



## ORIGINAL ARTICLE

# Metabolic dysfunction-associated steatohepatitis exhibits sex differences in people with HIV

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## Abstract

**Objectives:** People with HIV are at increased risk for metabolic dysfunction-associated steatohepatitis (MASH). Although sex differences are documented in the general population, their role in the context of HIV is less understood.

**Methods:** This was a multicentre cohort study including people with HIV without viral hepatitis coinfection. A FibroScan-AST (FAST) score >0.35 was used to diagnose MASH with significant liver fibrosis (stage F2–F4). We investigated sex-based differences in MASH trends as a function of age using a segmented linear mixed-effects model. Random effects accounted for clustering by the four sites. Adjusted models included ethnicity, diabetes, hypertension, and detectable HIV viral load.

**Results:** We included 1472 people with HIV (25% women). At baseline, the prevalence of MASH with fibrosis by FAST score was lower in women than in men (4.8% vs. 9.2%,  $p = 0.008$ ). Based on the adjusted model, male sex (+0.034;  $p = 0.04$ ), age per year (+0.003;  $p = 0.05$ ), detectable HIV viral load (+0.034;  $p = 0.02$ ), and hypertension (+0.03;  $p = 0.01$ ) were positively associated with MASH with fibrosis. Although men exhibited generally higher FAST scores, FAST scores increased in women during the critical biological age of presumed perimenopause to menopause (between 40 and 50 years), reaching levels similar to those in men by the age of 55 years.

**Conclusion:** Despite women with HIV having a lower prevalence of MASH with fibrosis than men, they exhibit an acceleration in FAST score increase around the perimenopausal age. Future studies should target adequate consideration of sex differences in clinical investigation of metabolic dysfunction-

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associated steatotic liver disease to fill current gaps and implement precision medicine for people with HIV.

#### KEYWORDS

FAST score, liver fibrosis, MASLD, perimenopause, sexual hormones, transient elastography

## INTRODUCTION

The advent of effective antiretroviral therapy (ART) has transformed HIV infection from a life-threatening condition to a chronic disease with manageable outcomes [1]. However, this prolonged lifespan has unveiled a spectrum of non-communicable comorbidities, among which metabolic dysfunction-associated steatotic liver disease (MASLD) is a significant concern [2]. MASLD is the most frequent chronic liver disease globally, affecting 32% of the general adult population [3, 4]. In people with HIV, MASLD is a prominent comorbidity, with a prevalence ranging between 25% and 55% across various countries [5, 6]. Importantly, higher rates of metabolic dysfunction-associated steatohepatitis (MASH) and liver fibrosis related to MASLD have been reported in people with HIV [7, 8]. Although the aetiology of MASLD in the general population is multifactorial, encompassing genetic predisposition, lifestyle factors, and metabolic syndrome components, the presence of HIV introduces additional layers of complexity that remain only partially understood. The intersection of HIV infection, ART, and metabolic dysregulation creates an intricate milieu that likely predisposes individuals to develop MASH with associated liver fibrosis [5].

Sex differences, encompassing biological, hormonal, and behavioural variations between men and women, play a significant role in the development and progression of metabolic diseases. Several studies in the general population have reported that the prevalence and severity of MASH is higher in men than in women during the reproductive years. However, MASH seems to occur at a higher rate in women after menopause, suggesting that oestrogen is protective. Sex differences also exist for the major risk factors of MASH with associated liver fibrosis, such as type 2 diabetes [9, 10]. Sex at birth, sex hormones, and gender habits interact with numerous factors, including cytokines, stress, and environmental factors, and alter the risk profiles and phenotypes of MASH [10]. In addition, liver cirrhosis and hepatocellular carcinoma exhibit sex differences, with a significantly higher incidence in men [9, 10].

The influence of sex on the pathogenesis and progression of MASH in people with HIV remains poorly understood yet may hold profound implications for personalized healthcare delivery. In the specific context

of HIV infection, sex-related factors may exert differential effects on hepatic lipid metabolism, insulin resistance, and immune-inflammatory responses, thereby modulating disease susceptibility and progression [11]. Additionally, socio-cultural factors, healthcare-seeking behaviours, and treatment adherence patterns may further shape the landscape of MASH in people with HIV across diverse gender identities. Finally, menopause tends to occur earlier in women with HIV than in the general population, potentially leading to a shorter lifespan of exposure to the protective benefits of oestrogens on the liver [12].

In an international cohort collaboration of people with HIV undergoing screening for hepatic steatosis and liver fibrosis with transient elastography, we aimed to determine sex-based differences in MASH trends as patients age. To achieve this, we employed the FibroScan-AST (FAST) score to diagnose MASH with significant liver fibrosis [13, 14].

## PATIENTS AND METHODS

### Study design and population

We conducted a cross-sectional retrospective analysis of four cohorts of people with HIV mono-infection: the LIVER disease in HIV (LIVEHIV) cohort [15] at the McGill University Health Centre, the Modena HIV Metabolic Clinic (MHMC) cohort, the Liver Pathologies in HIV in Palermo cohort, and the Royal Free Hospital cohort. Between January 2015 and December 2022, participants were identified through locally maintained prospective databases of people with HIV who underwent screening for MASLD. We included consecutive patients with confirmed HIV infection on ART and aged  $\geq 18$  years, with availability of liver stiffness measurement (LSM), controlled attenuation parameter (CAP) by vibration controlled transient elastography (Fibroscan<sup>®</sup>, Echosens, Paris, France), and relevant clinical and biochemical parameters. Exclusion criteria were (i) positivity for hepatitis C virus (HCV) antibody or hepatitis B virus surface antigen; (ii) evidence of other liver diseases; (iii) significant alcohol intake, defined as  $>30$  g/day in men

and >20 g/day in women [16]; and (iv) contraindications to and failure or unreliable measurement of transient elastography.

## Ethics

All participants provided informed written consent. The Research Ethics Board of the Research Institute of McGill University Health Centre (study code 14-182-BMD) and of MHMC (study code 254/12), the Ethics Committee of the “Paolo Giaccone” University Hospital (study code v.1.05.1.18), and the Royal Free Hospital ethical committee approved the study. The study was conducted according to the Declaration of Helsinki, and the manuscript was prepared according to the STROBE Statement checklist of items.

