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MAJOR ARTICLE

Fibroscan-aspartate aminotransferase (FAST) score predicts liver-related outcomes, but not extra-hepatic events, in a multicenter cohort of people with HIV

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Background: Nonalcoholic fatty liver disease (NAFLD) is frequent in people with HIV (PWH). The Fibroscan-aspartate aminotransferase (FAST) score was developed to identify patients with nonalcoholic steatohepatitis (NASH) and significant fibrosis. We investigated prevalence of NASH with fibrosis and the value of FAST score in predicting clinical outcomes in PWH.

Methods: Transient elastography (Fibroscan) was performed in PWH without viral hepatitis coinfection from four prospective cohorts. We used FAST>0.35 to diagnose NASH with fibrosis.

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Incidence and predictors of liver-related outcomes (hepatic decompensation, hepatocellular carcinoma) and extra-hepatic events (cancer, cardiovascular disease) were evaluated through survival analysis.

Results: Of the 1472 PWH included, 8% had FAST>0.35. On multivariable logistic regression, higher BMI (adjusted odds ratio [aOR] 1.21, 95% confidence interval [CI] 1.14-1.29), hypertension (aOR 2.24, 95% CI 1.16-4.34), longer time since HIV diagnosis (aOR 1.82, 95% CI 1.20-2.76) and detectable HIV viral load (aOR 2.22, 95% CI 1.02-4.85) were associated with FAST>0.35. 882 patients were followed for a median of 3.8 years (interquartile range 2.5-4.2). Overall, 2.9% and 11.1% developed liver-related and extra-hepatic outcomes, respectively. Incidence of liver-related outcomes was higher in patients with FAST>0.35 vs. FAST≤0.35 (45.1, 95% CI 26.2-77.7 vs. 5.0, 95% 2.9-8.6 per 1000 person-years). On multivariable Cox regression analysis, FAST>0.35 remained an independent predictor of liver-related outcomes (adjusted hazard ratio 4.97, 95% CI 1.97-12.51). Conversely, FAST did not predict extra-hepatic events.

Conclusion: A significant proportion of PWH without viral hepatitis coinfection may have NASH with significant liver fibrosis. FAST score predicts liver-related outcomes and can help risk stratification and management in this high-risk population.

Keywords: nonalcoholic steatohepatitis; liver fibrosis; hepatic decompensation; transient elastography; biomarkers.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the most frequent chronic liver disease worldwide, with a prevalence at 25% in the general population(1). NAFLD is an umbrella term encompassing a disease spectrum from simple hepatic steatosis to nonalcoholic steatohepatitis (NASH). NASH is the inflammatory subtype of NAFLD associated with liver fibrosis progression, development of cirrhosis, and need for liver transplant(2).

NAFLD is a frequent age-related comorbidity in people with HIV (PWH) in absence of viral hepatitis coinfection, with a prevalence ranging from 13 to 65%(3-7). This burden is likely driven by a multifactorial complex pathogenesis, including a high prevalence of traditional NAFLD risk factors such as overweight and diabetes, plus contribution from HIV-specific factors, such as persistent immune activation and HIV-related inflammation, lipodystrophy-induced dyslipidemia, past exposure to hepatotoxic d-drugs (didanosine and stavudine), and antiretroviral-associated weight gain induced by integrase strand transfer inhibitors(8, 9). The clinical picture of NAFLD in PWH seems more severe than the general population. In PWH with elevated transaminases, prevalence of NASH and liver fibrosis is at 42% and 22%, respectively, approximately double that of the general population(3).

NAFLD is often asymptomatic until patients develop hepatic decompensation, with significant morbidity and mortality(10). Early identification of patients with NASH and liver fibrosis is of paramount importance to tailor appropriate interventions and surveillance. The guidelines from the European AIDS Clinical Society recommend case-finding of liver fibrosis in people with HIV mono-infection with metabolic conditions or persistent elevated transaminases(11).

Transient elastography (TE) with controlled attenuation parameter (CAP) is a feasible and accurate tool to assess both hepatic steatosis and liver fibrosis in PWH(12). The Fibroscan-aspartate aminotransferase (AST) (FAST) score was developed to identify patients with histologic NASH, significant liver fibrosis (F2-F4) and elevated NAFLD activity score (NAS) associated with higher risk of end-stage liver disease(13). The FAST score has been validated in global clinical trials of NASH-targeted therapies and it has been used in two cross-sectional studies in PWH, but its prognostic value in PWH remains unknown(14, 15).

In an international cohort collaboration of PWH, we aimed to: i) investigate prevalence and associated cofactors of elevated FAST score; ii) determine the prognostic value of FAST score to predict liver-related and extra-hepatic outcomes; iii) compare the prognostic value of FAST score with non-invasive tests of liver fibrosis.

PATIENTS AND METHODS

Study design and population

We conducted a retrospective analysis of 4 cohorts of people with HIV mono-infection, the LIVEr disease in HIV (LIVEHIV) at the McGill University Health Centre (MUHC), Modena HIV Metabolic Clinic (MHMC), Liver Pathologies in HIV in Palermo and Royal Free Hospital Cohorts. Participants were identified through locally maintained prospective databases of PWH who underwent screening for NAFLD. We included consecutive patients with confirmed HIV infection on antiretroviral therapy (ART) and aged ≥18 years with availability of liver stiffness measurement (LSM) and CAP by TE and relevant clinical and biochemical parameters. Exclusion criteria were: (i) positivity for hepatitis C virus antibody or hepatitis B surface antigen; (ii) evidence of other liver diseases; (iii) significant alcohol intake, defined as >30g/day in men and >20g/day in women(16); (iv) presence of the clinical outcomes at study entry; (v) contraindications (pregnancy, pacemaker insertion) and failure or unreliable measurement of TE examination. The combined cohort of 1472 PWH included 475 patients from the LIVEHIV, 603 from the MHMC, 339 from the Liver Pathologies in HIV and 55 patients from the Royal Free Hospital Cohort.

Ethics

All participants provided informed written consent. The Research Ethics Board of the Research Institute of MUHC (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.

