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Increased BMI and Type 2 diabetes are the main predictors of NAFLD and advanced fibrosis in liver biopsies of patients with HIV mono-infection

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Summary: In patients living with HIV and without viral hepatitis or alcohol excess, obesity is the main predictor of fatty liver and type 2 diabetes of developing advanced fibrosis. Current non-invasive tests for fibrosis need optimisation in this population.

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Abstract

Background: Liver disease is an important cause of morbidity and mortality in people living with HIV (PLWH), of which non-alcoholic fatty liver disease (NAFLD) is an increasingly recognised cause. There is limited data investigating NAFLD in HIV mono-infection and histologically defined disease. We aimed to identify who is at risk of fibrosis, NAFLD and NASH among PLWH, and explore the diagnostic accuracy of non-invasive markers of fibrosis.

Methods: Retrospective cross-sectional international multicentre study including patients with HIV mono-infection, without chronic viral hepatitis or other known causes of chronic liver disease, who underwent liver biopsy for abnormal liver biochemistry and/or clinical suspicion of liver fibrosis.

Results: One hundred and sixteen patients from 5 centres were included. Sixty-three (54%) had NAFLD, of whom 57 (92%) had NASH. Overall, 36 (31%) had advanced fibrosis (≥F3) and 3 (3%) cirrhosis. Of the 53 cases without NAFLD, 15 (28%) had advanced fibrosis. Collagen proportionate area (CPA) was similar between cases with and without NAFLD (3% vs 2%). Body mass index (BMI) was independently associated with NAFLD (aOR 1.2 95% CI 1.08-1.34), and type 2 diabetes was independently associated with advanced fibrosis (aOR 3.42 95% CI 1.00-11.71)). The area under the curve for advanced fibrosis was 0.65 and 0.66 for both NAFLD Fibrosis Score (NFS) and FIB-4. Cut-off values of -1.455 (NFS) and 1.3 (FIB-4) have negative predictive values of 0.80 and 0.82, respectively.

Conclusion: Advanced fibrosis is strongly associated with type 2 diabetes in PLWH. Serological markers require further optimisation.

Key Words: NAFLD, NASH, fibrosis, HIV, histopathology

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List of Abbreviations

AIDS	Acquired immune deficiency syndrome
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ART	Antiretroviral therapy
AUROC	Area under the receiver operator curve
BMI	Body mass index
CAP	Controlled attenuation parameter
CPA	Collagen proportionate area
D-Drugs	Dideoxynucleoside analogues
FLIP	Fatty liver inhibition of progression
H&E	Haemotoxylin and eosin
HIV	Human immunodeficiency virus
IQR	Inter-quartile range
LR-	Negative likelihood ratio
LR+	Positive likelihood ratio
NAFL	Non- alcoholic fatty liver
NAFLD	Non-alcoholic fatty liver disease
NAS Score	Non-alcoholic steatohepatitis Score

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NASH	Non-alcoholic steatohepatitis
NASH CRN	NASH Clinical Research Network
NFS	NAFLD fibrosis score
NPV	Negative predictive value
PLWH	People living with HIV
PPV	Positive predictive value
RGB	Red green blue
SD	Standard deviation
SHIVER	Steatohepatitis in HIV Emerging Research group

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Introduction

Non-alcoholic fatty liver disease (NAFLD is defined by the presence of hepatic steatosis in the absence of secondary causes such as excessive alcohol consumption. It encompasses a large spectrum of disease from non-alcoholic fatty liver (NAFL, “simple steatosis”) to hepatocyte inflammation/ballooning defined as non-alcoholic steatohepatitis (NASH), fibrosis and cirrhosis.

A major limitation in the literature to date on NAFLD in people living with HIV (PLWH) has been little available data based on disease stage defined by liver histology. Despite the advances of non-invasive markers, NASH remains a histological diagnosis and the gold standard for fibrosis staging is liver biopsy. Therefore, accurate phenotyping of patients with NAFLD requires a histological diagnosis, and this is particularly needed in special populations such as PLWH in whom non-invasive markers have not been well validated[7] and the pathogenesis of liver disease is potentially more complex.

To facilitate research collaborations within this field, an international consortium of clinical academics with expertise on HIV-NAFLD was established - the Steatohepatitis in HIV Emerging Research (‘SHIVER’) Group. This study represents the inaugural project for the group. The primary objective was to assess the histopathological features of liver biopsies performed in HIV mono-infection and identify risk factors associated with fibrosis, NAFLD and NASH. The secondary objective was to assess the performance of non-invasive tests for the diagnosis of liver fibrosis in patients with HIV mono-infection using liver histology as a reference standard.

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Methods

Study Population

This study was a retrospective international, multicentre, cross-sectional study. Five centres from UK (Imperial College and Royal Free Hospital), Italy (University of Modena), USA (University of California San Francisco) and Canada (McGill University) collected liver biopsy samples from adult (age ≥18 years) cases with HIV mono-infection. Exclusion criteria were positive hepatitis C antibody or hepatitis B surface antigen, current or recent (within 6 months) alcohol excess defined as ≥21 units per week for men and ≥14 units per week for women, concurrent life-threatening illness, active malignancy, AIDS-defining illness or evidence of other chronic liver disease at the time of liver biopsy, including biliary disease, autoimmune hepatitis, Wilson's disease and hereditary haemochromatosis, long term exposure to steroids or amiodarone. Clinical data nearest to the time of liver biopsy and within 6 months was collected, including basic demographics and anthropometrics, liver biochemistry, HIV history including drug exposure and HIV-specific complications, and medical co-morbidities. The Triglyceride Glucose Index (TGI) was used as a surrogate marker of insulin resistance (TGI = ln(Triglycerides(mg/dl)*fasting glucose(mg/dL)).[8]

Liver biopsy and histology analysis

Liver biopsies were performed as part of the clinical evaluation of patients with unexplained elevations in liver transaminases and/or clinical suspicion of liver fibrosis, either by percutaneous or transjugular approach, with a minimum core of 10mm.