- high-density lipoprotein cholesterol <1.03 mmol/L (men) or <1.30 mmol/L (women) or lipid-lowering therapy [11].

## Transient elastography examination and FAST score

Transient elastography examinations were performed on patients after they had fasted for 4 hours by a maximum of two operators at each site using standard quality criteria [17]. A cut-off of LSM  $\geq 7.1$  kPa was used to define significant liver fibrosis, corresponding to histologic stage F2–F4 out of 4 [18, 19]. The FAST score was calculated with CAP, LSM, and aspartate transaminase (AST) level as previously described:

$$\text{FAST score} = \frac{\exp[-1.65 + 1.07 \times \ln(\text{LSM}) + 2.66 \times 10^{-8} \times \text{CAP}^3 - 63.3 \times \text{AST}^{-1}]}{1 + \exp[-1.65 + 1.07 \times \ln(\text{LSM}) + 2.66 \times 10^{-8} \times \text{CAP}^3 - 63.3 \times \text{AST}^{-1}]}$$

## Clinical and biological parameters

We collected data within 3 months from transient elastography, namely demographic information, time since HIV diagnosis (defined as the interval between the date of patient's first positive HIV test and the date of the visit), current exposure to ART classes (non-nucleoside reverse transcriptase inhibitors, protease inhibitors, integrase strand transfer inhibitors, nucleoside reverse transcriptase inhibitors), body mass index (BMI), liver serum biomarkers, lipid profile, and haematological and immune-virological parameters. Gender information in the cohorts was collected through self-reporting. Undetectable viral load was defined as HIV viral load <50 copies/mL. Sexual hormones, including estradiol, follicle-stimulating hormone (FSH), luteinizing hormone (LH), and total testosterone, were available for a subgroup of 323 patients. MASLD was defined as the presence of hepatic steatosis, defined as CAP  $\geq 270$  dB/m [13, 14], plus at least one of the following criteria:

- BMI  $\geq 25$  kg/m<sup>2</sup>
- previous diagnosis or treatment for type 2 diabetes
- blood pressure  $\geq 130/85$  mmHg or treatment for hypertension
- triglycerides >1.69 mmol/L or lipid-lowering therapy

FAST scores were categorized by 0.35 cut-off, which was proposed to rule out MASH with significant fibrosis. Specifically, a FAST score  $\leq 0.35$  has a sensitivity of 90% and specificity of 50% for MASH with F2–F4 fibrosis and non-alcoholic fatty liver disease (NAFLD) activity score  $\geq 4$  [13].

## Outcome measures

The primary study outcome was the FAST score, defined as a continuous variable. Based on the above-mentioned cut-off value of the FAST score, we also computed the prevalence of MASH with fibrosis.

## Statistical methods

We investigated sex-based differences in MASH with fibrosis trends as patients age. Because of the heterogeneous diets, lifestyles, and healthcare policies at each of the international centres, we used a random-effects model. This clustering considers patients within the same site as replicates that are more alike than subjects from other sites by introducing a random intercept. Since our hypothesis was that aging could play a role in the sex difference in MASH with fibrosis trends, and there may be a critical breaking point during menopause for women, we

extended our mixed-effects model to a segmented mixed-effect model. A segmented model allowed us to estimate a breakpoint where the slope could change. Models were adjusted for diabetes, hypertension, and having detectable HIV viral load. These covariates were selected a priori after group discussion and ensured no collinearity between variables. We excluded BMI from the model because of its collinearity with diabetes. Diabetes was chosen instead, based on previous literature demonstrating its strong association with histological advanced liver fibrosis in people with HIV [20]. Furthermore, BMI may be a less reliable predictor of MASH in the context of HIV, given the relatively high prevalence of lean MASLD in this population [21, 22]. We used a pairwise deletion analysis, with missing values <10% for included variables, and a two-sided level of significance of 0.05. All analyses were conducted using R.

## RESULTS

After applying the exclusion criteria, the combined cohort of 1472 people with HIV included patients from the LIVEHIV (32%), MHMC (41%), Liver Pathologies in HIV (23%), and Royal Free Hospital (4%) cohorts (Figure 1). The cohort included 373 women with HIV, representing 25% of the whole study population. Overall mean  $\pm$  standard deviation age was  $51.8 \pm 9.9$  years, the median CD4 cell count was 698 (interquartile range 494–844) cells/ $\mu$ L, and undetectable HIV viral load was observed in 1223 (83.1%) of people with HIV. The overall prevalence of MASLD by CAP was 25%. The prevalence of MASH with significant liver fibrosis by FAST score was 8.1%. The characteristics of the whole cohort are summarized in Table 1. When compared with men,

women with HIV were more frequently of non-white ethnicity and had longer time since HIV diagnosis, higher platelets, lower alanine aminotransferase (ALT) and AST, higher total and high-density lipoprotein cholesterol, and lower triglycerides. Women with HIV had higher FSH and LH levels and lower total testosterone. Women with HIV also had higher CAP and FAST scores than men, but no difference in LSM was observed. MASLD was present in 73 women and 294 men with HIV, resulting in an overall lower prevalence in women (19.6% vs. 26.8%,  $p = 0.006$ ). MASH with significant liver fibrosis was present in 18 women and 101 men with HIV, resulting in an overall lower prevalence in women (4.8% vs. 9.2%,  $p = 0.008$ ). This sex difference was significant in people with HIV with BMI  $>25$  kg/m<sup>2</sup> (prevalence 7.2% in women vs. 13.8% in men,  $p = 0.028$ ) but not observed in people with HIV with BMI  $<25$  kg/m<sup>2</sup> (prevalence 3.6% in women vs. 4.4% in men,  $p = 0.722$ ).