Clinical and biological parameters

We included patients with available data within 3 months from the TE examination, namely demographic information, time since HIV diagnosis, ART class, body mass index (BMI), liver serum biomarkers, lipid profile, hematological and immuno-virological parameters. Undetectable viral load was defined as HIV viral load <50 copies/mL.

Transient elastography examination and fibrosis biomarkers

TE examinations (Echosens, Paris, France) were performed on a 4-hour fasting patient by maximum two operators at each site using standard quality criteria(17). A cut-off of LSM ≥7.1 kPa was used to define significant liver fibrosis, corresponding to histologic stage F2-F4 out of 4(18, 19). NAFLD was defined as CAP ≥275 dB/m(20). Two fibrosis biomarkers were also computed: AST-to-Platelet Ratio Index (APRI)(21) and Fibrosis-4 index (FIB-4)(22, 23). A cut-off of APRI ≥0.5 and of FIB-4 ≥1.30 was used to identify PWH in whom significant liver fibrosis could not be excluded(21-23).

FAST score

The FAST score was calculated with CAP, LSM and AST level as previously described:

$$\text{FAST score} = \frac{\exp\left[-1.65+1.07\times\ln(\text{LSM})+2.66\times10^{-8}\times\text{CAP}^{3}-63.3\times\text{AST}^{-1}\right]}{1+\exp\left[-1.65+1.07\times\ln(\text{LSM})+2.66\times10^{-8}\times\text{CAP}^{3}-63.3\times\text{AST}^{-1}\right]}$$

FAST scores were categorized by 0.35 and 0.67 cut-offs, which were proposed to rule out or rule in NASH with significant fibrosis, respectively. Specifically, FAST score ≤0.35 has a sensitivity of 90% and specificity of 50% and FAST score ≥0.67 has a sensitivity of 50% and specificity of 90% for NASH with F2-F4 fibrosis and NAS ≥4(13).

Outcome measures

The primary study outcomes were: (i) prevalence of FAST score >0.35 and ≥0.67; (ii) incidence of liver-related outcomes, defined as the occurrence of any among classical hepatic decompensation, hepatocellular carcinoma, liver transplantation. Classical decompensation was defined as *de novo* ascites, variceal bleeding, or overt hepatic encephalopathy(24). Secondary

outcomes included incidence of extra-hepatic events, namely cardiovascular disease (myocardial infarction, arrhythmia, heart failure and coronary revascularization), extra-hepatic cancer and all-cause mortality. The study cohort was divided into two groups (Figure 1): (i) the prevalence cohort (whole study cohort) of 1472 patients with one valid TE examination and the initial study visit between January 2015 and January 2022; (ii) the incidence cohort included 882 patients with more than one valid TE examination in the same study period. The second valid TE examination was required to be within 1 year from the first TE and was used to confirm the FAST score category. In the incidence cohort, the duration of follow-up was calculated from the first reliable TE examination to the date of any clinical outcome, or last medical visit, until December 2022. Outcomes were verified against source documents and adjudicated by the same two investigators at each centre.

Statistical methods

In the prevalence cohort, multivariable logistic regression models were built to identify cofactors independently associated with FAST score >0.35. Results were reported as adjusted odds ratios (aOR) with 95% confidence interval (CI). In the incidence cohort, baseline (time zero) was set as the first visit after January 1st, 2015 when TE examination was performed. Incidence rates of liver-related outcomes and extra-hepatic events were estimated by dividing the number of participants developing the outcome by the number of person-years (PY) of follow-up. Kaplan-Meier plots and log-rank tests were used to illustrate time to outcomes by FAST score category. We employed area under the receiver operating curve (AUROC) to estimate the prognostic performance of FAST score and to compare it to LSM, FIB-4 and APRI. Prognostic performance was also assessed by sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive and negative likelihood ratio (LR). For liver-related outcomes, the Fine and Grey method was used to control for competing risks (all-cause mortality). The association between predictors and outcomes was assessed using multivariate Cox regression analysis reporting hazard ratio (HR) with 95% CI. Cofactors associated with FAST score >0.35 and predictors that could have an impact on an individual's risk of developing outcomes were selected *a priori* after group discussion or based on prespecified level of association ($p<0.05$) with the outcome variable. A complete case analysis was used, with missing values <10% for included variables. A two-sided level of significance of 0.05 was used. Statistical analyses were performed using STATA 17 (STATA Corp. LP, College Station, Texas, USA).

RESULTS

The baseline characteristics of the whole (prevalence) cohort comprising 1472 PWH are summarized in Table 1. Four hundred eighteen (28%) patients were overweight (BMI 25–29 Kg/m^2), and 269 (18%) were obese (BMI $\geq$30 Kg/m^2). The prevalence of significant liver fibrosis by LSM was 16%, while NAFLD was present in 25% of PWH.

Prevalence and associated cofactors of FAST score >0.35 in the prevalence cohort

Overall, 119 and 27 PWH had a FAST score >0.35 and ≥0.67, resulting in a prevalence of 8% and 2%, respectively. Table 2 shows the result of a multivariable analysis of cofactors associated with FAST score >0.35 in the prevalence cohort. After adjusting for age, male sex, white ethnicity and triglycerides, higher BMI, hypertension, longer time since HIV diagnosis and detectable HIV viral load were independently associated with FAST score >0.35.

Liver-related outcomes in the incidence cohort

In the incidence cohort, comprising of 882 patients, during a median follow-up of 3.8 years (interquartile range 2.5-4.2), there was a total of 124 events (Table 3). During the follow-up, 35 patients did not have a clinical visit for over 2 years. The cumulative incidence of liver-related outcomes was 2.9%, corresponding to an incidence rate of 8.9 per 1000 PY (95% CI, 6.1-13.1). Ascites was the most common liver-related outcome, followed by hepatic encephalopathy. Table 4 reports the association of predictors with liver-related outcomes by log-rank test. Incidence of liver-related outcomes was higher in patients with FAST >0.35 vs. FAST ≤0.35 (Figure 2). The competing risk analysis showed no competing risk event of all-cause mortality on liver-related outcomes. On multivariable Cox regression analysis, FAST >0.35 remained an independent predictor of liver-related outcomes, together with higher BMI and detectable HIV viral load (Table 5).