Liver samples were paraffin- fixed and formalin embedded, and stained with haemotoxylin and eosin (H&E) and Sirius Red. Liver biopsy slides were centrally read by an expert liver histopathologist (RG) blinded to clinical and biological data, scored according to the NASH Clinical Research Network (CRN) system (Non-alcoholic steatohepatitis (NAS) Score)[9] and classified as NAFLD or NASH according to the Fatty Liver Inhibition of Progression (FLIP) algorithm.[10] Fibrosis stage was defined by the Brunt classification.[11]

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Collagen proportionate area (CPA) was quantified on picro-Sirius Red stained formalin fixed paraffin embedded sections using previously published methods.[12] Image capture was carried out at x4 objective magnification on a Zeiss Azioskop 50 using a Zeiss Axiocam ICc5 camera. Image analysis was carried out by AH in a single centre (Royal Free Hospital, London, UK) on the captured images using a custom script for Zeiss Axiovison software: segmentation of tissue and collagen was achieved in a RGB colour-space, followed by a manual editing step to remove areas not related to pathological collagen deposition such as structural collagen in portal tracts and image artefacts. CPA was calculated as the amount of collagen expressed as a percentage proportion of overall biopsy tissue area as previously published.[12]

Non-invasive markers of liver fibrosis

The following non-invasive markers of fibrosis were calculated: FIB-4 = age (years) x AST (IU/L) / platelets (10^9/L) x √ALT (IU/ml);[13] NAFLD Fibrosis Score (NFS) = −1.675 + 0.037 – age (years) + 0.094 – body mass index (BMI, kg/m^2) + 1.13 × IFG/diabetes (yes = 1, no = 0) + 0.99 × AST/ALT ratio – 0.013 × platelet count (×10^9/l) – 0.66 × albumin (g/dl)[14].

Statistical analysis

Continuous variables were expressed as mean (SD) for parametric data or median (IQR) for non-parametric data, and ordinal variables as frequency (%). Groups with and without advanced (≥F3) fibrosis were compared using unpaired t-test or Mann- Whitney-U and chi squared tests as appropriate. Multivariate logistic regression models for NAFLD and advanced fibrosis were built using biologically relevant variables, presented as odds ratios with 95% confidence intervals (CI). Non-invasive markers of fibrosis were compared to CPA and the gold standard of NAS fibrosis stage, and the diagnostic accuracy was assessed with area under the receiver operator curve (AUROC), sensitivity, specificity, positive and negative predictive values (PPV and NPV), and positive and negative likelihood ratios (LR+ and LR-). Analyses were performed on IBM SPSS Statistics version 20.

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Results

Population Characteristics

116 patients were included in the study between August 2001 and February 2019 (Imperial College- including St Mary's Hospital and Chelsea and Westminster Hospital- n=39; Royal Free Hospital n=39; University of Modena and Reggio Emilia, Modena, Italy n=12; University of California San Francisco, USA n=14; McGill University Health Centre, Montreal, Canada n=12). The demographic and clinical characteristics of patients are included in Table 1. Mean (± SD) age was 48.4±10.4 years and they were mainly non-hispanic white (72.9%) males (93.2%) with suppressed HIV viral load (94.1%). The mean (± SD) BMI was 29.2±5.5 kg/m^2 with rates of diabetes, hypertension and dyslipidaemia at 21.2%, 44.9% and 39.8%, respectively.

Histopathological characteristics

63/116 (54.3%) had at least 5% macrovesicular steatosis consistent with the diagnosis of NAFLD, of which 57 (90.1% cases with NAFLD, 49.1% entire cohort) had NASH as defined by the FLIP algorithm (steatosis, ballooning and lobular inflammation).[10] Most cases (48/116, 41.4%) without NAFLD had non-specific mild lobular inflammation, and five cases had features most in keeping with a drug reaction. No vascular liver disease (e.g. nodular regenerative hyperplasia) was reported.

There was liver fibrosis in 102/116 (87.9%) cases, including 35 (30.2%) stage 1, 28 (24.1%) stage 2, 36 (31.0%) stage 3 and 3 (2.6%) with cirrhosis (Table 2). The subset of cases (53/116) without steatosis were also reported by the Ishak staging system, and included 21/53 (39.6%) stage 0, 9 (17.0%) stage 1, 8 (15.1%) stage 2, 11 (20.8%) stage 3, 1 (1.9%) stage 4, 2 (3.8%) stage 5 and 1 (1.9%) stage 6. The median CPA of all cases was 3.0% (2.0-5.0). CPA was identical between stages F0-F2 (2%) but then increased exponentially above F3 (F3 5.0% (3.0-7.8); F4 20.0% (4.0-20.0)) (Figure 1).

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BMI is the key predictor of NAFLD in HIV mono-infection

The characteristics of subjects with NAFLD were compared to those with no steatosis on liver biopsy. On univariate analysis, factors significantly associated with NAFLD were increased BMI (OR 1.15 95%CI 1.06-1.25 p=0.001), type 2 diabetes (OR 2.63 95%CI 1.00-6.90 p=0.050), hypertension (OR 2.43 95%CI 1.14-5.17 p=0.021), dyslipidaemia (OR 2.18 95%CI 1.01-4.70 p=0.047), increased CD4 (OR 1.02 95%CI 1.01-1.04 p=0.002), and increased CD8 (1.01 95%CI 1.00-1.02 p=0.009). Increased AST:ALT ratio (OR 0.008 95%CI 0.02-0.39 p=0.002) and previous use of D-drugs (OR 0.40 95%CI 0.19-0.88 p=0.022) were associated with no steatosis (Table 4). Two multivariate models were built to investigate for variables independently associated with NAFLD, both adjusted for age, BMI and CD4 nadir: Model 1 (Metabolic)- age (years), BMI (kg/m^2), type 2 diabetes, hypertension, dyslipidaemia, CD4 nadir (cells/µl); Model 2 (HIV)- age (years), BMI (kg/m^2), lifetime D-drug exposure, CD4 (cells/ µl), CD8 (cells/µl), CD4 nadir (cells/µl). BMI was the only variable independently associated with NAFLD (Model 1: aOR 1.20 95%CI 1.08-1.34 p=0.001; Model 2 aOR 1.20 95%CI 1.08-1.33 p=0.001). BMI remained the only independent predictor of NAFLD when TGI replaced type 2 diabetes in the model (supplementary Table 1). More subjects with NAFLD had advanced (≥F3) fibrosis (22.6% vs 42.9% p=0.022) but the CPA was similar between those with and without NAFLD (3.0% (2.0-5.0) vs 2.0% (2.0-5.0) p=0.445) (Table 3 and Figure 2).

Type 2 diabetes is an independent predictor of advanced fibrosis

A total of 39/116 (34%) subjects had advanced (≥F3) fibrosis on liver biopsy (Table 5). On univariate analysis, a diagnosis of NAFLD (OR 2.56 95%CI 1.14-5.78 p=0.023), type 2 diabetes (OR 5.26 95%CI 2.05-13.50 p=0.001), hypertension (OR 2.65 95%CI 1.20-5.85 p=0.016), dyslipidaemia (3.12 95%CI 1.40-6.94 p=0.005), duration of ART (OR 1.06 95%CI 1.00-1.11 p=0.036) and time since HIV diagnosis (1.08 95%CI 1.03-1.13 p=0.002) were associated with advanced fibrosis (Table 6).

