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Non-Alcoholic Fatty Liver Disease in HIV Infection

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Abstract

Non-alcoholic fatty liver disease is one of the most frequent chronic hepatic conditions worldwide. The spectrum of non-alcoholic fatty liver disease goes from hepatic steatosis to steatohepatitis, cirrhosis, and hepatocellular carcinoma. Risk factors for non-alcoholic fatty liver disease are metabolic, mainly obesity and the accompanying consequences. Treatment and prevention of non-alcoholic fatty liver disease should target those metabolic abnormalities. The frequency of and the factors associated with hepatic steatosis in HIV infection seem to be similar to those reported in the general population, though direct comparisons are lacking. Hepatic steatosis in HIV infection may also be secondary to antiretroviral drugs or HCV-related factors in HCV-coinfected subjects. However, more recent data suggest that hepatic steatosis in HIV infection represents true non-alcoholic fatty liver disease. As such, management of non-alcoholic fatty liver disease in HIV infection should follow the same principles as in the general population. **(AIDS Rev. 2017;19:35-46)**

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Key words

Antiretroviral therapy. HCV infection. HIV infection. NAFLD. Steatohepatitis.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is increasingly recognized as a leading cause of cirrhosis and hepatocellular carcinoma in Western countries[1-3]. It has been estimated that about one third of the general population have liver steatosis due to NAFLD. Most cases probably go unrecognized[1-3]. NAFLD is defined as significant steatosis in the absence of excessive alcohol consumption and other liver diseases[2,3]. NAFLD ranges from hepatic steatosis (HS) without other liver injury to non-alcoholic steatohepatitis (NASH), which may evolve to cirrhosis. NAFLD is the second most common cause for liver transplantation in the USA[4], and is projected to be the leading cause by 2020. This phenomenon might also be expected in other Western countries in the near future, paralleling the epidemic of obesity.

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NAFLD in the general population

Prevalence

The actual prevalence of NAFLD is largely unknown in the general population. Several estimations have been made using abdominal ultrasound or liver function tests in population-based samples of individuals. The rates of NAFLD have varied widely, ranging from 4 to