Extra-hepatic events in the incidence cohort

The cumulative incidence of extra-hepatic events and all-cause mortality was 11.1% and 1.1%, respectively, corresponding to incidence rates of 33.7 per 1000 PY (95% CI, 27.7-41.1) and 3.4 per 1000 PY (95% CI, 1.9-4.0) (Table 3). Table 4 reports the association of predictors with extra-hepatic outcomes by log-rank test. On multivariable Cox regression analysis, independent predictors of extra-hepatic events were older age, male sex and detectable HIV viral load (Table 5).

Performance of non-invasive scores to predict outcomes in the incidence cohort

The FAST score had the highest performance to predict occurrence of liver-related outcomes (AUROC 0.86, 95% CI 0.81-0.92; Figure 3). This was significantly higher than FIB-4 (AUROC 0.67, 95% CI 0.56-0.79; p<0.001), while there was no difference compared to LSM (0.77, 95% CI 0.67-0.86) and APRI (0.79, 95% CI 0.68-0.89). The 0.35 cut-off of FAST score had 0.50 sensitivity, 0.92 specificity, 0.15 PPV, 0.98 NPV, 5.94 LR+ and 0.55 LR-. The 0.67 cut-off had 0.15 sensitivity, 0.98 specificity, 0.18 PPV, 0.97 NPV, 6.94 LR+ and 0.87 LR-. As for extra-hepatic events, the performance of non-invasive tests was suboptimal to poor, ranging from 0.54 to 0.70. FIB-4 showed the highest performance, with an AUROC of 0.70 (95% CI, 0.64-0.76), which was higher than FAST score (0.54, 95% CI 0.48-0.60), LSM (0.63, 95% CI 0.53-0.66) and APRI (0.54, 95% CI 0.48-0.59) (p<0.001).

DISCUSSION

Our multicenter cohort study found that NASH with significant liver fibrosis detected by FAST score affected up to 8% of PWH who resulted at higher risk for clinical outcomes. Specifically, FAST score predicted liver-related outcomes, but not extra-hepatic events, underscoring the importance of validating prognostic scores specifically in the context of HIV infection. This study contributes to the existing literature of two studies in which the FAST score was evaluated cross-sectionally in PWH, being the first to generate longitudinal data with clinical outcomes, enlightening incidence, dynamics and predictors related to this score(14, 25).

NAFLD has become a global pandemic due the increasing trajectories of type 2 diabetes and obesity(26). The burden of the disease implies urgent public health strategies to halt the ascending epidemiological curve, including strategies to identify patients at high risk of clinical outcomes(1). Patients with NASH and significant liver fibrosis are at higher risk of liver-related outcomes, and also cardiovascular events and extra-hepatic cancers(27). Guidelines recommend case-finding of liver fibrosis in high risk populations, namely people with type 2 diabetes or metabolic syndrome(16, 28). These same populations are targeted in the ongoing global clinical trials to find NASH-targeted therapies able to halt the fibrogenetic process or resolve NASH(29). Recently, the FAST score has been developed as a diagnostic tool for NASH with significant liver fibrosis, to guide indication to NASH-targeted therapy and related ongoing clinical trials(13). Both NASH and significant liver fibrosis are frequent in PWH in absence of viral hepatitis coinfection, with a prevalence ranging from 7.3 to 67.6% and 3.6% to 33.3%, respectively(12, 30-34). Insulin resistance is the key metabolic mechanism in the development of NASH, but in PWH the pathogenesis seems more complex(2). Although traditional risk factors associated with insulin resistance are more frequent in PWH because of ongoing immune activation, the pathogenesis may involve several pathways specific and unique to the HIV infection and ART(8, 9). First, HIV itself may have a direct steatogenic effect(35). Second, several ARTs may be associated with unfavourable metabolic outcomes, such as weight gain, dyslipidemia and insulin resistance, or are associated with mitochondrial dysfunction. In detail, contemporary ART based on use of integrase inhibitors and tenofovir alafenamide has been related to greater weight gain(36, 37). However, the impact of these weight changes on metabolic outcomes is uncertain, as some studies suggest protective effect of integrase inhibitors on insulin resistance in PWH without metabolic abnormalities, while others imply that this class is associated with hepatic steatosis and excess incidence of cardiovascular disease(15, 38, 39). If ART would be directly involved in fatty liver, it may be regarded more as drug or toxic-related steatosis rather than NAFLD, but this deserves further demonstration. Finally, chronic HIV-related inflammation is associated with higher risk of non-AIDS-related events and may contribute to the systemic inflammatory *milieu* that characterizes NASH(2, 12).

Our study, including PWH undergoing routine evaluations for liver disease, found a prevalence of NASH with significant liver fibrosis up to 8%. This is a considerable proportion of cases given that, in the general population, reported prevalence of significant liver fibrosis due to

NASH ranges between 1 and 3%(40, 41). In our experience, cofactors associated with FAST >0.35 suggesting NASH with significant liver fibrosis, included metabolic conditions, namely hypertension and higher BMI, as well as HIV-related factors, namely time since HIV diagnosis and detectable HIV viral load. This finding points towards the complexity of NASH in PWH and confirms that multiple gameplayers contribute to liver fibrosis. Other two studies used the FAST score in PWH. In line with our findings, in 928 women with HIV Price and colleagues found a 6.3% prevalence of FAST >0.35 and that associated cofactors were waist circumference and detectable HIV viral load(14). In 282 PWH, Michel et al found that 12.3% had FAST >0.35, with associated cofactors being type 2 diabetes and ALT. However, this high proportion of NASH with significant liver fibrosis likely reflects the selection of study population, where alcohol abuse and viral hepatitis coinfection were not excluded(25).