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A multivariate model was built using the significant variables from multivariate analysis. Duration of ART and time since HIV infection could not be included together due to collinearity, therefore duration of ART was selected due to uncertainty between time of diagnosis and duration of infection. In the model type 2 diabetes had the strongest association with advanced fibrosis (aOR 3.42 95% CI 1.00-11.71 p=0.050). When the TGI was used in place of type 2 diabetes in the model, it was the only variable significantly associated with advanced fibrosis (aOR per 10 unit increase 1.04 95%CI 1.00-1.07 p=0.033, supplementary table 2).

In a sub-analysis on non-NAFLD subjects comparing those with (15/53) and without (38/53) advanced fibrosis (≥Ishak stage 3), the only significant difference between the groups on univariate analysis was longer time since HIV diagnosis (11.5 (6.8-18.3) vs 21.0 (13.0-26.0) years, p=0.005) but age was similar (49.0±12.0 vs 50.0±8.0, p=0.761).

Performance of non-invasive markers for advanced fibrosis

The diagnostic accuracy of non-invasive markers of fibrosis was evaluated using liver histology as a reference. FIB-4 and NAFLD Fibrosis Score (NFS) had poor diagnostic accuracy for advanced liver fibrosis, with The AUROC for FIB-4 and NAFLD Fibrosis Score (NFS) to detect advanced fibrosis were 0.65 (95%CI 0.53-0.76) and 0.66 (95%CI 0.56-0.80) (all subjects), 0.64 (95%CI 0.49-0.79) and 0.64 (0.49-0.79) (NAFLD subjects only), and 0.72 (95%CI 0.55-0.88) and 0.73 (95%CI 0.57-0.89) (Non-NAFLD subjects only) respectively (Figure 3).

A CPA value of ≥7.6% is predictive of long-term adverse outcomes.[12] Both FIB-4 and NFS performed more robustly at identifying cases with CPA≥7.6% than advanced fibrosis as defined by NAS CRN staging, where the AUROCs of FIB-4 and NFS for CPA≥7.6% were 0.84 (95%CI 0.74-0.93) and 0.81 (95%CI 0.70-0.91) (all subjects), 0.82 (95%CI 0.69-0.95) and 0.78 (95%CI 0.64-0.92) (NAFLD subjects only), and 0.88 (95%CI 0.75-1.00)8 and 0.83 (95%CI 0.68-0.98) (Non-NAFLD subjects) respectively (Figure 4).

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Validated cut-off values for ruling out advanced fibrosis are <1.3 for FIB-4[15] and <-1.455 for NFS.[14] Using these values, the sensitivity, NPV and LR- were 0.72, 0.80 and 0.66 for FIB-4 and 0.84, 0.82 and 0.43 for NFS (Table 7A). These tests were better at ruling out CPA≥7.6%, where the sensitivity, NPV and LR- were 0.93, 0.98 and 0.16 for FIB-4 and 0.93, 0.97 and 0.20 for NFS (Table 7B).

Discussion

This study reports the largest known sample of liver biopsies in HIV mono-infection, read by a central expert liver pathologist, in which the primary risk factor identified for NAFLD was BMI and for advanced fibrosis was type 2 diabetes.

NAFLD is common in PLWH, but understanding more clearly which patients progress to NASH and advanced fibrosis will help clinicians to appropriately risk stratify patients for further investigation such as a liver biopsy and initiate appropriate management.

In this study, subjects were selected from five centres in Europe and North America who had had a liver biopsy without other cause of chronic liver disease. Approximately half (54%) had macrovesicular steatosis consistent with NAFLD, only 4% had a drug reaction and interestingly 41% had non-specific features. Of the 63 subjects with NAFLD, 57 (90%) had NASH, and 58% of the whole cohort had ≥F2 fibrosis, reflecting the selection criteria in centres to biopsy patients with a high pre-test probability of more advanced disease.

CPA was used as a quantitative measure of fibrosis, which showed that the quantity of collagen deposition is similar between stages F0-F2 but then increases steeply from F3-F4. This helps to explain the decline in prognosis with F3 fibrosis whereas it matches population controls for at least 20 years for F2 and 30 years for F0-F1 fibrosis.[21] The subjects with cirrhosis (n=3) had a wide range in CPA scores (4.1%, 20.0% and 41.9%) illustrating an important limitation of current staging systems in which patients with cirrhosis are crudely grouped together despite large differences in collagen content.

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Fibrosis quantitation with CPA could have prognostic utility in identifying subjects at high risk of decompensation, which may aid the identification of patients at risk of hepatic complications.[12] This should also be considered in clinical trial design for antifibrotic drugs in NASH, both in subjects with and without HIV, where a continuous variable of fibrosis content may be a more sensitive measure of anti-fibrotic effect compared to semi-quantitative staging which suffers from significant inter-observer variability.[22] A limitation to implementing this technology has been lack of outcome data for CPA levels, but this has recently been addressed by a recent study showing CPA independently predicts clinical outcomes.[12]

The main feature differentiating patients with and without NAFLD was BMI, which was significantly increased in patients with NAFLD (30.9 vs 27.3 kg/m^2) and this remained independently associated in the multivariate analysis. Clearly, as in the general population, obesity is a hallmark characteristic of NAFLD in PLWH.[15][23][24] Some studies have shown an association with nucleoside reverse transcriptase inhibitor (NRTI) exposure and NAFLD,[25] possibly through an indirect effect on fat metabolism and redistribution, but this has not consistently been the case[6] and is not supported in this study, where ART exposure was not predictive of NAFLD. It is surprising that D-drug exposure tended towards a protective effect in the univariate and multivariate analysis, but in a cohort with median ART duration about 10 years and median D-drug exposure 0 months, the individual exposure of subjects was likely to have been very low towards the end of the era when they were prescribed, so the reported effect may be subject to confounding. There is emerging data on excess weight gain following use of integrase inhibitors, and further evaluation is required to understand if this may result in an increased risk of developing NAFLD.[26]

BMI was not associated with an increased risk of advanced fibrosis, whereas type 2 diabetes remained independently associated in the multivariate analysis. This was supported by the significant association with increased TGI, a surrogate marker of insulin resistance.[8] A paired biopsy study of NASH subjects (n=83) without HIV demonstrated similar results, in

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which fibrosis progression is independently linked to type 2 diabetes,[27] and a study using transient elastography in PLWH has also shown that liver fibrosis is significantly more common in patients with the metabolic syndrome, of which type 2 diabetes is a key feature.[28] Therefore in PLWH developing metabolic complications of obesity, particularly diabetes, represents increased risk for liver fibrosis and potential for higher liver-related morbidity.