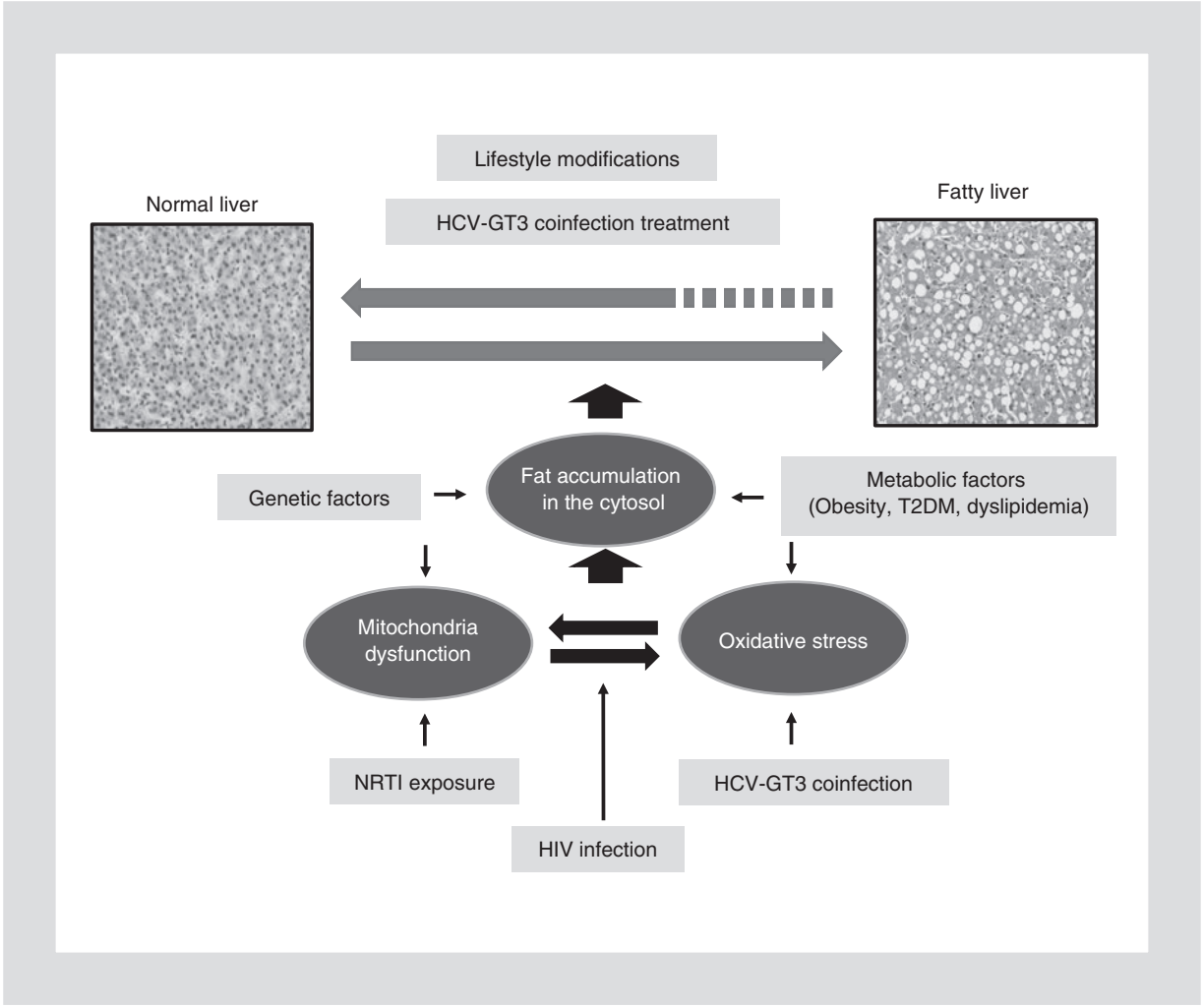

Figure 1. *Role of mitochondrial dysfunction and oxidative stress in the pathogenesis of NAFLD. Relationship with other pathogenic factors. GT3: genotype 3; NRTI: nucleoside reverse transcriptase inhibitor; T2DM: type 2 diabetes mellitus.*

46% in those studies[1,5]. The prevalence of NASH is estimated to be 3-5%[1,5]. These contradictory results may be due to uncontrolled selection biases, true variations among different populations, and different sensitivity of the screening tests. Studies conducted in individuals referred to tertiary care centers are usually based on liver biopsy or, alternatively, cumbersome imaging techniques such as liver magnetic resonance or spectrophotometry. Not surprisingly, those studies have reported higher rates of NAFLD[1].

Risk factors for NAFLD

The main risk factors for NAFLD in the general population are metabolic factors. The strongest ones are obesity, type 2 diabetes mellitus (T2DM), and dyslipidemia (Fig. 1). The prevalence of NAFLD increases to 80-90% in obese adults, 60% in patients with hyperlipidemia, and 69% in those with T2DM[1,2].

The prevalence of NAFLD varies with ethnicity. In the USA, Hispanics have the highest prevalence of NAFLD, followed by non-Hispanic Whites, with the lowest rate reported in African Americans[1]. In Asia, the situation seems to be equivalent to that of the West, with reported rates of approximately 30%, despite the lower body mass index (BMI) in Asians[2]. A genetic cause underlies this fact. The I148M allele (rs738409) of the patatin-like phospholipase domain containing the 3 (*PNPLA3*) gene is more prevalent in Hispanics (49%), the most susceptible group to NAFLD, whereas lower frequencies are observed in Caucasians (23%) and African Americans (17%)[1]. Another variant of the same gene, *PNPLA3* -S453I (rs6006460), which is common in African Americans

(10%) but rare in European Americans (0.3%) and Hispanics (0.8%), is associated with significantly lower liver fat content. These two sequence variations account for 72% of the observed ethnic differences in hepatic fat content[1].

Diagnosis of NAFLD

The definition of NAFLD requires the evidence of HS, either by imaging procedures or by histology, and that there are no causes for secondary hepatic fat accumulation, such as significant alcohol consumption, use of steatogenic medication, or hereditary disorders[2,3]. The criterion for considering HS as non-alcoholic is a daily alcohol consumption below 20 g in women and 30 g in men[2,3]. NASH is defined as the presence of HS and inflammation with hepatocyte injury (ballooning) with or without fibrosis[2,3].

Liver biopsy remains the gold standard for definitive NAFLD diagnosis as it allows determining steatosis, inflammation, and fibrosis[2,3]. Liver biopsy limitations are manifold and include sampling error, complications of the procedure, interobserver variability, high cost, and limited availability[2,6]. These limitations may make biopsy a non-recommended first-line diagnostic tool. In addition, liver biopsy is unsuitable to follow-up the course of NAFLD as it would be a repeated invasive approach. Given that steatosis and fibrosis could be assessed and followed by non-invasive blood tests and transient elastography (TE), liver biopsy might be justified only for selected cases, i.e. in those with suspicion of severe NAFLD lesions not clarified by non-invasive testing. However, the precise diagnosis of NASH still needs histologic evaluation, as non-invasive methods cannot fully identify the characteristics of NASH.

Non-invasive diagnosis of hepatic steatosis

There are two kinds of non-invasive diagnostic procedures for the diagnosis of HS: blood markers and imaging tests. Nearly 80% of patients with HS have normal-range ALT levels (males < 40 IU/l and females < 31 IU/l). In addition, ALT values do not correlate with histological findings. Because of this, ALT levels are unhelpful in both the diagnosis and grading of disease severity of NAFLD[7]. In cases having abnormal liver function tests, mildly raised alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels and/or gamma-glutamyl transferase (GGT) are usually observed.

A number of panels based on blood tests have been proposed to predict HS[8]. However, their performance is not better than that of imaging tests[8]. SteatoTest® incorporates 12 variables in an undisclosed formula, including a2-macroglobulin, haptoglobin, and apolipoprotein A1. It showed a negative predictive value (NPV) of 93% and positive predictive value (PPV) of 63% in a French cohort[9]. The results were validated in morbid obese subjects[10]. The fatty liver index is an algorithm derived from an Italian cohort[11], which was validated considering abdominal ultrasound (US) as the reference technique. The fatty liver index is calculated using BMI, waist circumference, triglycerides, and GGT. Its main indication is for epidemiological studies in an attempt to avoid US.