In the incidence cohort, we found an incidence rate of liver-related and extra-hepatic outcomes of 8.9 per 1000 PY and 33.7 per 1000 PY, respectively. Longitudinal data characterizing the natural history of NASH in PWH are scarce. Our findings indicate that NASH is a progressive disease in this population and may lead to adverse liver-related outcomes. FAST score, LSM, FIB-4 and APRI were associated with the development of liver-related outcomes by survival analysis. We also confirmed that FAST >0.35 was an independent predictor of liver-related outcomes, together with BMI and detectable HIV viral load. This finding underlines the diversified pathogenic hits leading to end-stage liver disease in PWH. The AUROC analysis showed that FAST score had the highest performance to predict liver-related outcomes, with high NPV. Interestingly, we did not observe a significant difference between FAST score or LSM and APRI. As APRI requires no specialty equipment to calculate, it could be used for risk stratification in clinic settings without easy access to elastography. As previously reported in the general population, extra-hepatic outcomes accounted for the majority of adverse outcomes(27). Interestingly, FIB-4 was the only non-invasive test associated with the development of extra-hepatic events, likely because it incorporates age. On multivariable analysis, classical clinical factors were predictors of extra-hepatic events, namely age, male sex and detectable HIV viral load. The AUROC analysis demonstrated that all non-invasive tests had a suboptimal performance to predict extra-hepatic events. In the HIV-negative NASH, some studies reported that LSM, FIB-4 and APRI predicted extra-hepatic events(42-44), while others did not(45). A recent study of 1773 NAFLD patients showed that liver fibrosis stage was not associated with incident cardiac events and nonhepatic cancers(46). This evidence indicates that more prospective studies on NAFLD are needed. It is also possible that, in PWH, more complex drivers beyond liver fibrosis affect extra-hepatic events.

Our study has several strengths, including the large collaborative cohort design and the availability of longitudinal data for 60% of the patients. We wish to acknowledge several limitations of our study. First, due to the relatively short follow-up period and limited number of outcomes, we were not able to study the influence of specific ART drugs on clinical outcomes. Second, we did not have available histology as the gold standard of reference to diagnose

NASH(2). Third, clinical outcomes were collected retrospectively, with the potential risk of missing relevant variables. Fourth, the cohort was overall predominantly male, white, with relatively low BMI, which may limit generalizability.

In conclusion, PWH have high prevalence of NASH with significant liver fibrosis and can develop clinical outcomes at a significant rate. Our data support the notion that this population should enter clinical pathways for case-finding of those at higher risk for liver-related outcomes, as recommended by guidelines(11). FAST score has good prognostic accuracy and can identify PWH at low risk of liver-related outcomes, thus can be used in clinical practice to prioritize management and treatment. In consideration of these data, we advocate that PWH should not be excluded and be instead prioritized for inclusion in global trials of NASH-targeted therapies(47).

NOTES

Authors contributions: GS contributed to conception, study design, data and interpretation of the data, statistical analysis and first draft of the manuscript. JM and GG contributed to conception, study design, data and interpretation of the data. AVR contributed to statistical analysis. DK, CG, AA, BL, AB, MD, AC, GM contributed to data and interpretation of data. All authors approved the final version of the article. Part of this work has been presented at the International Liver Meeting of the European Association for the Study of the Liver (EASL) (London, UK; June 2022).

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Conflict of interest*:* GS has acted as speaker for and received payment or honoraria from Merck, Gilead, Abbvie, Novonordisk, Pfizer, and Intercept served as an advisory board member for Pfizer, Merck, Novonordisk, Gilead and Intercept and has received unrestricted research funding from Theratec. BL has acted as a speaker and advisory board member for ViiV, Gilead, and Merck, and received research funding from ViiV, Merck, and Gilead. ET has participated to advisory boards for and reports payment or honoraria from Boehringer, Pfizer, NovoNordisk, Alexion and Orphalan and acted as a speaker for NovoNordisk and Orphalan. AC has served as an advisory board member for Gilead, Janssen, Merck, ViiV, GSK and Astra Zeneca, acted as a speaker for and received payment or honoraria from Gilead and Tillots and support for attending meetings from Pfizer and Nordic pharma. GG received a research grant and speaker honoraria from Gilead, ViiV, Merck and Jansen and attended advisory boards of Gilead, ViiV and Merck. JM, DK, CG, ASA, JF, LRB, AVR, SB, AB, GM have nothing to disclose.

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Table 1. Baseline characteristics of the cohort by FAST score status (n=1472).

	Whole cohort (n=1472)	**FAST score >0.35** (n=119)	**FAST score ≤0.35** (n=1353)	**p-value**
Age (years)	51.8 (9.9)	53.8 (8.1)	51.6 (10.1)	0.019
Male sex (%)	1099 (74.7)	101 (84.9)	998 (73.8)	<0.001
Ethnicity (%)				
White	945 (64.2)	96 (80.7)	849 (62.7)	<0.001
Black	371 (25.2)	9 (7.6)	362 (26.8)	
IDU (%)	130 (8.8)	4 (3.4)	126 (9.3)	<0.001
MSM (%)	614 (41.7)	47 (39.5)	567 (41.9)	0.560
Hypertension (%)	342 (23.2)	40 (33.6)	302 (22.3)	<0.001
Diabetes (%)	221 (15.0)	24 (20.2)	197 (14.6)	0.101
Time since HIV diagnosis (years)	17.2 (9.5)	20.4 (8.8)	17.0 (9.5)	0.001
Undetectable HIV viral load (≤ 50 copies) (%)	1223 (83.1)	91 (76.4)	1132 (83.7)	0.047
BMI (Kg/m^2) °	25.1 (4.4)	28.6 (5.3)	24.8 (4.2)	<0.001
CD4 (cell/uL)	706.0 (319.4)	687.3 (364.4)	707.7 (315.3)	0.419
Creatinine (mmol/L)	0.96 (0.31)	0.97 (0.21)	0.96 (0.32)	0.761
Platelets (10^9/L)	227.5 (68.0)	213.9 (57.4)	228.8 (68.7)	0.076
Albumin (g/L)	41.4 (4.8)	43.4 (4.7)	41.2 (4.8)	0.011
ALT (IU/L)	29.0 (22.0)	63.1 (48.9)	24.8 (13.3)	<0.001
AST (IU/L)	25.1 (15.6)	52.0 (33.7)	22.8 (8.0)	<0.001
Total cholesterol (mmol/L)	4.7 (1.1)	4.7 (1.0)	4.7 (1.1)	0.855
HDL cholesterol (mmol/L)	1.3 (0.4)	1.1 (0.4)	1.3 (0.4)	0.008