An interesting observation in our study was the high rate of non-specific findings in cases with no evidence of significant steatosis, including 60% with some evidence of fibrosis and 28% with ≥ Ishak stage 3 fibrosis, representing a group with significant underlying liver damage but no known cause of chronic liver disease. This included the patients with cirrhosis, possibly through 'burnt-out' NASH, but this observation does raise further questions about the natural history of liver fibrosis in patients with HIV, particularly since the time since HIV diagnosis was the main variable associated with fibrosis in subjects without steatosis. There were insufficient cases to do a multivariate analysis in this sub-group which warrants further investigation, particularly with longitudinal follow-up.

How, therefore, should we select patients to send for further evaluation including liver biopsy? Targeted screening for NAFLD should certainly be considered in PLWH who are obese, a practice that becomes even more important in those with accompanying metabolic complications especially type 2 diabetes. Current guidelines for NAFLD recommend risk stratification of patients at risk of NAFLD and liver fibrosis with non-invasive markers, including FIB-4 and NFS.[15] The accuracy of these serological markers of fibrosis were evaluated in this population of PLWH and the AUROC values were poor. A recent large cross-sectional study by Boursier et al (n=452) in NAFLD subjects in the general population validating these markers against a liver biopsy gold standard also showed these tests only have a modest AUROC for diagnosing advanced fibrosis - 0.732 for NAFLD and 0.780 for FIB-4.[29] However, these scores are primarily applied using cut-off values designed to optimise the negative predictive value (NPV) of the test, a practice that has been

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successfully applied to stratify patients for referral to secondary care from the community.[30] Using previously validated cut-offs for FIB-4[31] and NFS[14] in this study, there was a NPV of 0.80 and 0.82 for FIB-4 and NFS. The performance of these markers was similar to the Boursier study where the optimised cut-offs -1.036 (NFS) and 1.515 (FIB-4) had NPV of 0.81 and 0.82 respectively.[29] The performance improved in the prediction of CPA≥7.6% (NPV 0.98 for FIB-4 and 0.97 for NFS), but overall about a quarter of cases with advanced fibrosis could be mis-classified as low risk, supporting concerns about the accuracy of these markers in HIV-associated NAFLD.[7]

The main limitations of the study are its retrospective design with data from a selected population and heterogeneous indications for liver biopsy, and a lack of elastography data. However, the strength of the study is the large, multi-centre international collection of centrally- reviewed liver biopsy data in a field where to date only a few, small studies with liver biopsy in patients with HIV mono-infection have been published. Although significant alcohol excess was an exclusion criterion, we did not have data on moderate alcohol use. However, our cut-off approximates to that used in NAFLD trials.

Conclusion

In HIV mono-infection, advanced liver fibrosis is strongly associated with type 2 diabetes, and NAFLD to elevated BMI. Liver fibrosis may be evident in PLWH and with no known established cause of chronic liver disease. The biochemical markers of fibrosis (FIB-4 and NFS) require further validation in this population.

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Conflicts of Interest

JBM has received consulting fees and conference support from Intercept Pharma and Norgine, and research funding from ViiV.

GS has acted as speaker for Merck, Gilead, Abbvie, Novonordisk served as an advisory board member for Merck, Gilead, Intercept, Allergan and Novartis has received research funding from Merck, Theratec and Pfizer.

PI has received speaker´s or consultancy fees from Gilead, Abbvie, MSD, ViiV and holds a research grant from Gilead (NoCo).

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Table Legends

Table 1: Study population demographic data. Results are presented as mean (SD) or median (IQR) according to distribution. ART: antiretroviral therapy; NRTI: nucleoside reverse transcriptase inhibitors; NNRTI: non-nucleoside reverse transcriptase inhibitors; PI: protease inhibitors; II: integrase inhibitors; ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; LDL: low-density lipoprotein; HDL: high-density lipoprotein; TGI: Triglyceride Glucose Index.

Table 2: Summary of histological characteristics of study population liver biopsies. Data presented as number (%). *NASH is defined as the presence of steatosis, ballooning and lobular inflammation. [+] NAS score is only reported on subset of cases with NAFLD (n=63). [#] Non-alcoholic Steatohepatitis (NAS) Score and fibrosis stage reported according to the NASH CRN system.

Table 3: Demographic and clinical characteristics of subjects according to the presence of NAFLD on liver biopsy (defined as macrovesicular steatosis ≥5%). Data is presented as mean (SD) or median (IQR) according to distribution. ART: antiretroviral therapy; NRTI: nucleoside reverse transcriptase inhibitor; NNRTI: non-nucleoside reverse transcriptase inhibitor; PI: protease inhibitor; TGI: Triglyceride Glucose Index; VL: HIV viral load.

Table 4: Univariate and multivariate analysis of risk factors for NAFLD. Model 1 (Metabolic): age (per year), BMI (per kg/m^2), diabetes, hypertension, dyslipidaemia, CD4 nadir (per 10 cells/µl); Model 2 (HIV): BMI (per kg/m^2), diabetes, lifetime D-Drugs, CD4 (per 10 cells/µl), CD8 (per 10 cells/µl), CD4 nadir (per 10 cells/µl). *Past exposure in lifetime.

Table 5: Demographic and clinical characteristics of subjects according to the presence of advanced fibrosis (defined as ≥F3 Fibrosis by Brunt classification). Data is presented as mean (SD) or median (IQR) according to distribution. Groups compared by Mann Whitney test (discrete data) and Chi-Square (categorical). P<0.05 considered statistically significant. ART: antiretroviral therapy; NRTI: nucleoside reverse transcriptase inhibitor; NNRTI: non-nucleoside reverse transcriptase inhibitor; PI: protease inhibitor; TGI: Triglyceride Glucose Index; VL: HIV viral load; NAFLD: Non-alcoholic fatty liver disease; NFS: NAFLD fibrosis score.

Table 6: Univariate and multivariate analysis of risk factors for advanced fibrosis. Multivariate model: Body mass index (BMI, per kg/m^2), NAFLD (non-alcoholic fatty liver disease, ≥5% steatosis on liver biopsy), Type 2 Diabetes, hypertension, dyslipidaemia, duration of antiretroviral therapy (ART, per year).

Table 7: Diagnostic accuracy of FIB-4 and NFS for A. Advanced Fibrosis; B. Collagen proportionate area (CPA) >7.6%.