The NAFLD liver fat score was derived from a Finnish cohort using magnetic resonance spectroscopy as the reference test[12]. This score includes the presence of the metabolic syndrome, T2DM, fasting serum insulin, and AST/ALT ratio. It yielded 95% sensitivity (Se) and specificity (Sp). Variants in the *PNPLA3* rs738409 improved the accuracy for the prediction in less than 1%. When imaging tests are not available, this might be a test to consider in clinical practice.

Among imaging techniques, US, controlled attenuation parameter (CAP) and computed tomography (CT)- or magnetic resonance imaging (MRI)-based techniques are used for the detection of HS. Ultrasound is routinely available, applicable in most patients, and easy to use. A meta-analysis reported that US had a Se of 85% and an Sp of 94% for the detection of NAFLD[13]. However, US can detect HS only if more than 33% hepatocytes are steatotic. Thus, US imaging cannot identify moderate or mild HS.

Controlled attenuation parameter is a technique for the measurement of HS integrated into TE. A meta-analysis of nine studies including 11 patient cohorts showed Se and Sp of 78 and 79% for ≥ S1 (11-33% steatotic hepatocytes), 85 and 79% for ≥ S2 (34-66% steatotic hepatocytes), and 83 and 79% for S3 (> 66% steatotic hepatocytes), respectively. Diagnostic accuracies were 82% for ≥ S1, 87% for ≥ S2, and 86% for S3[14]. The cutoff of 238 dB/m to define the presence of HS involving < 10% of hepatocytes has been considered the optimal cutoff to discriminate S1[15,16]. It has shown a NPV of 81% for ≥ S1 in a prospective study[16]. CAP cut-offs yielding PPV > 80% have been described: ≥ 263 dB/m for ≥ S1; ≥ 311 dB/m for ≥ S2; and ≥ 400 dB/m for S3[16]. However, CAP cut-offs to define HS stages have varied among studies[14]. In addition, one of the main issues

Table 1. Diagnosis of advanced fibrosis in NAFLD patients[20]

Non-invasive test	Parameters	Cut-off	Se (%)	Sp (%)	PPV (%)	NPV (%)
Transient elastography, KPa	–	< 7.9 > 9.6	91.1 75.0	75.3 91.6	52.0 72.4	96.6 92.6
AST/ALT ratio	ALT, AST	< 0.8 > 1.0	39.7 20.6	79.7 90.1	37.9 39.4	80.9 78.4
APRI	AST/platelet count	0.5 1.5	65.1 6.3	72.3 97.0	42.3 40.0	86.9 76.9
FIB-4	Age, AST, ALT, platelet	< 1.30 > 2.67	65.1 20.6	80.2 95.5	50.6 59.1	88.0 79.4
NAFLD fibrosis score	Age, AST/ALT ratio, albumin, BMI, impaired fasting glucose or DM, platelet	≤ 1.455 > 0.676	73.3 18.3	69.5 96.3	43.6 61.1	89.0 78.6
BARD score	DM, BMI, AST/ALT ratio	2.0	61.9	65.8	36.1	84.7

ALT: alanine aminotransferase; APRI: AST to platelet ratio index; AST: aspartate aminotransferase; BARD: BMI, AST/ALT ratio, diabetes mellitus; BMI: body mass index; DM: diabetes mellitus; FIB-4: fibrosis-4 index.

of studies validating the use of CAP to predict HS is heterogeneity of populations. Thus, individuals with different etiologies of chronic liver diseases are pooled along with NAFLD patients in those studies[14]. For these reasons, CAP measurement needs further validation before being incorporated into clinical practice.

Hepatic steatosis can be diagnosed by CT- or MRI-based procedures. These are not routinely used in the assessment of HS because they are time-consuming, costly, associated with radiation exposure (in the case of CT), or limited in claustrophobic or big individuals (when using MRI). Nevertheless, MRI-determined proton density fat fraction and proton magnetic resonance spectroscopy (H-MRS) could be the most accurate non-invasive tools for the quantification of steatosis[2,17].

Non-invasive diagnosis of fibrosis in NAFLD

A number of panels of routinely available blood tests have been developed to predict fibrosis in the setting of NAFLD. They aim at discriminating advanced fibrosis (F ≥ 3) and generally reach NPVs > 80% with poor PPVs (Table 1).