### Sex differences in MASH with significant liver fibrosis

The multivariable model with fixed effects showed that male sex, per year increase in age, white ethnicity, detectable HIV viral load, and hypertension were positively associated with MASH with liver fibrosis (Table 2). Although men had generally higher FAST scores, FAST scores increased in women during the critical biological age of presumed perimenopause to menopause (between 40 and 50 years), reaching levels similar to those of men by the age of 55 years. The breaking point was 50.5 years for women and 52.0 years for men. As the trends plateaued for men, women experienced similar levels of MASH with fibrosis (Figure 2). Indeed, the prevalence

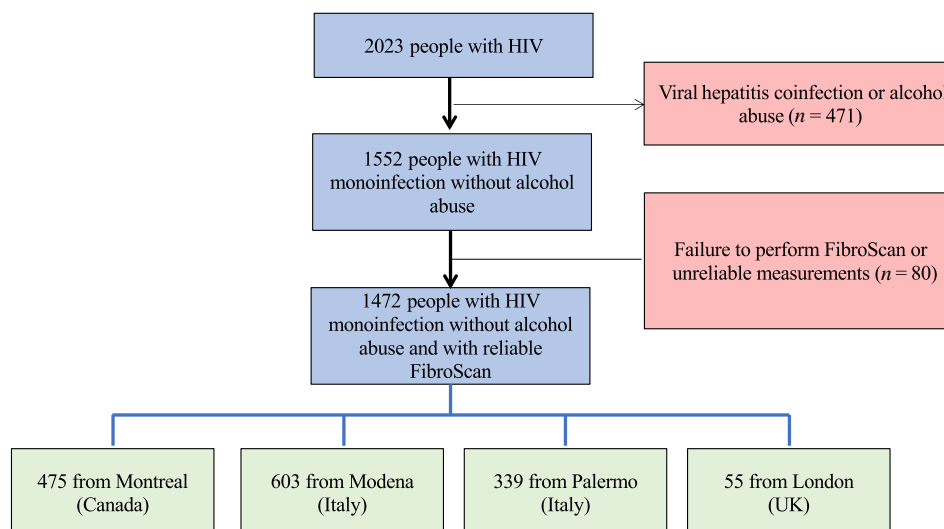


FIGURE 1 Flow chart displaying the selection of study participants.

TABLE 1 Characteristics of study population ( $n = 1472$ ) by biological sex category.

Characteristics	Whole cohort ( $n = 1472$ )	Female sex ( $n = 373$ )	Male sex ( $n = 1099$ )	<i>p</i> -value
Age (years)	51.8 ± 9.9	51.5 ± 10.2	51.9 ± 9.9	0.457
Non-white ethnicity	527 (35.4)	214 (57.4)	313 (28.4)	<0.001
BMI (kg/m <sup>2</sup> )	25.1 (4.4)	25.2 (5.8)	25.1 (3.9)	0.790
BMI >25	673 (45.7)	164 (44.0)	509 (46.3)	0.618
IDU	130 (8.8)	23 (6.2)	107 (9.7)	0.508
Hypertension	342 (23.2)	95 (25.5)	247 (22.5)	0.237
Diabetes	621 (42.2)	147 (39.4)	474 (43.1)	0.209
Years since HIV diagnosis	17 (9–25)	19 (10–27)	17 (9–25)	<0.001
Undetectable viral load	1223 (83.1)	303 (81.3)	920 (83.7)	0.209
CD4 Abs (cells/μL)	698 (494–844)	700 (484–835)	697 (496–846)	0.257
Nadir CD4 (cells/μL)	217 (187–250)	250 (190–250)	210 (174–250)	0.330
Platelets (10 <sup>9</sup> /L)	227 (68)	237 (82)	224 (62)	0.010
ALT (IU/L)	23 (17–34)	18 (14–25)	25 (18–37)	<0.001
AST (IU/L)	22 (18–27)	20 (17–25)	22 (19–28)	<0.001
Total cholesterol (mmol/L)	4.7 ± 1.1	4.8 ± 1.1	4.7 ± 1.1	0.040
HDL-C (mmol/L)	1.3 ± 0.4	1.5 ± 0.5	1.2 ± 0.4	<0.001
Triglycerides (mmol/L)	1.3 (0.9–2.0)	1.0 (0.8–1.5)	1.4 (1.0–2.2)	<0.001
Current antiretroviral regimen				
NNRTIs	442 (30.0)	100 (26.8)	342 (31.1)	0.117
NRTIs	1253 (85.1)	307 (82.3)	946 (86.1)	0.08
Protease inhibitors	553 (37.6)	150 (40.2)	403 (36.7)	0.222
Integrase inhibitors	699 (47.5)	187 (50.1)	512 (46.6)	0.236
Past exposure to didanosine	181 (12.3)	41 (11.0)	140 (12.7)	0.375
Past exposure to stavudine	315 (21.4)	70 (18.7)	245 (22.3)	0.151
Estradiol (pg/mL) <sup>a</sup>	23 (12–32)	28 (20–35)	22 (18–27)	0.246
FSH (mIU/mL) <sup>a</sup>	9.0 (5.5–39.3)	65.9 (17.8–105.7)	7.2 (4.8–10.9)	<0.001
LH (IU/mL) <sup>a</sup>	8.5 (5.7–20.3)	33.8 (12.6–49.1)	7.1 (5.2–10.2)	<0.001
Total testosterone (nmol/L) <sup>a</sup>	4.6 ± 2.3	0.27 ± 0.20	5.0 ± 2.1	<0.001
LSM (kPa)	4.9 (4.0–6.1)	4.8 (3.9–6.1)	4.9 (4.1–6.2)	0.133
CAP (dB/m)	239.4 ± 57.4	230.8 ± 54.6	242.3 ± 58.0	<0.001
FAST score	0.08 (0.04–0.16)	0.06 (0.03–0.13)	0.08 (0.04–0.17)	<0.001

Note: Continuous variables are expressed as mean ± standard deviation for normally distributed data or as median (interquartile range) for non-normally distributed data. Categorical variables are expressed as frequencies (%). The *p*-values refer to Student's *t*-test or the  $\chi^2$  test between female and male sex.

<sup>a</sup>Estradiol, FSH, LH, and total testosterone were available in 323 patients (105 women and 218 men).