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Figure Legends

Figure 1: Collagen Proportionate Area (CPA) per fibrosis stage (Brunt). Data presented as median ±IQR: F0 2.0% (1.8-4.0); F1 2.0% (1.0-3.3); F2 2.0% (2.0-4.0); F3 5.0% (3.0-7.8); F4 20.0% (4.0-20.0).

Figure 2: Collagen proportionate area (CPA) in subjects with and without NAFLD.

Figure 3: ROC curve assessing the performance of the non-invasive markers for detecting (≥F3) advanced fibrosis: A. All Cases. AUROC FIB-4: 0.659; NFS 0.688; combined 0.716; B. NAFLD cases only. AUROC FIB-4: 0.655; NFS: 0.684; combined 0.662; C. Non-NAFLD cases only. AUROC FIB-4: 0.722; NFS: 0.679. NFS: NAFLD Fibrosis Score.

Figure 4: ROC curve assessing the performance of the non-invasive markers for detecting CPA ≥7.6% (n=14/116). A. All Cases. AUROC FIB-4: 0.836; NFS: 0.805; combined 0.784; B. NAFLD cases only. AUROC FIB-4 0.819; NFS 0.780; C. Non-NAFLD cases only. AUROC FIB-4 0.878; NFS 0.830.

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Characteristic	No. (%) or Median (IQR) N=116
Age, years	48.4 (10.9)
Male, n (%)	110 (93.2)
Ethnicity	
White-European, n(%)	86 (72.9)
White- Hispanic, n(%)	5 (4.2)
Black, n(%)	13 (11.0)
Other, n(%)	12 (10.3)
Body Mass Index, kg/m^2	29.2 (5.5)
Diabetes, n(%)	25 (21.2)
Hypertension, n(%)	53 (44.9)
Dyslipidaemia, n(%)	47 (39.8)
Time Since HIV, years	13.0 (7.0-21.0)
CD4 Nadir, Cells/mm^3	162.5 (36.8-277.5)
Time from Diagnosis to ART, months	11 (1-45)
Duration of ART, years	9.0 (5.0-17.0)
NRTI, months	158 (63-216)
NNRTI, months	41 (6-95)
PI, months	22 (0-116)
II, months	0 (0-1)
D-Drugs, months	0 (0-54)
Platelets, x10^9/L	206 (66)
ALT, IU/L	68 (45-107)
AST, IU/L	46 (32-63)
ALP, U/L	91 (74-111)
Bilirubin, µmol/L	10 (7-17)

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Albumin, g/L	43.1 (5.2)
Cholesterol, mmol/L	4.8 (1.1)
Triglycerides, mmol/L	1.9 (1.2-3.1)
LDL cholesterol, mml/L	2.7 (2.1-3.4)
HDL cholesterol, mmol/L	1.2 (0.5)
Fasting glucose, mmol/L	5.9 (2.1)
TGI	505.6 (445.8-608.2)
Detectable HIV Viral Load (%)	7 (5.9)
CD4 (cells/µl)	638.1 (297.3)
CD8 (cells/µl)	875 (586-1209)
CD4:CD8	1.0 (0.5-1.0)

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Histological Feature or Diagnosis	N (%)
Steatosis	
None (<5%)	53 (45.7)
Mild (5-33%)	34 (29.3)
Moderate (34-66%)	28 (24.1)
Severe (>66%)	1 (0.9)
NASH*	57 (49)
Ballooning 0/1/2	39(33.6)/ 49(42.2)/ 28(24.1)
Inflammation 0/1/2/3	14(12.1)/ 72(62.1)/22(19.0)/ 8(6.9)
NAS Score[+]	4.1 (1.2)
NAS <3	2 (3.2)
NAS 3-4	41 (65.1)
NAS >4	20 (31.7)
Drug Reaction	5 (4.3)
Fibrosis[#]	
None	14 (12.1)
F1	35 (30.2)
F1a	*3 (2.6)*
F1b	*21 (18.1)*
F1c	
F2	*11 (9.5)*
F3	28 (24.1)
F4	36 (31.0)
	3 (2.6)
Non-specific changes	48 (41.4)

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Characteristic	No. (%) or Median (IQR)		p
	Steatosis<5% N=53	Steatosis≥5% N=63	
Age (Years)	49.3 (10.9)	47.7 (11.0)	0.458
Male	49 (92.5)	61 (96.8)	0.289
Black Ethnicity	9 (17.0)	4 (6.3)	0.071
Body Mass Index (kg/m^2)	27.3 (5.5)	30.9 (5.0)	***<0.001***
Diabetes	7 (13.2)	18 (28.6)	***0.045***
Hypertension	18 (34.0)	35 (55.6)	***0.020***
Dyslipidaemia	16 (30.2)	31 (49.2)	***0.045***
Time Since HIV (Years)	14.0 (7.0-21.5)	11.5 (6.8-21.3)	0.565
CD4 Nadir (Cells/mm^3)	143.0 (39.0-238.5)	189.5 (32.3-294.3)	0.419
Time from Diagnosis to Treatment (Months)	11.5 (2.0-37.8)	11.0 (1.0-50.5)	1.000
Duration of ART (Years)	10.0 (6.0-17.5)	8.0 (4.8-16.3)	0.415
NRTI (months)	180 (80-253)	122 (62-204)	0.076
NNRTI (Months)	33 (8-94)	50 (4-107)	0.448
PI (Months)	57 (0-130)	3 (0-106)	0.061
II (Months)	0 (0-0)	0 (0-12)	***0.027***
Previous D-Drugs (Y/N)	31 (58.5)	23 (36.5)	***0.021***
Platelets (x10^9/L)	193 (71)	217 (58)	***0.047***
ALT (U/L)	52 (32-76)	78 (59-137)	***<0.001***
AST (U/L)	39 (27-62)	50 (41-63)	***0.019***
AST:ALT	0.87 (0.41)	0.65 (0.23)	***<0.001***
ALP (U/L)	96 (75-112)	89 (72-108)	0.174
Bilirubin (µmol/L)	12.0 (7.0-18.0)	10.0 (7.0-16.3)	0.507
Albumin (g/L)	42.9 (6.8)	43.3 (3.4)	0.709