Transient elastography (TE), FibroScan® (Echosens, Paris), can be applied to assess liver fibrosis by liver stiffness measurement. A meta-analysis has evaluated the diagnostic value of TE in determining liver fibrosis in different studies on chronic liver diseases. Depending on the range of pretest probability of fibrosis, spanning from 25 to 75%, the PPV for the detection of significant fibrosis (F ≥ 2) was 55-92% and for the detection of cirrhosis was 76-97%[18]. In a more recent meta-analysis and systematic review, the overall pooled estimates of the diagnostic accuracy of TE were: F ≥ 2, Se 79% and Sp 75%; F ≥ 3, Se 85% and Sp 85%; and F4, Se 92% and Sp 92%[19]. In a large prospective study of NAFLD patients, TE had high NPV and modest PPV to detect advanced fibrosis and cirrhosis[20]. The area under the receiver-operating characteristics curve of TE for F ≥ 3 was 0.93 and for F4 was 0.95. Liver stiffness measurement was not affected by HS, necroinflammation, or obesity. Discordance between TE and histology occurred in patients with short liver biopsy lengths and mild or no fibrosis. In addition, TE had superior performance to blood tests in diagnosing advanced fibrosis (Table 1). Measurement failures were observed in 25% of obese patients[20]. In this regard, the XL probe is associated with fewer liver stiffness measurement failures (1.1 vs. 16.0%) than the M probe and is accurate for the diagnosis of F ≥ 2 fibrosis and cirrhosis[7]. However, even with the XL probe, 10% of patients with a BMI > 28 kg/m^2 have a difference of ≥ 2 fibrosis stages between TE and liver biopsy[7].

Acoustic radiation force impulse is another imaging tool for non-invasive assessment of liver fibrosis. One of its advantages is its integration into a conventional

B-mode ultrasound machine, which allows non-invasive assessment of fibrosis during a standard US examination. The performance of acoustic radiation force impulse in NAFLD is similar to that in other chronic liver diseases, with diagnostic accuracies of 66-86% for significant fibrosis and 91-98% for advanced fibrosis and liver cirrhosis[2].

Non-invasive diagnosis of NASH

Unfortunately there is no available simple blood test or imaging modality that can differentiate simple HS from NASH. The risk of NASH increases with the number of metabolic risk factors. Of centrally obese patients with hypertension and diabetes, 70% have NASH on liver biopsy[7]. Therefore, until accurate tests become available, metabolic risk factor profiling could be used to identify NASH patients for further investigations.

A number of blood biomarkers have been tested to diagnose NASH, such as cytokeratin (CK)-18, terminal peptide of procollagen III (PIIINP), interleukin-6 (IL-6), tumor necrosis factor (TNF)-α, chemokine monocyte chemotactic protein 1 (MCP-1), and RANTES or fibroblast growth factor 21 (FGF21)[2]. None of them is ready for clinical use; CK-18 is the most extensively validated. In a meta-analysis of 11 studies, CK-18 showed a pooled Se of 66% and Sp of 82% for the diagnosis of NASH[19]. When CK-18 level cut-offs optimized for Se were used, Se improved to 82%. Similarly, when cut-offs optimized for Sp were applied, SP reached 98%[19]. However, there is considerable variability in the suggested cut-offs and their respective diagnostic accuracy among studies. In clinical practice, this makes it very difficult to choose which threshold to use[19].

Progression of NAFLD

The natural history of NAFLD is largely unknown. The main reasons for gaps in the knowledge of NAFLD course are the methods applied to assess histologic progression. Thus, few paired liver biopsy studies have evaluated the histological changes in the NAFLD spectrum. In these studies, HS seemed to progress to NASH and cirrhosis infrequently[1,2]. In a cohort of 40 patients with simple HS after 11 years, 12 (30%) patients had abnormal liver tests, but none of them progressed to NASH or cirrhosis. Of the 14 (35%) deceased patients, none died of a liver-related cause[1]. Fibrosis progression seemed to be restricted to patients with NASH, whereas individuals with HS showed a benign course[1,2]. In this regard, it has been classically considered that individuals with HS infrequently develop cirrhosis; thus, it has been found that overall 1.2% of subjects with HS will end up developing cirrhosis, and death from liver-related causes will occur in only 0.6% of them after approximately 20 years[2,21]. In contrast, once NASH is present, fibrosis progression happens commonly. It is estimated that fibrosis progresses in 20-40% individuals with NASH, leading to cirrhosis in up to 30% of cases[2]. After roughly 20 years, liver-related mortality rises to 18% for those with NASH[1]. However, more recent studies have shown that HS could evolve to NASH and fibrosis in a proportion of patients higher than previously reported[2,22]. Among 108 patients with a follow-up of 6.6 years, progression to NASH was seen in 44% of patients with baseline HS[22]. Strikingly, no significant difference in fibrosis progression was found comparing individuals with HS and NASH at baseline, namely 37 vs. 43%, respectively. All patients with baseline HS who showed fibrosis progression had NASH on the follow-up biopsy[22]. These results are in agreement with two smaller previous studies[2]. Finally, in a meta-analysis of studies on follow-up biopsies, fibrosis progressed one stage after 14 years among subjects with HS and after seven years among those with NASH at baseline[23]. This means a non-negligible rate of fibrosis progression for patients with HS, albeit slower than for NASH. Aside from the low sample size of studies with paired liver biopsies, the main limitation of those studies is the selection bias inherent in the decision to perform a second biopsy. Indeed, patients with follow-up biopsies could have been mainly those with suspected progressive disease.