Abbreviations: Abs, absolute; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CAP, controlled attenuation parameter; FAST, Fibroscan-AST; HDL-C, high-density lipoprotein cholesterol; FSH, follicle-stimulating hormone; IDU, injection drug use; LH, luteinizing hormone; LSM, liver stiffness measurement; NNRTIs, non-nucleoside reverse transcriptase inhibitors; NRTIs, nucleoside reverse transcriptase inhibitors.

of MASH with fibrosis was not significantly different after the age of 55 years, being 9.8% and 6.3% in men and women, respectively ( $p = 0.216$ ). In the subgroup of 105 women (representing 28.2% of the women in the study) with available hormonal measurements, those who had MASH with fibrosis presented with higher levels of total testosterone than did women without MASH with fibrosis ( $0.40 \pm 0.27$  nmol/L vs.  $0.23 \pm 0.18$ ;

$p = 0.048$ ). No difference in FSH, LH, or estradiol was observed.

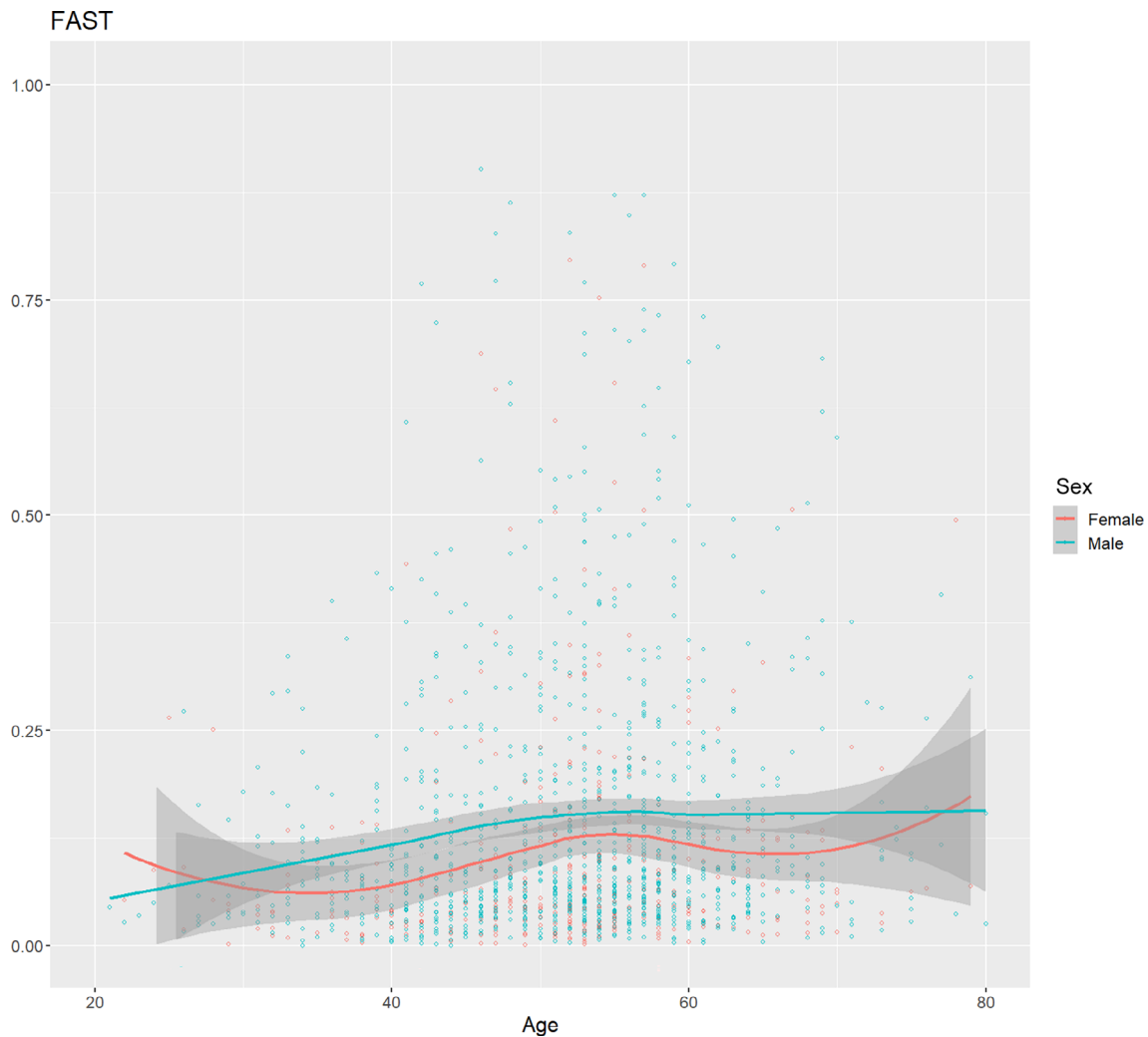
## DISCUSSION

In our study, which comprised a large cohort of people with HIV, we demonstrated that male sex is associated with

**TABLE 2** Linear mixed-effects model and segmented random-effects multivariable model.

Variable	Unadjusted	Standard error	p-value	Adjusted	Standard error	p-value
Male sex	0.032	0.009	0.005	0.034	0.017	0.044
Age (per year)	0.002	0.001	0.002	0.003	1.953	0.051
Presence of diabetes	-	-	-	0.008	0.018	0.668
Detectable HIV viral load	-	-	-	0.034	0.014	0.018
Presence of hypertension	-	-	-	0.033	0.013	0.014
White ethnicity	-	-	-	0.033	0.012	0.007
Difference in slopes between men and women	-	-	-	0.001	0.003	0.77

Note: The segmented random-effects model estimated that the breaking point was 50.5 years for women and 52.0 years for men (not statistically different  $p = 0.88$ ).



**FIGURE 2** Overall trajectory of FibroScan-AST (FAST) score by biological sex category predicted from mixed-effects regression model over age.