Triglycerides (mmol/L)	1.7 (1.4)	2.5 (2.6)	1.6 (1.2)	<0.001
Statin use (%)	253 (17.2)	30 (25.2)	223 (16.5)	0.016
Current antiretroviral regimen (%)				
NNRTI	442 (30.0)	26 (21.8)	416 (30.7)	0.042
NRTI	1253 (85.1)	98 (82.4)	1155 (85.4)	0.376
Protease inhibitors	553 (37.6)	39 (32.8)	514 (38.0)	0.260
Integrase inhibitors	699 (47.5)	62 (52.1)	637 (47.1)	0.293
Past exposure to didanosine (%)	181 (12.3)	24 (20.2)	157 (11.6)	<0.001
Past exposure to stavudine (%)	315 (21.4)	40 (33.6)	275 (20.3)	<0.001
LSM (kPa)	5.8 (3.6)	12.5 (8.5)	5.2 (2.0)	<0.001
CAP (dB/m)	239.4 (57.4)	306.1 (57.1)	233.5 (53.6)	<0.001
FIB-4	1.2 (0.7)	1.9 (1.2)	1.1 (0.6)	<0.001
APRI	0.3 (0.2)	0.7 (0.6)	0.3 (0.3)	<0.001

Legend: Continuous variables are expressed as mean (standard deviation) and categorical variables are expressed as frequencies (%). The p-values refer to Student t-test or χ^2 test between FAST score >0.35 and FAST score $\leq$0.35. Abbreviations: ALT, alanine aminotransferase; APRI, AST-to-Platelet Ratio Index; AST, aspartate aminotransferase; BMI, body mass index; CAP, controlled attenuation parameter; FAST, Fibroscan-AST; FIB-4, Fibrosis-4 index; HDL, high-density lipoprotein; HIV, human immunodeficiency virus; IDU, injection drug use; IU, international units; LSM, liver stiffness measurement; MSM, men having sex with men; NAFLD, nonalcoholic fatty liver disease; NNRTI, non-nucleoside reverse transcriptase inhibitors; NRTI, nucleoside reverse transcriptase inhibitors.

Table 2. Univariable and multivariable analysis of cofactors associated with FAST score >0.35 in the prevalence cohort (n=1472).

	Univariable		Multivariable
Variable	**OR** (95% CI)	**p-value**	**aOR** (95% CI)
Age (per 10 years)	1.26 (1.04-1.53)	0.019	0.83 (0.58-1.18)
Male sex (yes vs. no)	2.00 (1.19-3.34)	0.009	1.80 (0.81-4.00)
White ethnicity (yes vs. no)	2.39 (1.31-4.37)	0.005	2.23 (0.98-5.08)
Type 2 diabetes (yes vs. no)	1.28 (0.85-1.94)	0.10	-
Hypertension (yes vs. no)	2.62 (1.59-4.32)	<0.001	2.24 (1.16-4.34)
BMI (per Kg/m^2)	1.16 (1.12-1.21)	<0.001	1.21 (1.14-1.29)
Time since HIV diagnosis (per 10 years)	1.48 (1.20-1.81)	<0.001	1.82 (1.20-2.76)
HIV viral load >50 cp/mL (yes vs. no)	1.59 (0.89-2.83)	0.117	2.22 (1.02-4.85)
CD4 count (per cells/µL)	0.99 (0.99-1.00)	0.510	-
Past exposure to didadosine (yes vs. no)	1.90 (0.74-4.91)	0.185	-
Past exposure to stavudine (yes vs. no)	1.95 (0.88-4.33)	0.102	-
HDL cholesterol (per mmol/L)	0.61 (0.31-1.22)	0.165	-

Triglycerides (per mmol/L)	1.38 (1.18-1.62)	<0.001	1.19 (0.99-1.44)

Odds ratios (OR) and 95% confidence intervals (CI) are shown for each variable analyzed in univariable and multivariable logistic regression analysis. Abbreviations: aOR, adjusted odds ratio; BMI, body mass index; HDL, high-density lipoprotein; HIV, human immunodeficiency virus.

Table 3. Incidence of liver-related outcomes, extra-hepatic events and all-cause mortality in the incidence cohort (n=882).

	Cases	**Incidence rate** (per 1000 PY, 95% CI)
Any event	124	41.3 (34.5-49.4)
Liver-related outcomes	26	8.9 (6.1-13.1)
Ascites	13	
Hepatic encephalopathy	7	
Variceal bleeding	5	
Hepatocellular carcinoma	1	
Extra-hepatic events	98	33.7 (27.7-41.1)
Cardiovascular event	74	
Extra-hepatic cancer	24	
All-cause mortality	10	3.4 (1.9-4.0)
Myocardial infarction	3	
Lung cancer	2	
Trauma	2	
Esophageal cancer	1	
Pneumonia	1	
Sepsis	1	

Abbreviations: CI, confidence interval; PY, person-years.

Table 4. Association of predictors with liver-related outcomes and extra-hepatic events by log-rank test (n=882).