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Cholesterol (mmol/L)	4.7 (1.0)	4.9 (1.1)	0.288
Triglycerides (mmol/L)	1.8 (1.1-2.5)	2.1 (1.4-3.3)	0.126
LDL (mml/L)	2.6 (0.9)	2.8 (1.0)	0.186
HDL (mmol/L)	1.2 (0.4)	1.1 (0.5)	0.265
Fasting Glucose (mmol/L)	5.7 (2.5)	6.1 (1.8)	0.367
TGI	480.5 (405.4-587.0)	514.5 (460.7-656.8)	***0.040***
Detectable VL (%)	1 (1.9)	6 (9.5)	0.082
CD4 (cells/µl)	541 (261)	720 (303)	***0.001***
CD8 (cells/µl)	739 (494-1025)	900 (673-1339)	***0.019***
CD4:CD8	1.0 (0.4-1.0)	0.9 (0.5-1.0)	0.816
CPA (%)	2.0 (2.0-5.0)	3.0 (2.0-5.0)	0.445
≥F2 Fibrosis	27 (50.9)	40 (63.5)	0.173
≥F3 Fibrosis	12 (22.6)	27 (42.9)	***0.022***

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	Univariate		Multivariate			
			Model 1		Model 2	
	OR (95% CI)	p	aOR (95% CI)	p	aOR (95% CI)	p
Age	0.99 (0.95-1.02)	0.455	0.96 (0.91-1.01)	0.128	1.01 (0.96-1.06)	0.681
BMI	1.15 (1.06-1.25)	***0.001***	1.20 (1.08-1.34)	***0.001***	1.20 (1.08-1.33)	***0.001***
Black Ethnicity	0.33 (0.10-1.15)	0.081				
Diabetes	2.63 (1.00-6.90)	***0.050***	1.07 (0.27-4.21)	0.928		
Hypertension	2.43 (1.14-5.17)	***0.021***	3.14 (0.98-10.11)	0.055		
Dyslipidaemia	2.18 (1.01-4.70)	***0.047***	1.28 (0.41-3.99)	0.668		
Duration of ART	0.98 (0.94-1.03)	0.470				
D-Drugs*	0.40 (0.19-0.88)	**0.022**			0.36 (0.13-1.02)	0.053
CD4	1.02 (1.01-1.04)	***0.002***			1.00 (1.00-1.00)	0.177
CD8	1.01 (1.00-1.02)	***0.009***			1.00 (1.00-1.00)	0.094
CD4 Nadir	1.02 (0.99-1.04)	0.226	1.00 (1.00-1.00)	0.940	1.00 (1.00-1.00)	0.744

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Characteristic	F<3 N=77	F≥3 N=39	P
Age, years	47.2 (10.5)	50.9 (11.5)	0.082
Male, n (%)	73 (94.8)	37 (94.8)	0.988
Black Ethnicity, n (%)	12 (15.6)	1 (2.6)	
Body Mass Index, kg/m2	28.8 (5.5)	30.1 (5.5)	0.242
Diabetes n (%)	9 (11.7)	16 (41.0)	***<0.001***
Hypertension n (%)	29 (37.7)	24 (61.5)	***0.015***
Dyslipidaemia n (%)	24 (31.2)	23 (59.0)	***0.005***
Time Since HIV (Years)	10.5 (5.0-18.8)	20.0 (11.0-24.0)	***0.001***
CD4 Nadir (Cells/mm^3)	171.0 (48.0-299.3)	137.0 (19.5-216.0)	0.175
Time from Diagnosis to Treatment (Months)	6.5 (1.0-37.8)	14.0 (2.0-78.0)	0.157
Duration of ART (Years)	8.0 (4.0-15.0)	13.0 (6.0-20.0)	***0.038***
NRTI (months)	132 (52-226)	172 (72-211)	0.377
NNRTI (Months)	26 (2-88)	64 (28-112)	0.054
PI (Months)	23 (0-110)	22 (0-120)	0.594
Previous D-Drugs (Y/N)	33 (45.2)	21 (53.8)	0.150
Platelets (x10^9/L)	213 (57)	193 (144-252)	0.122
ALT (U/L)	67 (41-101)	73 (52-137)	0.176
AST (U/L)	44 (30-62)	54 (42-72)	***0.017***
AST:ALT	0.74 (0.36)	0.75 (0.31)	0.852
ALP (U/L)	93 (74-111)	90 (75-102)	0.377
Bilirubin (µmol/L)	10.0 (6.9-17.1)	11.0 (7.5-18.6)	0.484
Albumin (g/L)	43.2 (5.7)	42.8 (4.2)	0.699
Cholesterol (mmol/L)	4.9 (1.0)	4.6 (1.2)	0.172

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Triglycerides (mmol/L)		1.8 (1.2-3.1)	2.0 (1.2-3.3)	0.711
LDL (mml/L)		2.8 (0.8)	2.4 (1.2)	***0.044***
HDL (mmol/L)		1.2 (0.4)	1.1 (0.7)	0.626
Fasting Glucose (mmol/L)		5.4 (1.1)	7.1 (3.1)	***<0.001***
TGI		475.4 (431.4-574.3)	534.0 (505.0-764.6)	***0.001***
Detectable VL (%)		3 (3.9)	4 (10.3)	0.174
CD4 (cells/µl)		626 (262)	661 (357)	0.555
CD8 (cells/µl)		902 (683-1264)	764 (514-1044)	***0.050***
CD4:CD8	1.0 (0.4-1.0)	1.0 (0.7-1.0)	0.539	
NAFLD	36 (46.8)	27 (69.2)	***0.022***	
NFS	-1.9 (-3.0 – 1.0)	-0.7 (-2.2 – 0.2)	***0.002***	
FIB-4		**1.1 (0.8-1.8)**	**1.7 (1.0-2.1)**	*0.008*

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	Univariate		Multivariate	
			Model 1	
	OR (95% CI)	p	aOR (95%CI)	p
Age	1.03 (1.00-1.07)	0.085		
BMI	1.04 (0.97-1.12)	0.242		
Black Ethnicity	0.14 (0.02-1.14)	0.066		
NAFLD	2.56 (1.14-5.78)	***0.023***	2.47 (0.96-6.39)	0.062
Type 2 Diabetes	5.26 (2.05-13.50)	***0.001***	3.42 (1.00-11.71)	***0.050***
Hypertension	2.65 (1.20-5.85)	***0.016***	0.99 (0.34-2.89)	0.983
Dyslipidaemia	3.12 (1.40-6.94)	***0.005***	1.88 (0.69-5.13)	0.221
Duration of ART	1.06 (1.00-1.11)	***0.036***	1.03 (0.97-1.09)	0.394
D-Drugs*	1.82 (0.80-4.12)	0.152		
CD4	1.71 (0.76-3.86)	0.195		
CD8	1.00 (1.00-1.00)	0.1240.205***0.002***		
CD4 Nadir	1.00 (1.00-1.00)			
Time since HIV infection	1.08 (1.03-1.13)			