MASH with significant liver fibrosis, as diagnosed by the FAST score. However, the prevalence among women was no longer significantly different after menopausal age, reaching levels of MASH with fibrosis similar to those in

men. Given that biological sex is not typically a factor in the clinical decision-making process for MASH, these findings indicate that women with HIV may face an increased risk of MASH with fibrosis after the critical perimenopause

biological age. Our data support the notion that MASH exhibits sex differences in people with HIV, underscoring the need for targeted diagnostic interventions in women with HIV, tailored to their age and menopause status.

With the aging of the population living with HIV, liver diseases have become a significant cause of morbidity and mortality [1]. The successful implementation of effective antiviral therapies for HCV and hepatitis B virus has shifted the focus to MASLD, which has now become an emerging condition in people with HIV [2]. MASLD is the most frequent chronic liver disease globally, affecting one in three adults [3]. Due to a complex and multifaceted pathogenesis, MASLD is more prevalent in people with HIV, with reported figures as high as 55% [5, 6]. These figures surpass those seen in the general population, suggesting a contribution from both HIV and ART. In our study, the overall prevalence of MASLD (25%) was somewhat lower than reported in previous studies [2, 23–28]. This is due to the higher CAP cut-off we used, based on recent literature, and to additional metabolic criteria previously not required to define NAFLD. Furthermore, there may be some cases of burnt-out MASH in our cohort, leading to low CAP values [29]. Finally, in our international cohort, people with HIV undergo consecutive screening for MASLD, without selection based on elevated liver transaminases or specific risk factors for MASLD, as was the case in previous studies. Importantly, the most severe form of MASLD, namely MASH with significant liver fibrosis, is also more frequent in people with HIV. Two previous studies in people with HIV reported a prevalence of MASH with fibrosis by FAST score of 6.3% in women and 12.3% in a mixed-gender cohort, aligning closely with our findings and exceeding rates observed in the general population [30, 31].

In the general population, sex differences in MASH prevalence and severity have been reported, with men exhibiting a higher prevalence than women. However, age-specific sex differences and the effect of menopause was infrequently considered in studies. Data suggest that MASLD prevalence and incidence are higher in men than in women before the age of 50 years or menopause but tend to increase in women after this age [10]. Women aged >50 years with MASLD are 1.2 times more likely to develop MASH than are age-matched men and are more likely to progress to advanced liver fibrosis [32]. Transcriptomic and plasma profiling studies suggest that MASLD may follow a distinct biological trajectory in women aged >50 years [33, 34]. Additionally, men with MASLD have a higher risk of liver cirrhosis and hepatocellular carcinoma than women, likely due to the protective role of oestrogens against MASLD. Indeed, postmenopausal women receiving hormone replacement therapy have a

lower prevalence of MASLD than those not receiving hormone replacement therapy [10].

The influence of biological sex on the pathogenesis and progression of MASH may be amplified in the context of HIV infection. Studies have shown that women suffer more than men from the socioeconomic consequences of HIV infection, which can affect healthcare-seeking behaviour and treatment adherence [35]. Women with HIV also tend to experience poorer treatment outcomes and are at greatest risk for significant weight gain with HIV treatment, potentially increasing their risk of progressive MASH [11]. Additionally, women with HIV tend to experience menopause at an earlier age [12] and have lower total estradiol levels [36] than the general population, potentially reducing the exposure to the protective benefits of oestrogens on the liver. The absence of oestrogen increases the likelihood of various metabolic derangements, an effect compounded in people with HIV due to their predisposition to insulin resistance and HIV-related metabolic perturbations [37]. Interestingly, liver fibrosis may begin accelerating earlier in women with HIV than in the general population. A study in people with HIV/HCV coinfection found that liver fibrosis begins to accelerate in perimenopause, highlighting a previously unrecognized group of women at increased risk for advanced fibrosis and associated complications [38].

Our study used the FAST score as a non-invasive biomarker of histologic MASH with significant liver fibrosis (F2–F4) and elevated NAFLD activity score [13]. MASH is the inflammatory subtype of MASLD associated with liver fibrosis progression, development of cirrhosis, and need for liver transplant [39]. In MASH, the presence of significant liver fibrosis predicts major adverse events, including all-cause mortality, end-stage liver complications, and cardiovascular events [40]. The FAST score has been used in several studies in people with HIV, confirming its prognostic utility to predict clinical outcomes [14, 23, 30]. Interestingly, this biomarker may more accurately depict liver damage in HIV-associated MASH as it incorporates AST. Although both AST and ALT are markers of liver injury, ALT is located in the hepatocellular cytosol, whereas AST is mainly found within the mitochondria [41]. Consequently, AST may better reflect liver damage in the context of HIV infection and signal mitochondrial toxicity, which is a common feature of both MASH and ART-associated hepatotoxicity [42, 43].

Our study found that the overall prevalence of MASH with significant liver fibrosis was higher in men (9.2%) than in women (4.8%) with HIV. However, during the critical biological age from presumed perimenopause to menopause, this gap narrowed. This resulted in a similar prevalence of MASH with fibrosis in men (9.8%) and women (6.3%) after the age of 55 years. Although male

sex was independently associated with a higher FAST score, the difference in prevalence diminished and became not significant after age 55 due to slower progression in men and faster progression in women between the critical biological age of 40 to 50 years. Interestingly, in a subgroup of women with available hormonal measurements, those with MASH with fibrosis had higher testosterone levels than did those without. This aligns with recent evidence from the general population, where higher testosterone levels conferred a 2-fold higher risk of MASH and MASH with fibrosis [44].

Beyond biological sex, our study identified age, white ethnicity, detectable HIV viral load, and hypertension as significant factors associated with MASH with significant liver fibrosis. Age is well-established as a risk factor for MASH and liver fibrosis [4]. White ethnicity carries a higher risk of MASH than does Black ethnicity due to a combination of genetic, environmental, and lifestyle factors [45]. A detectable HIV viral load is associated with a higher risk of developing MASH through several inter-related mechanisms [5]. Ongoing viral replication contributes to chronic inflammation and immune activation, which can exacerbate liver inflammation and fibrosis, key components of MASH. HIV-related alterations in lipid metabolism and insulin resistance promote hepatic fat accumulation and progression to MASH. People with HIV with a detectable viral load may also experience higher levels of oxidative stress and mitochondrial dysfunction, further promoting liver cell injury and inflammation [46]. Hypertension also contributes to MASH by exacerbating metabolic dysfunction and systemic inflammation [45]. In the context of HIV, the frequent occurrence of hypertension compounds the metabolic disturbances common in these patients, such as insulin resistance and dyslipidaemia [5]. Furthermore, hypertension can lead to increased arterial stiffness and endothelial dysfunction, which can aggravate liver injury and fibrosis in MASH [47].