	Liver-related outcomes		Extra-hepatic events	
	Incidence rate (per 1000 PY, 95% CI)	**p-value**	**Incidence rate** (per 1000 PY, 95% CI)	**p-value**
Age				
<50 years	6.2 (3.1-12.4)	0.108	12.4 (7.6-20.2)	<0.001
≥50 years	11.1 (7.0-17.7)		50.7 (40.9-63.0)	
Sex				
Female	6.1 (2.6-14.7)	0.309	11.0 (5.7-21.2)	<0.001
Male	10.0 (6.5-15.4)		42.6 (34.6-52.4)	
Type 2 diabetes				
No	7.7 (5.0-12.0)	0.178	30.6 (24.5-38.1)	0.027
Yes	19.9 (8.9-44.3)		63.1 (40.2-98.9)	
Hypertension				
No	8.5 (5.4-13.3)	0.563	30.9 (24.4-39.1)	0.123
Yes	10.9 (5.2-22.8)		45.0 (31.2-64.7)	
BMI				
≤25 Kg/m^2	3.8 (1.6-9.1)	0.042	31.7 (23.4-42.8)	0.466
>25 Kg/m^2	12.0 (7.3-20.0)		27.3 (19.5-38.2)	
Time since HIV diagnosis				
<15 years	5.6 (2.9-10.8)	0.034	22.4 (16.2-31.1)	<0.001
≥15 years	13.2 (8.2-21.2)		47.3 (36.8-60.7)	
HIV viral load				
<50 cp/mL	6.2 (3.6-10.6)	<0.001	27.0 (20.8-35.0)	<0.001
≥50 cp/mL	21.2 (12.0-37.3)		72.4 (53.3-98.3)	
Triglycerides				
<1.7 mmol/L	3.8 (1.7-8.5)	<0.001	25.4 (18.7-34.7)	0.029
≥1.7 mmol/L	21.2 (13.2-34.0)		43.6 (31.3-60.7)	

FAST score				
≤0.35	5.0 (2.9-8.6)	<0.001	32.8 (26.6-40.6)	0.706
>0.35	45.1 (26.2-77.7)		41.7 (23.7-73.4)	
LSM				
<7.1 kPa	5.0 (2.9-8.8)	<0.001	32.2 (25.8-40.3)	0.511
≥7.1 kPa	27.1 (16.1-45.8)		40.7 (26.5-62.4)	
CAP				
<275 dB/m	4.5 (2.7-8.7)	0.002	36.3 (28.9-45.8)	0.164
≥275 dB/m	18.3 (11.4-29.5)		28.0 (19.1-41.2)	
APRI				
<0.5	4.3 (2.1-7.3)	<0.001	41.8 (22.7-71.3)	0.313
≥0.5	24.1 (13.1-39.3)		29.0 (13.1-40.4)	
FIB-4				
<1.3	5.2 (3.1-8.8)	0.018	20.5 (9.8-33.4)	<0.001
≥1.3	15.1 (9.3-24.9)		45.1 (24.1-78.1)	

Incidence rates per 1000 person-years (PY) and 95% confidence intervals (CI) are shown for each variable and compared by log-rank test. Abbreviations: APRI, aspartate aminotransferase-to-Platelet Ratio Index; BMI, body mass index; CAP, controlled attenuation parameter; FAST, Fibroscan-aspartate aminotransferase; FIB-4, Fibrosis-4 index; HIV, human immunodeficiency virus; LSM, liver stiffness measurement.

Table 5. Multivariable analysis of predictors of liver-related and extra-hepatic outcomes in the incidence cohort (n=882).

	Liver-related outcomes	**Extra-hepatic outcomes**
Variable	**aHR** (95% CI)	**aHR** (95% CI)
Age (per 10 years)	1.05 (0.69-1.61)	1.79 (1.45-2.21)
Male sex (yes vs. no)	1.22 (0.40-3.73)	3.53 (1.61-7.74)
Type 2 diabetes (yes vs. no)	-	0.86 (0.46-1.61)
BMI (per Kg/m^2)	1.07 (1.01-1.14)	-
HIV viral load >50 cp/mL (yes vs. no)	4.88 (1.91-12.46)	4.48 (2.80-7.17)
Triglycerides (per mmol/L)	-	1.08 (0.94-1.23)
FAST >0.35 (yes vs. no)	4.97 (1.97-12.51)	-

Adjusted hazard ratios (aHR) and 95% confidence intervals (CI) are shown for each variable analyzed in multivariable Cox regression analysis. Abbreviations: BMI, body mass index; FAST, Fibroscan-aspartate aminotransferase; HIV, human immunodeficiency virus.

FIGURE LEGEND

Figure 1. Flow chart displaying the selection of study participants and the two study cohorts (prevalence and incidence).