In studies with more representative population-based samples, NAFLD has usually been evaluated using US or liver enzymes, both inaccurate methods. In the National Health and Nutrition Examination Survey (NAHNES), the presence of NAFLD was assessed using US among 11,154 participants in the USA[24]. Baseline blood tests, NAFLD fibrosis score, AST to platelet ratio index (APRI), and FIB-4 score were used to stage fibrosis. Patients with advanced fibrosis had an increased likelihood of death. However, the increase in the risk of death was almost entirely due to cardiovascular causes. This result is in agreement with other studies on the natural history of liver-biopsy proven NAFLD[2].

Table 2. Prevalence of and factors associated with fatty liver in HIV infection

Ref.	n	Diagnosis	HIV/HCV coinfection	Fatty liver	Factors independently associated with fatty liver	Comments
[73]	112	Biopsy	100%	40%	Caucasian race, weight > 86 kg, hyperglycemia, d4T use	
[44]	183	Biopsy	100%	69%	Use of ddI or d4T, HCV-GT3	
[51]	395	Biopsy	100%	61%	BMI, HCV-GT3, HCV VL, fibrosis, ferritin	HCV-GT3: BMI, HCV VL. Non-HCV-GT3: BMI, fibrosis, ferritin
[74]	154	Biopsy	100%	72%	TG, ART for ≥ 4 years	UVA metabolic factors, CD4
[75]	137	Biopsy	100%	67%	Infl activity	
[76]	148	Biopsy	100%	67%	BMI, HCV-GT3, infl activity	
[50]	283	Biopsy	100%	23%	HCV-GT3, HDL, hip circumference, infl activity, ALT, FPG, HCV VL	Non-HCV-GT3: BMI, TG, ZDV, ddX, infl activity HCV-GT3: HCV VL, BP, cholesterol, infl activity
[77]	222	Biopsy	100%	23%	BMI, T2DM, HCV-GT3:	
[78]	225	US	40%	108/830 (13%)	BMI, alcohol, lipohypertrophy, HIV VL, HCV VL	108 cases with severe steatosis-117 controls, of 830 patients with US
[27]	216	US/biopsy	Excluded	31% AA 14% vs. Caucasians 35%	WC, TG, HDL	BMI highly correlated with WC and excluded from MVA
[28]	225	CT	Excluded	37%	WC, ALT/AST ratio, male sex, NRTI	BMI and WC both included in MVA
[26]	465	CT	12%	13%	VAT, HOMA, ddX, PNPLA3, ALT	
[29]	505	CAP	32%	40%	BMI	UVA with metabolic factors
[68]	300	CAP	Excluded	48%	BMI and ALT	

AA: African Americans; ALT: alanine aminotransferase; ART: antiretroviral therapy; AST: aspartate aminotransferase; BMI: body mass index; BP: blood pressure; CT: computed tomography; d4T: stavudine; ddI: didanosine; ddX: dideoxynucleosides; FPG: fasting plasma glucose; GT3: genotype 3; Infl activity: histologic inflammatory activity; MVA: multivariate analysis; NRTI: nucleoside reverse transcriptase inhibitors; Ref.: references; TG: triglycerides; US: ultrasonography; UVA: univariate associations; VAT: abdominal visceral adipose tissue volume; VL: viral load; WC: waist circumference.

NAFLD in HIV infection

Prevalence

Studies evaluating the prevalence of HS in HIV-infected individuals have yielded contradictory results. Thus, a large range of frequencies of HS has been reported, from 15 to 72%, in HIV infection (Table 2)[25-30]. This variability approaches that observed in general population studies[1,5]. However, there are no direct comparisons between HIV-infected patients and a population-based sample from the same area. To our knowledge, only one study roughly approximated that comparison[26]. In this study, 254 HIV-uninfected and 465 HIV-infected men who have sex with men (MSM) were included. The prevalence of HS measured by CT was 19% for MSM without HIV infection and 13% for MSM with HIV infection. These rates of HS are at the lower end of those reported for the general population[1,5] and HIV-infected patients (Table 2). This finding

may be partly explained by the large proportion of African American MSM participating in the study, as black race may protect from HS[1,27], as stated above. In this regard, the selection of MSM participants and the racial mixture of this study are limitations that preclude drawing generalizable conclusions. Thus, studies that compare the prevalence of and factors associated with HS in HIV-infected patients vs. the general population are necessary.

HIV infection, antiretroviral therapy, and NAFLD

Mitochondria have a central role in the first steps of NAFLD pathophysiology (Fig. 1). Thus, loss of mitochondrial function affects the fatty acid beta-oxidation, which leads to the accumulation of fatty acids in the cytosol. This effect is enhanced when there are higher circulating levels of fatty acids, which may determine an increased demand of mitochondrial oxidation[31]. In fact, beta-oxidation has been found to be impaired in patients with fatty liver[32]. Drugs that inhibit beta-oxidation enzymes can lead to microvesicular HS. In addition, abnormal mitochondrial function initiates the production of reactive oxygen species, which induces oxidative stress and liver injury, and decreases beta-oxidation of fatty acids, leading to accumulation of fat in the cytosol[33].