Overall, this study contributes to gender medicine science and takes a step forward in the direction of precision medicine intended as a personalized, predictive, preventive, and participatory medicine approach. Nevertheless, we wish to acknowledge several limitations of our study. First, we did not use the gold standard of liver biopsy to diagnose MASH. Instead, we relied on the FAST score, which is based on transient elastography. This method provides a single point-of-care evaluation of both hepatic fibrosis and steatosis and has been validated in people with HIV. In addition, non-invasive diagnostic techniques offer a patient-centred approach, reduce selection bias, improve overall study coverage, and are cost effective. Second, we used critical biological age as a

proxy for menopause due to the lack of precise estimates of menopausal status. Third, we lacked extensive hormonal data to provide a biological and physiopathological validation of our epidemiological findings. Fourth, due to significant limitations in sample size, we were unable to include non-binary and transgender people in our analysis. Future research should specifically target these populations to ensure comprehensive understanding. Fifth, the effect size for the sex difference was 0.034. Although this was a significant statistical finding, further investigation is necessary to determine whether a 0.034 increase in FAST score is clinically meaningful. Sixth, as a cross-sectional study, our research is limited by its inability to establish causality. Capturing data at a single point in time makes it challenging to determine the direction and temporal sequence of observed associations.

In conclusion, our data support the characterization of MASH as a disease with significant sex differences in people with HIV. Despite the observation that women with HIV have a lower prevalence of MASH with significant liver fibrosis than do men with HIV, our findings reveal a concerning acceleration in the increase of FAST scores in women around the perimenopausal age. This suggests that hormonal changes associated with menopause may exacerbate liver disease progression in women with HIV, highlighting the need for heightened monitoring and tailored interventions during this critical biological period. These insights emphasize the importance of considering gender-specific factors in the management and treatment of MASH in people with HIV to improve outcomes and reduce disparities in liver disease progression between men and women.

## AUTHOR CONTRIBUTIONS

Dana Kablawi contributed to study design, data collection and interpretation of the data, and the first draft of the manuscript. Tyler Thomas contributed to statistical analysis and interpretation of the data. Thierry Fotsing Tadjou, Jovana Milic, Felice Cinque, Wesal Elgretli, Claudia Gioè, Bertrand Lebouché, Emmanuel Tsochatzis, Jemima Finkel, Sanjay Bhagani, Antonio Cascio, Giovanni Guaraldi, and Giovanni Mazzola, contributed to data collection and interpretation of data. Sahar Saeed contributed to the conception, study design, statistical analysis, data collection and interpretation of the data. Giada Sebastiani contributed to conception, study design, statistical analysis, data collection and interpretation of the data, and the first draft of the manuscript. All authors approved the final version of the article. Part of this work was presented at the Conference on Retroviruses and Opportunistic Infections (CROI) (Seattle, WA, USA; February 2023).



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## CONFLICT OF INTEREST STATEMENT

Jovana Milic received speaker honoraria from Gilead and ViiV. Bertrand Lebouché has acted as a speaker and advisory board member for ViiV, Gilead, and Merck, and received research funding from ViiV, Merck, and Gilead. Emanuel Tsochatzis has participated to advisory boards for Boehringer, Pfizer, Novo Nordisk, Alexion and Orphan and acted as a speaker for Novo Nordisk and Orphan. Antonio Cascio has served as an advisory board member for Gilead, Janssen, Merck, ViiV, GSK and Astra Zeneca, acted as a speaker for Gilead and

Tillots and support for attending meetings from Pfizer and Nordic pharma. Giovanni Guaraldi received a research grant and speaker honoraria from Gilead, ViiV, Merck and Jansen and attended advisory boards of Gilead, ViiV and Merck. Sahar Saeed attended advisory boards of Novo Nordisk. Giada Sebastiani has acted as speaker for Merck, Gilead, Abbvie, Novo Nordisk, Pfizer, served as an advisory board member for Pfizer, Merck, Novo Nordisk, Gilead, and has received unrestricted research funding from Theratechnologies Inc. Dana Kablawi, Tyler Thomas, Thierry Fotsing Tadjjo, Felice Cinque, Wesal Elgretli, Claudia Gioe, Jemima Finkel, Sanjay Bhagani, Giovanni Mazzola have nothing to disclose.

## DATA AVAILABILITY STATEMENT

According to stipulations of the patient consent form signed by all study participants, ethical restrictions imposed by our institutional ethics review boards (Institutional Ethics Review Board Biomedical B Research Ethics Board of the McGill University Health Centre), and legal restrictions imposed by Canadian law regarding clinical trials, anonymized data are available upon reasonable request. Please send data access requests to Sheldon Levy, Biomedical B (BMB) Research Ethics Board (REB) Coordinator Centre for Applied Ethics, 5100, boul. de Maisonneuve Ouest, 5th floor, Office 576, Montréal, Québec, H4A 3 T2, Canada.