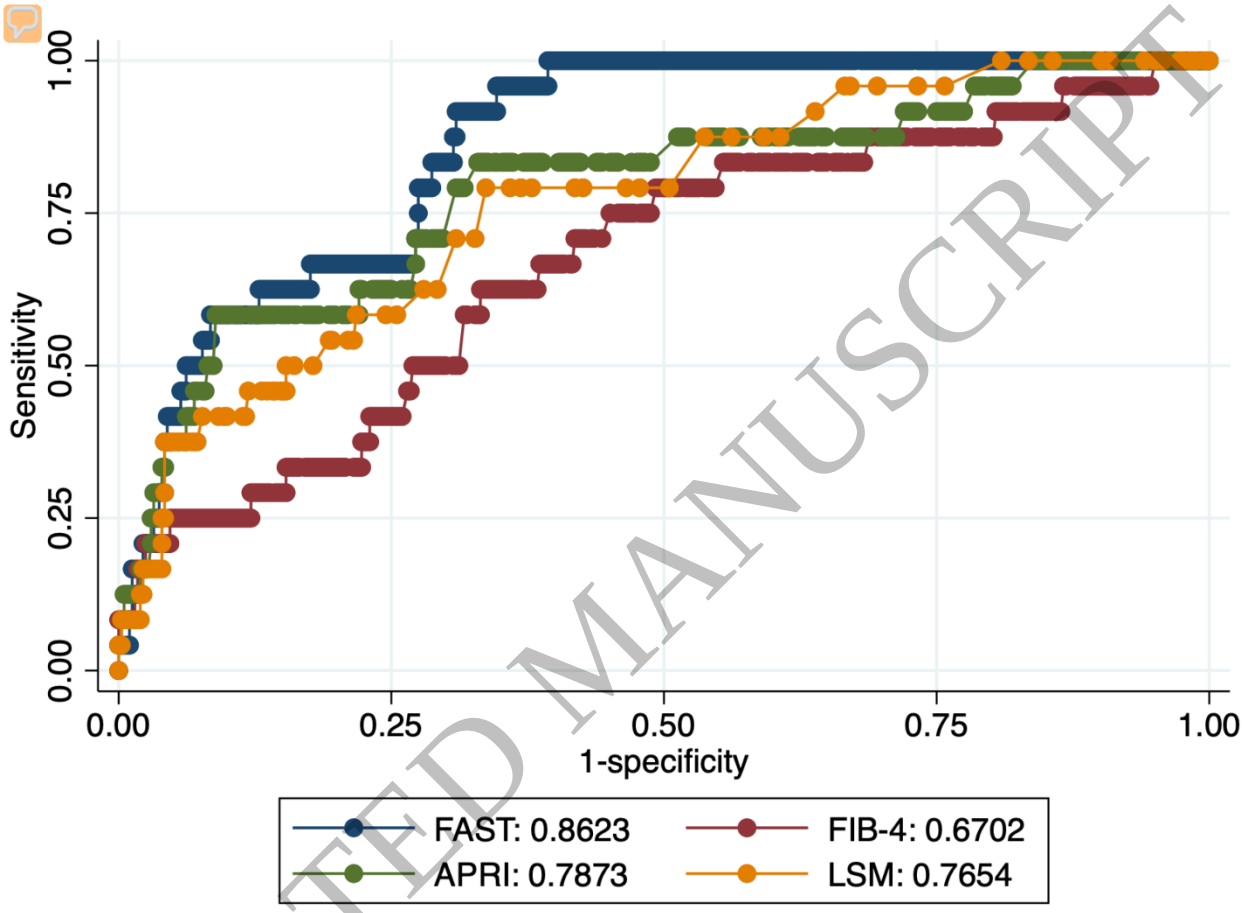


Figure 2. Survival curves of incidence of liver-related outcomes by cut-off of FAST score. The p-value refers to log-rank test.

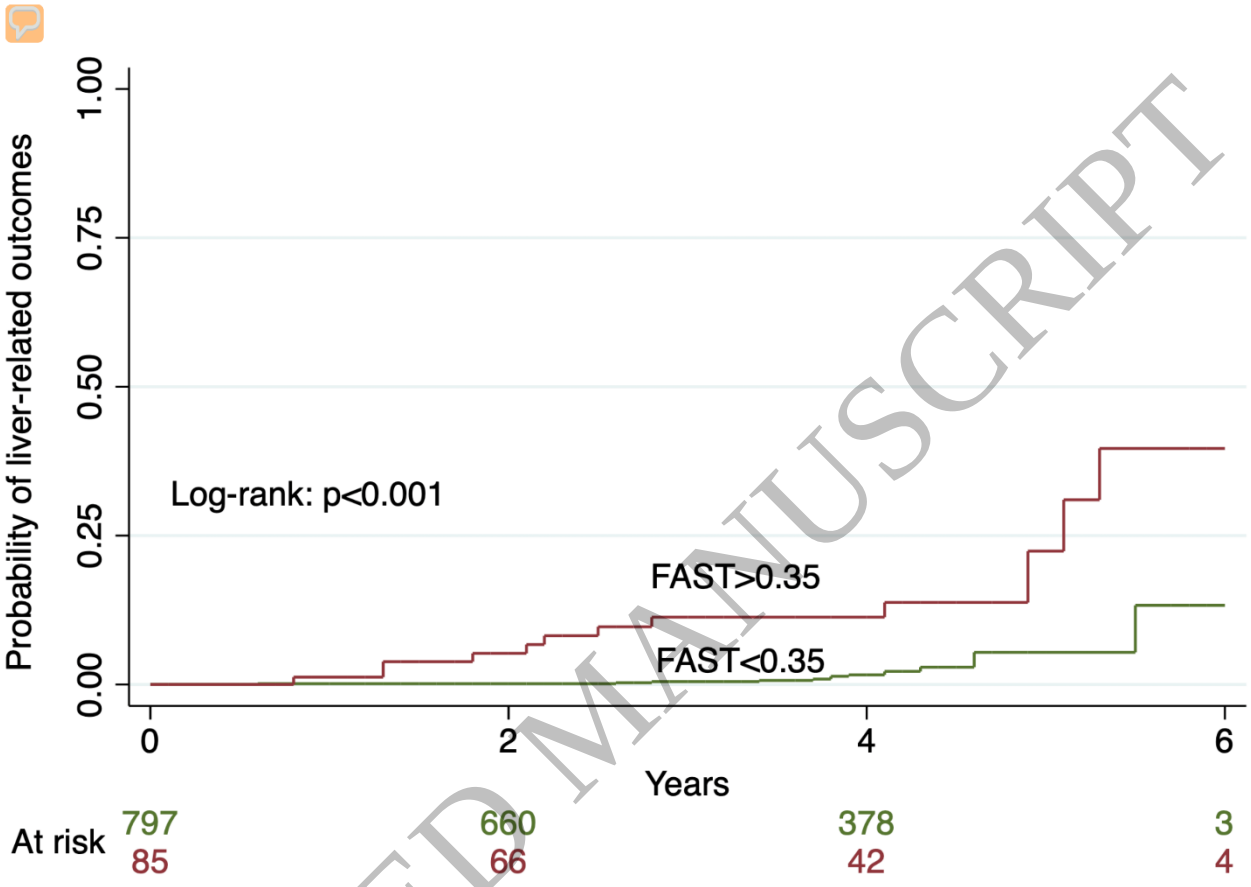
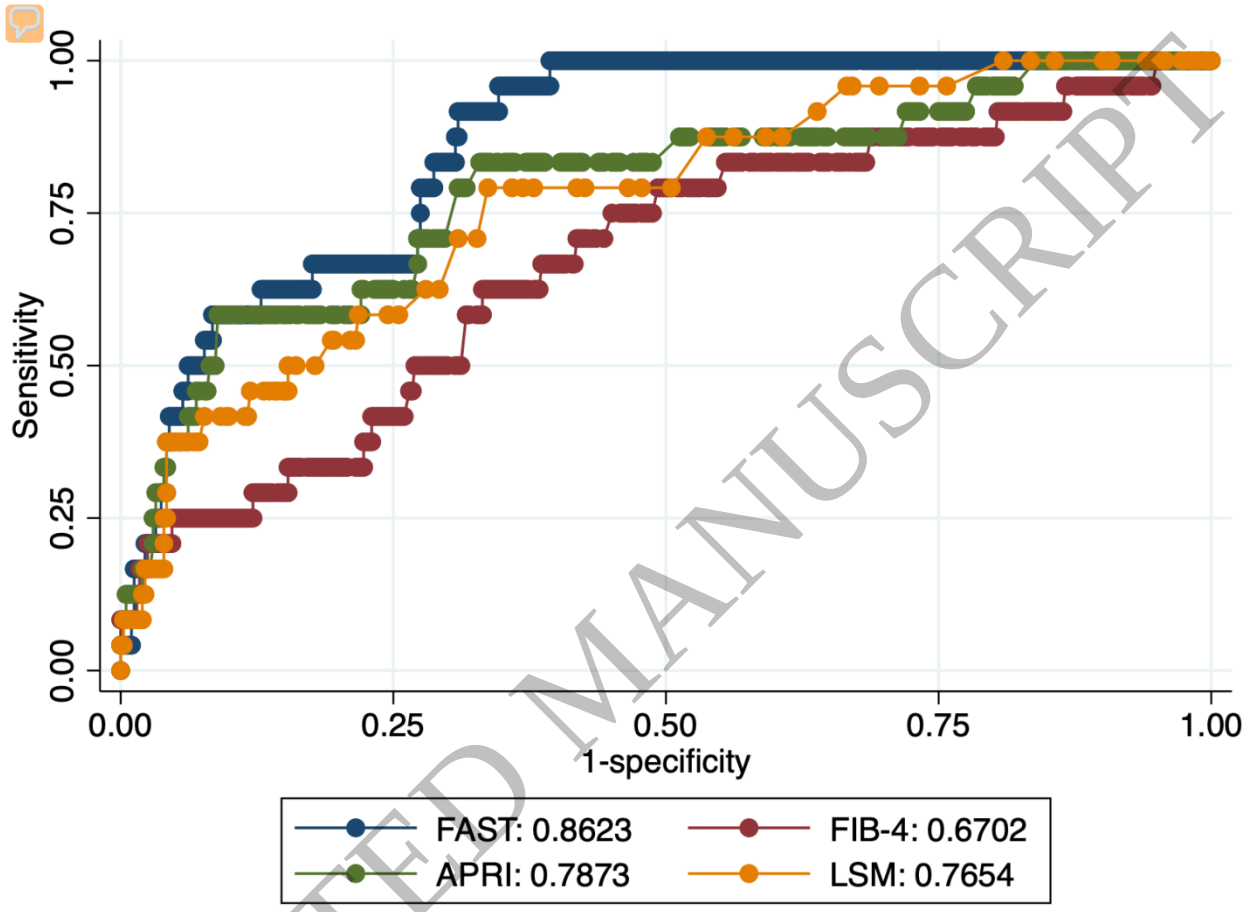