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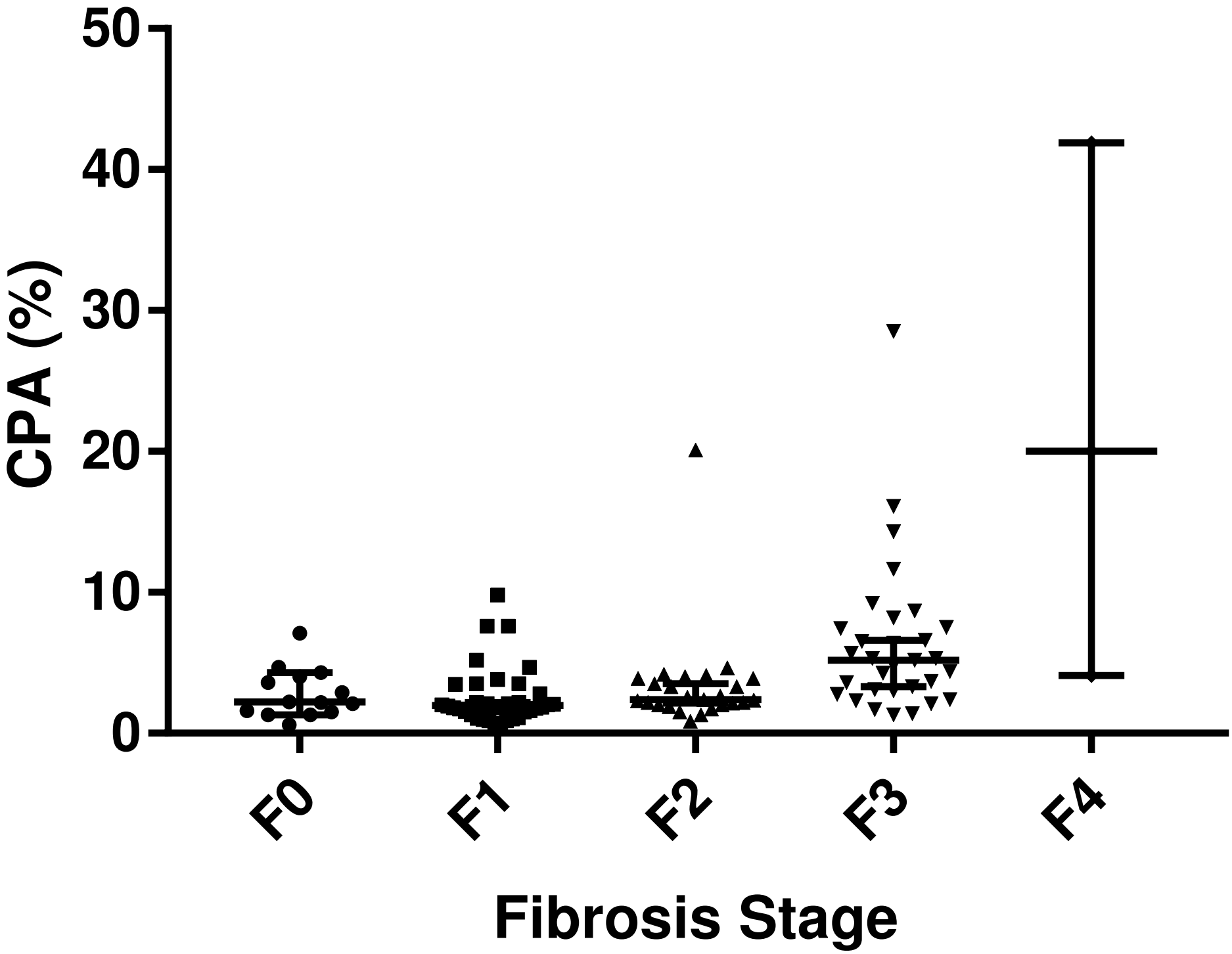
	Cut-off	Sensitivity	Specificity	PPV	NPV	LR+	LR-
FIB-4	1.3	0.72	0.43	0.46	0.80	1.26	0.66
NFS	-1.455	0.84	0.36	0.40	0.82	1.32	0.43

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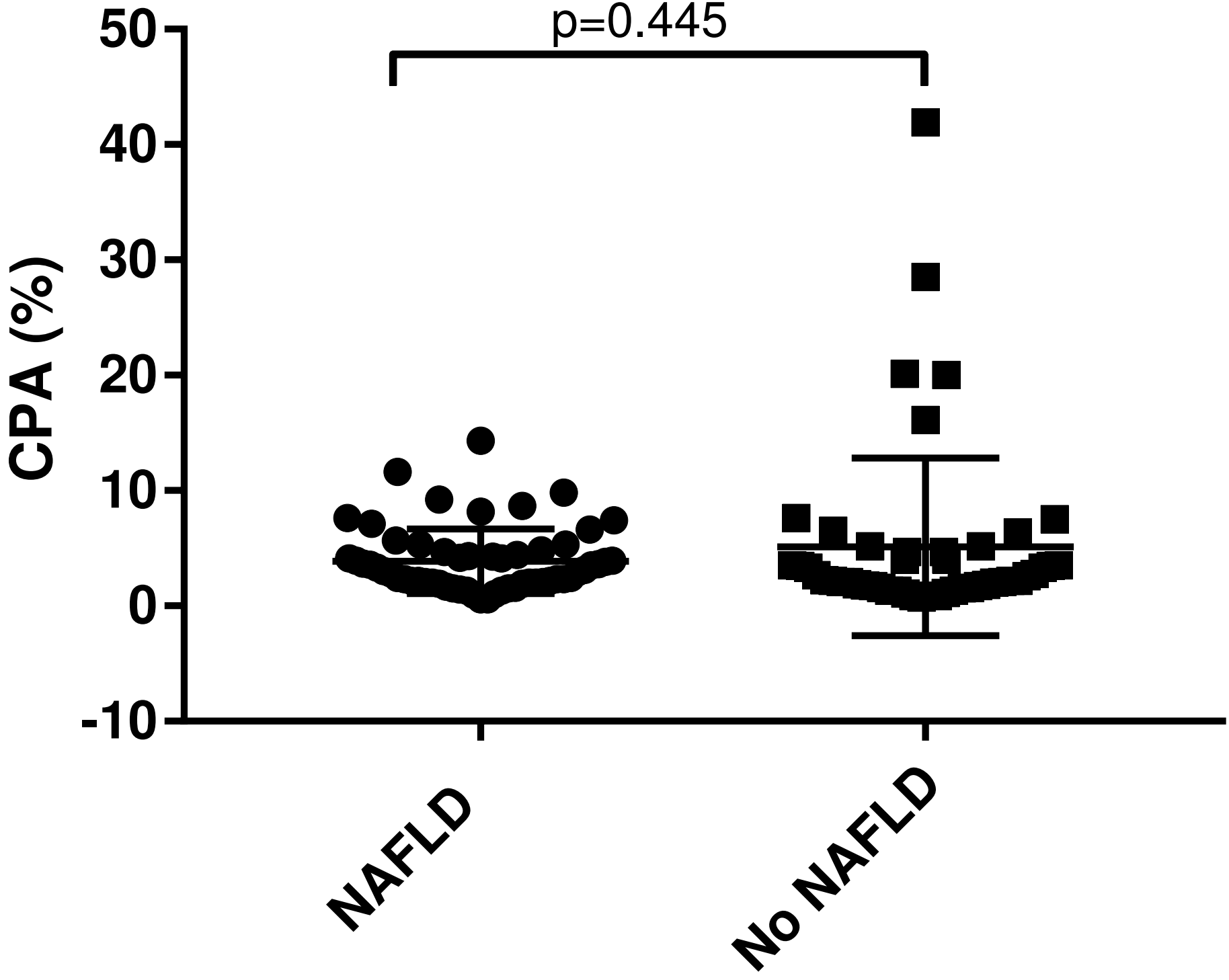
	Cut-off	Sensitivity	Specificity	PPV	NPV	LR+	LR-
FIB-4	1.3	0.93	0.44	0.24	0.98	1.65	0.16
NFS	-1.455	0.93	0.35	0.18	0.97	1.44	0.20