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## REFERENCES

- Collins LF, Palella FJ Jr, Mehta CC, et al. Aging-related comorbidity burden among women and men with or At-risk for HIV in the US, 2008-2019. *JAMA Netw Open*. 2023;6(8):e2327584. doi:10.1001/jamanetworkopen.2023.27584
- Gawrieh S, Vilar-Gomez E, Woreta TA, et al. Prevalence of steatotic liver disease, MASLD, MetALD and significant fibrosis in people with HIV in the United States. *Aliment Pharmacol Ther*. 2024;59(5):666-679. doi:10.1111/apt.17849
- Riazi K, Azhari H, Charette JH, et al. The prevalence and incidence of NAFLD worldwide: a systematic review and meta-analysis. *Lancet Gastroenterol Hepatol*. 2022;7(9):851-861. doi:10.1016/S2468-1253(22)00165-0
- Rinella ME, Neuschwander-Tetri BA, Siddiqui MS, et al. AASLD practice guidance on the clinical assessment and management of nonalcoholic fatty liver disease. *Hepatology*. 2023;77:1797-1835. doi:10.1097/HEP.0000000000000323
- Cervo A, Shengir M, Patel K, Sebastiani G. NASH in HIV. *Curr HIV/AIDS Rep*. 2020;17(6):601-614. doi:10.1007/s11904-020-00531-0

6. Kalligeros M, Vassilopoulos A, Shehadeh F, et al. Prevalence and characteristics of nonalcoholic fatty liver disease and fibrosis in people living with HIV Mono-infection: a systematic review and meta-analysis. *Clin Gastroenterol Hepatol*. 2023;21:1708-1722. doi:10.1016/j.cgh.2023.01.001
7. Benmassaoud A, Ghali P, Cox J, et al. Screening for nonalcoholic steatohepatitis by using cytokeratin 18 and transient elastography in HIV mono-infection. *PLoS One*. 2018;13(1):e0191985. doi:10.1371/journal.pone.0191985
8. Pembroke T, Deschenes M, Lebouché B, et al. Hepatic steatosis progresses faster in HIV mono-infected than HIV/HCV co-infected patients and is associated with liver fibrosis. *J Hepatol*. 2017;67(4):801-808. doi:10.1016/j.jhep.2017.05.011
9. Burra P, Bizzaro D, Gonta A, et al. Clinical impact of sexual dimorphism in non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH). *Liver Int*. 2021;41(8):1713-1733. doi:10.1111/liv.14943
10. Lonardo A, Nascimbeni F, Ballestri S, et al. Sex differences in nonalcoholic fatty liver disease: state of the art and identification of research gaps. *Hepatology*. 2019;70(4):1457-1469. doi:10.1002/hep.30626
11. Soti S, Corey KE, Lake JE, Erlandson KM. NAFLD and HIV: do sex, race, and ethnicity explain HIV-related risk? *Curr HIV/AIDS Rep*. 2018;15(3):212-222. doi:10.1007/s11904-018-0392-1
12. Van Ommen CE, King EM, Murray MCM. Age at menopause in women living with HIV: a systematic review. *Menopause*. 2021;28(12):1428-1436. doi:10.1097/GME.0000000000001871
13. Newsome PN, Sasso M, Deeks JJ, et al. FibroScan-AST (FAST) score for the non-invasive identification of patients with non-alcoholic steatohepatitis with significant activity and fibrosis: a prospective derivation and global validation study. *Lancet Gastroenterol Hepatol*. 2020;5(4):362-373. doi:10.1016/S2468-1253(19)30383-8
14. Sebastiani G, Milic J, Kablawi D, et al. Fibroscan-aspartate aminotransferase score predicts liver-related outcomes, but not extrahepatic events, in a multicenter cohort of people with human immunodeficiency virus. *Clin Infect Dis*. 2023;77(3):396-404. doi:10.1093/cid/ciad203
15. Cinque F, Saeed S, Kablawi D, et al. Role of fatty liver in the epidemic of advanced chronic liver disease among people with HIV: protocol for the Canadian LIVEHIV multicentre prospective cohort. *BMJ Open*. 2023;13(8):e076547. doi:10.1136/bmjopen-2023-076547
16. European Association for the Study of the Liver (EASL), European Association for the Study of Diabetes (EASD), European Association for the Study of Obesity (EASO), European Association for the Study of the Liver (EASL). EASL-EASD-EASO clinical practice guidelines on the management of metabolic dysfunction-associated steatotic liver disease (MASLD). *J Hepatol*. 2024;S0168-8278(24)00329-5. doi:10.1016/j.jhep.2024.04.031
17. Castera L. Noninvasive methods to assess liver disease in patients with hepatitis B or C. *Gastroenterology*. 2012;142(6):1293-1302 e4. doi:10.1053/j.gastro.2012.02.017
18. Lemoine M, Assoumou L, de Wit S, et al. Diagnostic accuracy of noninvasive markers of steatosis, NASH, and liver fibrosis in HIV-Mono-infected individuals at risk of nonalcoholic fatty liver disease (NAFLD): results from the ECHAM study. *J Acquir Immune Defic Syndr*. 2019;80(4):e86-e94. doi:10.1097/QAI.0000000000001936
19. Kleiner DE, Brunt EM, van Natta M, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology*. 2005;41(6):1313-1321. doi:10.1002/hep.20701
20. Maurice JB, Goldin R, Hall A, et al. Increased body mass index and type 2 diabetes are the Main predictors of nonalcoholic fatty liver disease and advanced fibrosis in liver biopsies of patients with human immunodeficiency virus Mono-infection. *Clin Infect Dis*. 2021;73(7):e2184-e2193. doi:10.1093/cid/ciaa1302
21. Cervo A, Milic J, Mazzola G, et al. Prevalence, predictors, and severity of lean nonalcoholic fatty liver disease in patients living with human immunodeficiency virus. *Clin Infect Dis*. 2020;71(10):e694-e701. doi:10.1093/cid/ciaa430
22. Mohammed SS, Aghdassi E, Salit IE, et al. HIV-positive patients with nonalcoholic fatty liver disease have a lower body mass index and are more physically active than HIV-negative patients. *J Acquir Immune Defic Syndr*. 2007;45(4):432-438. doi:10.1097/QAI.0b013e318074efe3
23. Bischoff J, Gu W, Schwarze-Zander C, et al. Stratifying the risk of NAFLD in patients with HIV under combination antiretroviral therapy (cART). *EClinicalMedicine*. 2021;40:101116. doi:10.1016/j.eclinm.2021.101116
24. Frías M, Corona-Mata D, Moyano JM, et al. Lack of associations of microRNAs with severe NAFLD in people living with HIV: discovery case-control study. *Front Endocrinol (Lausanne)*. 2023;14:1230046. doi:10.3389/fendo.2023.1230046
25. Fernandez-Botran R, Plankey MW, Ware D, Bordon J. Changes in liver steatosis in HIV-positive women are associated with the BMI, but not with biomarkers. *Cytokine*. 2021;144:155573. doi:10.1016/j.cyto.2021.155573
26. Duarte M, Tien P, Ma Y, Noworolski SM, Korn N, Price JC. Controlled attenuation parameter accurately detects liver steatosis in people with HIV. *AIDS*. 2022;36(15):2147-2152. doi:10.1097/QAD.0000000000003351
27. Price J, Ma Y, Adimora A, et al. Multisite prospective liver disease and reproductive ageing (LIVRA) study in US women living with and without HIV. *BMJ Open*. 2022;12(4):e055706. doi:10.1136/bmjopen-2021-055706
28. Yanavich C, Perazzo H, Li F, et al. A pilot study of microbial signatures of liver disease in those with HIV mono-infection in Rio de Janeiro, Brazil. *AIDS*. 2022;36(1):49-58. doi:10.1097/QAD.0000000000003084
29. van der Poorten D, Samer CF, Ramezani-Moghadam M, et al. Hepatic fat loss in advanced nonalcoholic steatohepatitis: are alterations in serum adiponectin the cause? *Hepatology*. 2013;57(6):2180-2188. doi:10.1002/hep.26072
30. Price JC, Ma Y, Kuniholm MH, et al. Human immunodeficiency virus is associated with elevated FibroScan-aspartate aminotransferase (FAST) score. *Clin Infect Dis*. 2022;75(12):2119-2127. doi:10.1093/cid/ciac337
31. Michel M, Labenz C, Wahl A, et al. Prevalence and risk factors of nonalcoholic steatohepatitis with significant fibrosis in people with HIV. *AIDS*. 2022;36(12):1665-1674. doi:10.1097/QAD.0000000000003312
32. Balakrishnan M, Patel P, Dunn-Valadez S, et al. Women have a lower risk of nonalcoholic fatty liver disease but a higher risk of progression vs men: a systematic review and meta-analysis. *Clin Gastroenterol Hepatol*. 2021;19(1):61-71.e15. doi:10.1016/j.cgh.2020.04.067

