	ART (*n*)	Altered HSI
#1	F	17.8	V	24.2 (N)	0.91	F0–F1	340	0.31	A	4	Und
#2	M	11.3	II	23.4 (O)	0.94	F0–F1	217	1.27	A	5	No
#3	F	10.5	I	25.0 (O)	0.91	F0–F1	402	0.92	N	3	Und
#4	F	9.2	I	17.6 (N)	0.84	F0–F1	328	1.02	A	1	U
#5	F	18.0	V	23.2 (N)	U	F0–F1	360	0.86	A	2	Yes
#6	M	18.0	V	26.8 (O)	1.07	F0–F1	302	0.74	A	4	U
#7	M	26.1	V	21.9 (N)	0.89	F0–F1	158	U	C	9	No
#8	F	26.6	V	21.2 (N)	U	F0–F1	200	U	A	9	Und
#9	F	33.5	V	18.2 (N)	0.87	F0–F1	216	U	B	12	No
#10	M	27.8	V	43.3 (Ob)	U	F2	291	U	B	10	Yes
#11	F	26.0	V	23.3 (N)	U	F0–F1	639	U	B	7	Yes

Continuous variables are expressed as medians and interquartile rates. ART, historical number of antiretroviral treatments used since HIV diagnosis; BMI, body mass index; F, female; M, male; N, normal BMI; n, number; NAFLD, nonalcoholic fatty liver disease; O, overweigh; Ob, obesity; PHIV, people with HIV;PNPLA3, patatin-like phospholipase domain-containing 3 protein;U, unknown;Und, undetermined.

Although nonsignificant, overweight was more common among participants with NAFLD, who had a significantly higher BMI. Differences in HIV-related parameters between the groups were nonsignificant, except for the $CD4^+/CD8^+$ T-cells ratio, which decreased among those diagnosed with NAFLD ($P = 0.04$). Time from diagnosis, CDC HIV stage, number of regimens and ART regimens were not associated with the prevalence of NAFLD.

Performance of the clinic-analytical scores for the screening of nonalcoholic fatty liver disease in people with HIV

Overall, no differences in laboratory parameters such as glucose, aminotransferases, cholesterol, triglycerides, platelets or bilirubin values were observed among PHIV, independent of the NAFLD diagnosis. Regarding screening scores, results were normal for most HIV-infected participants, presenting only five out of 30 PHIV with HIS score available with an altered index (NAFLD diagnosis confirmed by imaging techniques in three) and two out of 31 with an altered APRI (none of them had fibrosis by TE). FIB-4 and TyG scores were normal in all cases.

Discussion

In this exploratory study using noninvasive imaging techniques, the prevalence of NAFLD among youths living with HIV since childhood was surprisingly high (28.9%) compared to an uninfected cohort. The fact that no clear relation to overweight and the metabolic syndrome defining factors could be established, together with the extremely poor performance of the scores based on clinical and analytical parameters, are worrisome, as identification of patients at risk remains extremely challenging among youths.

Studies in industrialized countries suggest that NAFLD represents the most common chronic liver disease in children, with a prevalence ranging from around 10% in children, 17% in teenagers, to 40–70% among obese children [51]. Among adults in the general population, the numbers go up to 30% in adults and 80% among obese patients [52]. Compared to these data, the prevalence of NAFLD in our series, integrated mainly by teenagers and youths, is two times higher than expected by age group [51,52] supporting previous results in PHIV [53]. The first hypothesis to explain these findings is the presence of HIV infection and ART treatment, as underlined by the fact that NAFLD prevalence was much lower among the recruited uninfected controls. Mitochondrial dysfunction can be caused by ART and has been described to contribute to the pathogenesis of NAFLD and lead to ROS production, lipid peroxidation and cytokine release that induce liver inflammation and fibrosis [54]. This hypothesis is in line with the limited studies addressing the prevalence of NAFLD among PHIV [26,40], based on abnormal noninvasive markers [16] and noninvasive imaging techniques [41], suggesting abnormalities in around half of the studied populations [36,41]. The sample size of the mentioned studies is small, and methodologies cannot be compared. None of the studies is based on biopsies, which is meant to be the gold standard for diagnosis. However, these findings are worrisome, and especially considering that compared to other westernized countries, the Mediterranean region is usually considered a low-risk area for metabolic disorders [55]. According to the literature, the expected prevalence of NAFLD/NASH in the area would be lower compared to the one reported in areas such as North America [56–58]. Although results are worrisome, the longitudinal evolution of fatty liver is unknown, and studies have proven that changes in diet and behavior can induce disease regression [58]. Prompt diagnosis and management may prevent disease progression, as the rate of success is higher when lifestyle changes are promoted early in life [59,60]. The hypothesis of a direct