The HIV infection itself could alter mitochondrial function. Although the mechanism is not well established, the increased oxidative stress observed in HIV patients[34] could underlie this effect. Loss of mitochondrial DNA (mtDNA) has been documented in peripheral blood mononuclear cells (PBMC) from antiretroviral therapy (ART)-naive patients[35,36]. This loss of mtDNA has been suggested to occur in the liver of HIV-infected individuals[37]. Studies comparing the prevalence of HS in HIV-infected ART-naive patients with individuals from the general population would be necessary to further support this hypothesis.

Nucleoside reverse transcriptase inhibitors (NRTI) may produce mitochondrial toxicity[38]. The molecular mechanisms underlying this toxicity are not clearly defined and can be different from drug to drug. Most NRTIs affect mitochondrial replication though the inhibition of the mtDNA polymerase pol-gamma[38]. Other mechanisms of mitochondrial injury, such as inhibition of mitochondrial RNA expression or inhibition of the mitochondrial adenylate kinase and adenosine nucleotide translocator, have been proposed for these drugs[38-40]. Currently used NRTIs, such as tenofovir or abacavir, cause less mitochondrial toxicity[41]. Other anti-HIV drugs such as protease inhibitors (PI) and non-NRTIs (NNRTI) also seem to interfere with the mitochondria[38]. In particular, efavirenz induces bioenergetic stress in hepatic cells by inhibiting mitochondrial function through an independent mtDNA replication mechanism[38].

Most studies have failed to find an association between HS and ART or individual antiretroviral drugs (Table 2). A paired liver biopsy study even found that reduced progression of HS was associated with ART[42]. Cumulative exposure to dideoxynucleoside analogs was independently associated with an increased likelihood of HS in both HIV/HCV-coinfected and HIV-monoinfected individuals in some studies[26,28,43,44]. The use of efavirenz was associated with an increased risk of HS progression in a paired biopsy study among HIV/HCV-coinfected individuals, but this relationship did not stand multivariate analysis[43]. Efavirenz use also showed a trend to an association with increases in CAP values during follow-up[45]. It is conceivable that drugs with a safer metabolic profile, such as maraviroc or integrase inhibitors, might reduce the risk of HS. However, no association of HS with such antiretroviral drugs remained after controlling for metabolic factors[29,45]. A clinical trial designed to clarify the potential effect of efavirenz vs. raltegravir on HS is underway (Clinicaltrials.gov ID: NCT01900015).

HCV coinfection

Hepatic steatosis is frequently found in chronic HCV infection[46]. The interaction between the viral cycle and the host lipid metabolism is involved in the development of HS, and many aspects of this interaction have been investigated (Fig. 1)[47,48].

In a large meta-analysis, monoinfected patients harboring HCV genotype 3 (HCV-GT3) showed a higher probability of HS compared with patients infected with other HCV genotypes[49]. Overall, about 70% of HCV-GT3-infected patients suffered from HS versus 30% of non-genotype 3-monoinfected patients[48,49]. Some studies in HIV/HCV coinfection have similarly reported an association of HS with HCV-GT3 (Table 2). In fact, in a meta-analysis only pooling data from studies with a prevalence of HCV-GT3 > 30%, an association between HS and HCV-GT3 infection was found[25]. In the setting of HCV-GT3 coinfection, HS has been related with HCV replication (Table 2). Indeed, HS may disappear after successful interferon-based therapy against

Table 3. Single nucleotide polymorphisms associated with NAFLD in genome-wide studies performed in HIV-uninfected populations

SNP	Allele 1/ Allele 2	Chr	Gene	References
rs738409	G*/C	22	*PNPLA3*	[55,59-62]
rs1227756	A/G*	10	*COL13A1*	[58]
rs887304	G/A*	12	*EFCAB4B*	
rs6591182	T*/G	11	*EHBP1L1*	
rs2645424	C/T*	8	*FDFT1*	[55]
rs780094	G/A*	2	*GCKR*	
rs12137855	C*/T	1	*LYPLAL1*	
rs2228603	C/T*	19	*NCAN*	
rs4240624	A*/G	8	*PPP1R3B*	
rs222054	G/C*	4	*GC*	[57]
rs7324845	A*/G	13	*LCP1*	
rs12743824	C*/A	1	*LPPR4*	
rs11864146	G/A*	16	*SLC38A8*	
rs58542926	C/T*	19	*TM6F2*	[62]
rs5764455	G/A*	22	*PARVB*	[60]
rs6006473	C/T[a]	22	*PARVB*	
rs6006611	G*/A	22	*PARVB*	
rs2143571	G/A*	22	*SAMM50*	
rs3761472	T/C*	22	*SAMM50*	
rs738491	C/T*	22	*SAMM50*	

*Risk allele reported.
Chr: chromosome; *COL13A1*: collagen alpha-1 (XIII) chain isoform 15; *EFCAB4B*: EF-hand calcium binding domain 4B; *EHBP1L1*: EH domain-binding protein 1-like protein 1; *FDFT1*: farnesyl-diphosphate farnesyltransferase 1; *GC*: vitamin D-binding protein; *GCKR*: glucokinase (hexokinase 4) regulator; *LCP1*: lymphocyte cytosolic protein 1; *LPPR4*: lipid phosphate phosphatase-related protein type 4; *LYPLAL1*: lysophospholipase-like 1; *NCAN*: neurocan; *PARVB*: parvin beta; *PNPLA3*: patatin-like phospholipase domain containing 3; *PPP1R3B*: protein phosphatase 1, regulatory subunit 3B; *SAMM50*: sorting and assembly machinery component 50 homolog; *SLC38A8*: putative sodium-coupled neutral amino acid transporter 8; SNP: single nucleotide polymorphism; *TM6SF2*: transmembrane 6 superfamily member 2.