33. Vandel J, Dubois-Chevalier J, Gheeraert C, et al. Hepatic molecular signatures highlight the sexual dimorphism of non-alcoholic Steatohepatitis (NASH). *Hepatology*. 2021;73(3):920-936. doi:10.1002/hep.31312
34. Eng PC, Forlano R, Tan T, Manousou P, Dhillon WS, Izzu-Engbeaya C. Non-alcoholic fatty liver disease in women - current knowledge and emerging concepts. *JHEP Rep*. 2023;5(10):100835. doi:10.1016/j.jhepr.2023.100835
35. Rodrigo C, Rajapakse S. HIV, poverty and women. *Int Health*. 2010;2(1):9-16. doi:10.1016/j.inhe.2009.12.003
36. Coburn SB, Dionne-Odom J, Alcaide ML, et al. The association between HIV status, estradiol, and sex hormone binding globulin among premenopausal women in the Women's interagency HIV study. *J Womens Health (Larchmt)*. 2022;31(2):183-193. doi:10.1089/jwh.2021.0276
37. Kaspar MB, Sterling RK. Mechanisms of liver disease in patients infected with HIV. *BMJ Open Gastroenterol*. 2017;4(1):e000166. doi:10.1136/bmjgast-2017-000166
38. Sarkar M, Dodge JL, Greenblatt RM, et al. Reproductive aging and hepatic fibrosis progression in human immunodeficiency virus/hepatitis C virus-Coinfected women. *Clin Infect Dis*. 2017;65(10):1695-1702. doi:10.1093/cid/cix643
39. Chalasani N, Younossi Z, Lavine JE, et al. The diagnosis and management of nonalcoholic fatty liver disease: practice guidance from the American Association for the Study of Liver Diseases. *Hepatology*. 2018;67(1):328-357. doi:10.1002/hep.29367
40. Taylor RS, Taylor RJ, Bayliss S, et al. Association between fibrosis stage and outcomes of patients with nonalcoholic fatty liver disease: a systematic review and meta-analysis. *Gastroenterology*. 2020;158(6):1611-1625.e12. doi:10.1053/j.gastro.2020.01.043
41. Zoppini G, Cacciatori V, Negri C, et al. The aspartate aminotransferase-to-alanine aminotransferase ratio predicts all-cause and cardiovascular mortality in patients with type 2 diabetes. *Medicine*. 2016;95(43):e4821. doi:10.1097/MD.0000000000004821
42. Dornas W, Schuppan D. Mitochondrial oxidative injury: a key player in nonalcoholic fatty liver disease. *Am J Physiol Gastrointest Liver Physiol*. 2020;319(3):G400-G411. doi:10.1152/ajpgi.00121.2020
43. Schank M, Zhao J, Moorman JP, Yao ZQ. The impact of HIV- and ART-induced mitochondrial dysfunction in cellular senescence and aging. *Cells*. 2021;10(1):174. doi:10.3390/cells10010174
44. Sarkar MA, Suzuki A, Abdelmalek MF, et al. Testosterone is associated with nonalcoholic Steatohepatitis and fibrosis in premenopausal women with NAFLD. *Clin Gastroenterol Hepatol*. 2021;19(6):1267-1274.e1. doi:10.1016/j.cgh.2020.09.045
45. Fu H, Yu H, Zhao Y, Chen J, Liu Z. Association between hypertension and the prevalence of liver steatosis and fibrosis. *BMC Endocr Disord*. 2023;23(1):85. doi:10.1186/s12902-023-01318-1
46. Ivanov AV, Valuev-Elliston VT, Ivanova ON, et al. Oxidative stress during HIV infection: mechanisms and consequences. *Oxid Med Cell Longev*. 2016;2016:8910396. doi:10.1155/2016/8910396
47. Brandes RP. Endothelial dysfunction and hypertension. *Hypertension*. 2014;64(5):924-928. doi:10.1161/HYPERTENSIONAHA.114.03575

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