Figure 3. Area under the receiver operating characteristics of Fibroscan-aspartate aminotransferase (FAST) score, liver stiffness measurement (LSM), Fibrosis-4 index (FIB-4) and aspartate aminotransferase-to-Platelet Ratio Index (APRI) to predict liver-related outcomes.



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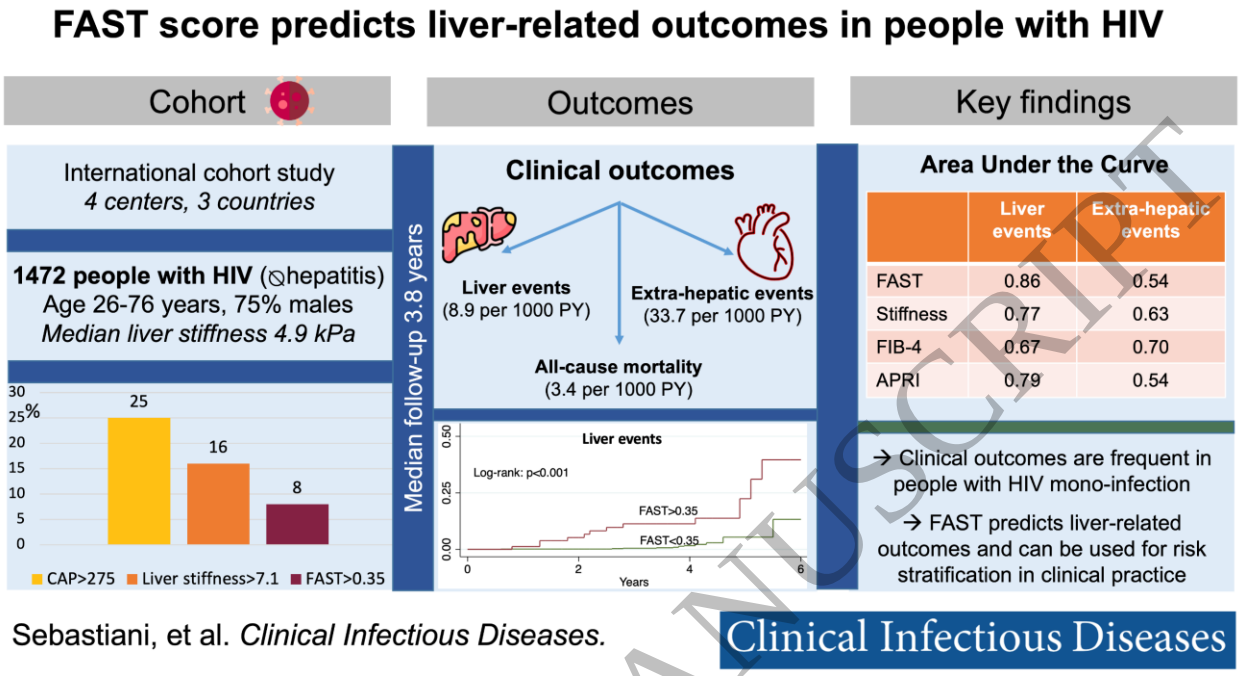
Graphical Abstract



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