HCV-GT3 in HIV-coinfected patients[50]. In contrast, the characteristics of HS associated with other HCV genotypes may be similar to those observed in metabolically induced HS[50-52].

The natural history of HCV is altered by HIV infection, with a faster histological and clinical progression[53,54]. However, this does not seem to be the case for HS. A meta-analysis comprising 1,989 coinfected patients and 1,540 monoinfected individuals reported no differences in the prevalence of HS between these populations[25]. In a large study, HS rates were similar for the whole study group and for HCV/HIV-coinfected patients. Moreover, HS among HIV/HCV-coinfected individuals was related with the same metabolic factors as the global study population[29]. No specific HCV-associated variable was associated with HS in that study.

Genetic factors

The development of NAFLD in the HIV-uninfected population is strongly influenced by genetic factors (Fig. 1)[55,56]. Several genome-wide association studies (GWAS) have been carried out in different ethnic groups to analyze the genetic basis of NAFLD[55,57-62]. These studies identified some single nucleotide polymorphisms (SNP), which are located within or close to different genes, independently associated with NAFLD (Table 3). Among them, the association between rs738409 within the *PNPLA3* gene and NAFLD was the most uniformly replicated in the general population of different ethnicities[55,59-62]. In spite of this, the magnitude and strength of the effect of the variant of NAFLD varied widely among populations of similar ethnic background[63]. Interestingly, PNPLA3 is a lipase highly expressed in human liver and adipose tissue. The *PNPLA3*_rs738409 SNP modifies the enzyme catalytic binding site, leading to cellular triglyceride accumulation[64]. This mechanism could explain the etiology of HS and its relation to *PNPLA3*_rs738409 SNP. However, this hypothesis has not been confirmed in any *in vivo* experiment performed so far[65].

There are few studies focused on the genetics of HS in HIV infection. The SNPs linked to NAFLD in the

general population, such as *PNPLA3*, *NCAN*, *GCKR*, *LYPLAL1,* and *PPP1R3B*, were evaluated in the MACS cohort[26]. The *PNPLA3*_rs738409 variant was related to HS in HIV-infected MSM, but not in HIV-uninfected MSM[26]. This association in HIV infection was not replicated in a larger sample of patients[30]. This contradictory result could be due to the different proportion of HCV-coinfected individuals in those studies. Moreover, other authors have reported a lack of association between this SNP and HS in HIV/HCV-coinfected patients[66,67].

Recently, the association of almost all SNPs previously found to be related with NAFLD by GWAS in the general population was subject to validation in HIV-infected individuals[30]. In this study, an independent association with HS was found for rs12743824 and rs738491 linked to *LPPR4* and *SAMM50* genes, respectively. These results need to be validated by other authors. In fact, further studies, including specific GWAS in the HIV-infected population, are necessary to completely explore the genetic basis of NAFLD in this setting.

Metabolic factors

As mentioned above, metabolic abnormalities are strongly associated with NAFLD in the general population (Fig. 1). In HIV infection, a variety of metabolic factors were associated with HS measured by CAP in a large prospective cross-sectional study[29]. However, only BMI was related with HS after multivariate analysis[29]. In another large prospective cross-sectional study, again BMI was independently associated with HS measured by CAP[68]. Other studies have linked the likelihood of HS to waist circumference, abdominal visceral adiposity, serum triglyceride levels, high-density lipoprotein levels or insulin resistance (Table 2). In HIV/HCV coinfection, metabolic factors associated with HS evaluated by biopsy were increased weight and T2DM[25].

In studies conducted in HIV/HCV coinfection with paired liver biopsies, BMI[42] and fasting plasma glucose levels[43] were associated with HS progression. More recently, increases in liver steatosis measured by CAP were only associated with elevations of BMI in HIV-infected individuals, with or without HCV coinfection[45]. All the findings confirm an important role of metabolic factors, mainly BMI, in the development and progression of HS in the HIV-infected population.

Management of NAFLD

Treatment

First-line therapy for NAFLD includes lifestyle modification, aiming at weight loss through diet, exercise, and behavioral changes (Fig. 1)[3]. Lifestyle interventions are safe and improve insulin resistance, which is considered a major mechanism in the pathogenesis of NAFLD. A meta-analysis of randomized clinical trials on diet and exercise showed a consistent benefit for patients who achieved the targeted weight loss. Improvements in transaminase levels and histological parameters (e.g. steatosis, hepatocyte ballooning, necroinflammation) were observed[69]. A significant reduction in histological severity of NASH was associated with a weight loss of more than 7% sustained over 48 weeks[69], suggesting that the degree of weight loss may be important. However, lack of patient compliance and relapse are the main limitations of this approach.