relationship between liver damage and HIV-infection plus ART treatment in this cohort is plausible and has been pointed out in previous studies [27,61]. However, the fact that apart from the $CD4^+/CD8^+$ ratio, no HIV-related variables were independently associated with NAFLD, does not support the role of chronic inflammation or metabolic abnormalities secondary to ART as an underlying cause. Advanced age, years from HIV infection [28], higher viral load, increased liver enzymes and long exposure to NRTIs [29] and especially the use of ddI/d4T, have been previously described as risk factors for fibrosis progression among people with HIV [35]. However, none of these factors were identified as risk factors for NAFLD in our cohort, nor time since diagnosis or ART exposure. The etiology of NAFLD is not well understood and is most probably multifactorial, including genetic, behavioral, diet-related, and inflammatory factors. While the association between NAFLD and metabolic syndrome is beyond question [29,34,62] and has been described also in the context of HIV infection [35], several authors describe the presence of steatosis in patients not presenting any of the metabolic syndrome defining factors [63–67]. Although in this study the differences in terms of the prevalence of overweight among PHIV with and without NAFLD was nonsignificant, BMI was significantly higher in participants with NAFLD. Probably, this reflects how the reduced sample size impaired our ability to analyze weight as a dichotomous variable. However, as most scores include biochemical and clinical parameters related to BMI, these subjects would never be identified as subjects at risk when classical scores are used. Similarly, hypertransaminasemia can be present or not at diagnosis [31,62]. This issue is even more challenging between children, adolescents and youth patients, in which scores have not been validated. In children, in fact, even the definition of metabolic syndrome is controversial [68,69].

Imaging techniques are not the gold standard for diagnosis of NAFLD but allow a noninvasive approach and permit close follow-up of patients, including monitoring disease regression. The main limitations are a low sensitivity for early stages of the disease, as changes suggestive of steatosis cannot be appreciated if the fat content is lower than 20–30%, and the interobserver variability, partially overcome with the use ofstandardized parameters such as p-SWE or CAP. Cirrhosis associated with NAFLD is hard to detect, and there are no ultrasound markers for steatohepatitis. Small participants may require the use of an adapted probe. On the other side, the adipose panniculus of participants with overweight might lead to low image quality. Both fibrosis and steatosis can modify the attenuation and dispersion of ultrasound waves, and this is in fact one of the limitations of the imaging techniques when evaluating the liver. None of these limitations should interfere with its sensitivity for the identification of higher-risk patients with no metabolic syndrome, and thus, to our view these techniques are ideal among individuals living with HIV.

Furthermore, our results suggest a very low sensitivity of usual NAFLD scores, with no concordance with imaging diagnosis, which induces us to recommend against the use of scores for children and youths. Together with the limitation regarding diagnosis, which did not include liver biopsy and thus cannot be considered certain, the main limitation of our study is the small sample size. The reduced number of participants included impaired our ability to analyze HIV-related risk factors. However, despite the reduced strength of the study, we found statistically significant differences regarding prevalence between cases and uninfected controls.

As genetic and lifestyle-related factors might determine the risk for NAFLD, controls were recruited among siblings and uninfected partners, to achieve a comparable cohort in terms of epidemiological, genetic and lifestyle-related factors, leaving HIV status as the only differentiating factor. However, the potential effect of intrauterine ART exposure is always a concern. The pubertal status assessment was not included in the protocol and therefore puberty could not be assessed as a potential confounder for NAFLD diagnosis [70]. Data regarding alcohol consumption rely only on clinical anamnesis.

Despite all limitations, the unexpected higher prevalence of NAFLD observed in this cohort of PHIV, together with an 8% of fibrosis are extremely worrisome, and comparable to previous studies [53]. These findings emphasize the need to systematically screen for liver disease among PHIV, regardless of age or the presence of risk factors. According to our results, the absence of metabolic factors or abnormal liver enzymes does not rule out the possibility of NAFLD. Due to the bad performance of classic scores in this population, noninvasive ultrasound techniques are nowadays the best option for screening, reserving biopsy for diagnosis confirmation when progression is suspected. Furthermore, noninvasive imaging techniques could be informative during the follow-up, and to evaluate long-term evolution. There is an urgent need to include liver assessment into routine clinical practice, to achieve a prompt diagnosis before the disease progresses to fibrosis or steatohepatitis. The development of new and more sensitive diagnostic procedures, as well as biomarkers to predict progression to severe forms of the disease is mandatory.

Conclusion

Larger and longitudinal studies addressing the evolution of liver disease in PHIVare needed. Clinicians should be aware of the risk and consider the need for screening. According to our results, the performance of scores based on clinical and analytical parameters for the identification of patients at risk is poor among youths. Despite their limitations, imaging techniques should be considered when available. As specific therapeutic measures are under research, intensifying prevention of metabolic risk factors since childhood seems mandatory to avoid future comorbidities.

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Summary: Based on noninvasive imaging techniques, the prevalence of nonalcoholic fatty liver disease is high among children and youths acquiring HIV perinatally compared to controls. Scores based on clinical and analytical parameters do not identify individuals at risk.

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Conflicts of interest

There are no conflicts of interest.

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