Bariatric surgery is the most reliable method for achieving substantial, sustained weight loss in patients with morbid obesity. Recent studies have shown an improvement in NASH components and fibrosis in paired liver biopsies conducted before and 1-2 years after surgery[2].

As NAFLD is frequently associated with obesity, T2DM, arterial hypertension and dyslipidemia, optimal management of the associated diseases is essential and one of the first steps in the management of NAFLD, in order to minimize the risk for metabolic and cardiovascular complications. In patients whose lifestyle modifications fail or in those with advanced disease, a liver-targeted pharmacotherapy might be needed[2,3]. However, no pharmacological treatment targeting NASH has been approved so far.

Several pharmacological agents like metformin, statins, angiotensin II receptor antagonists, polyunsaturated fatty acids, ursodeoxycholic acid, and pentoxifylline have been tested for NAFLD. However, none of these agents has proven to lead to a significant histological improvement[2].

Thiazolidinediones and vitamin E are therapies with the widest evidence of potential benefit in NASH patients[2,3]. In a clinical trial, adults with NASH, without cirrhosis and without T2DM, were randomized to receive pioglitazone, vitamin E, or placebo for 96 weeks[70]. The primary end-point was histological improvement of at least two points in the NAFLD activity score (NAS) with no worsening of fibrosis. Pioglitazone significantly improved each individual component of NAS, but it

did not achieve a statistically significant effect compared with placebo. Vitamin E compared to placebo showed significantly more often an improvement of at least two points in NAS. However, a significant improvement in fibrosis could not be detected with either pioglitazone or vitamin E. In addition, there are safety issues for both pioglitazone and high-dose vitamin E. Taking all these facts into account, the use of both drugs should be individualized in patients with NASH[3].

Few clinical trials have assessed drug therapy for NAFLD in HIV infection. In a phase IIa clinical trial including HIV-infected patients with abdominal fat accumulation, tesamorelin, a growth hormone-releasing analog, was associated with reductions in visceral fat[71]. Modest reductions in HS measured by MRI were also observed in that trial. A specific phase IIa clinical trial to evaluate the effects of tesamorelin on HS determined by MRI in HIV-infected patients is underway (clinicaltrials.gov ID: NCT02196831). A synthetic conjugate of arachidic acid and cholic acid, a fatty acid plus a bile acid (Aramchol®), showed reductions in MRI-measured HS in a phase IIa clinical trial and is entering phase IIb now (clinicaltrials.gov ID: NCT02279524). A proof-of-concept, investigator-initiated, phase IIa trial testing the effect of Aramchol® in HIV-infected patients with lipodystrophy and NAFLD is currently recruiting patients (clinicaltrials.gov ID: NCT02684591). To date, there is no data on the efficacy of drug therapy to manage NASH in HIV infection.

Prevention

The prevention of NAFLD should be a result of the prevention of obesity and associated metabolic conditions. Environmental factors, such as excessive intake of calories, processed food, and a sedentary lifestyle, play a key role in liver fat accumulation, NAFLD, and progressive liver disease. In addition, non-modifiable inherited factors are known to contribute to the predisposition to NAFLD. Thus, children with a high risk for adult NAFLD could be identified early in life and undergo interventions to reduce excess adiposity and insulin resistance[72]. In one study, metabolic factors at childhood, such as high insulin levels and high BMI, along with male sex and low birth weight, were predictors of adulthood NAFLD. In that study, taking into account genetic variants in *PNPLA3* and *TM6SF2* genes enhanced the prediction of adult NAFLD[72].

In HIV infection, prevention of NAFLD should follow the same principles as prevention of NAFLD in the general population. Secondary HS associated with ART is not epidemiologically relevant nowadays because ART with greatest mitochondrial toxicity has been abandoned. The potential role of efavirenz *in vivo* still needs elucidation. Secondary HS mediated by HCV-GT3 will unlikely be a problem in the near future as very effective treatment against HCV infection is now available. Finally, alcohol abuse, which is generally difficult to quantify precisely, needs to be taken into account as a cause of secondary HS.

Conclusions

Hepatic steatosis is frequent in HIV infection. In studies among HIV/HCV-coinfected individuals, mostly carried out in patients selected to undergo liver biopsy, there was probably an important contribution of viral and drug or toxic-related causes on HS (Table 2). However, in studies measuring HS by non-invasive methods, with larger and unselected study populations, the characteristics of HS closely correspond to those of NAFLD in the general population (Table 2). It is unclear whether NAFLD prevalence is higher in HIV infection than in the general population, but metabolic risk factors seem similar for both groups. Accordingly, the management of NAFLD in HIV infection should primarily address obesity, T2DM, and dyslipidemia.

The natural history of NAFLD in HIV infection is unclear. There are no data on the rates of progression to end-stage liver disease and hepatocellular carcinoma. In this regard, information on liver outcomes in HIV-infected patients has been mostly determined by the presence of HCV coinfection. Once HCV eradication is reached in the near future, the true impact of NAFLD on morbidity and mortality could be ascertained. Long-term prospective cohort studies could clarify these issues. However, a better understanding of NAFLD prognosis in HIV infection will require improved non-invasive diagnostic tools to accurately detect the emergence of NASH and the progression of fibrosis.

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