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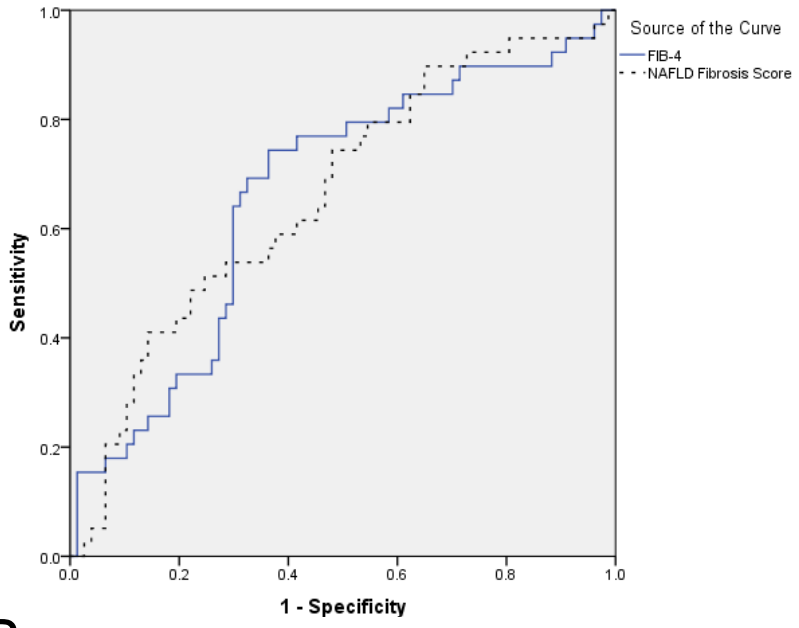


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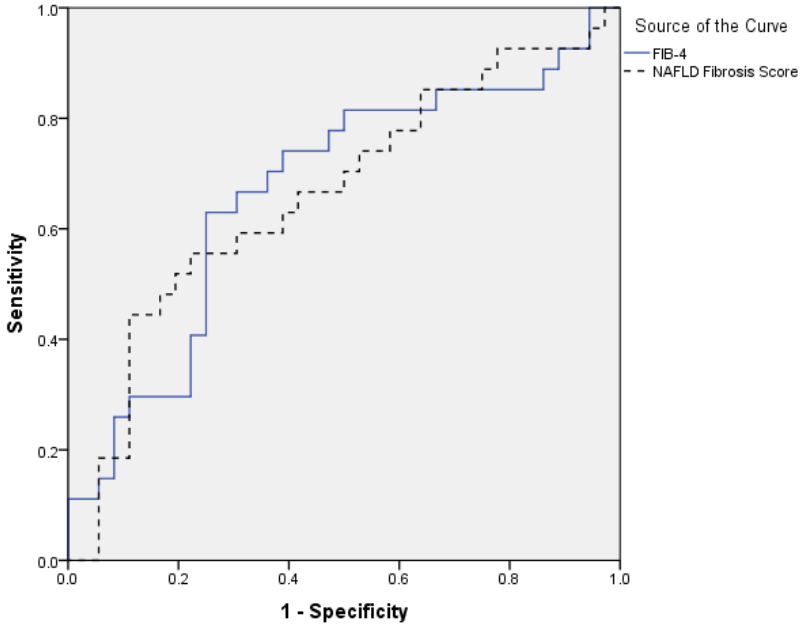


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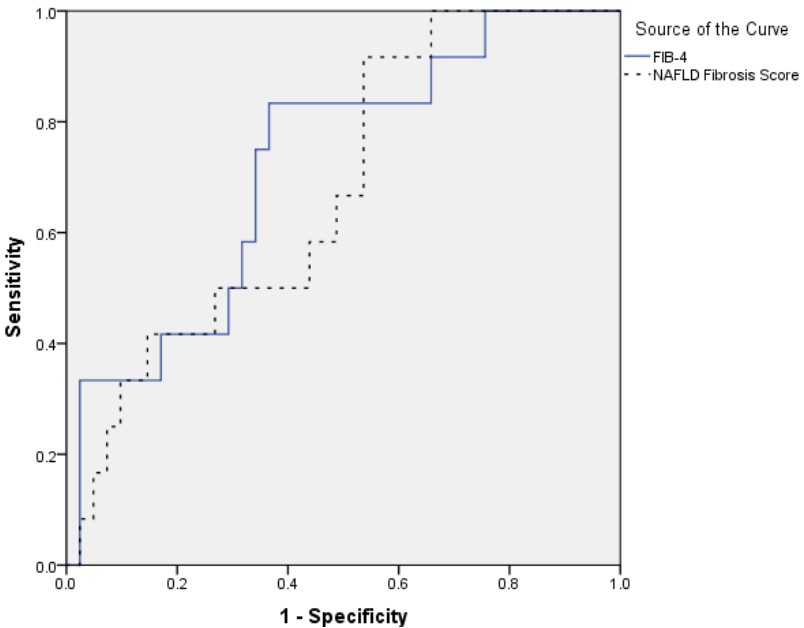
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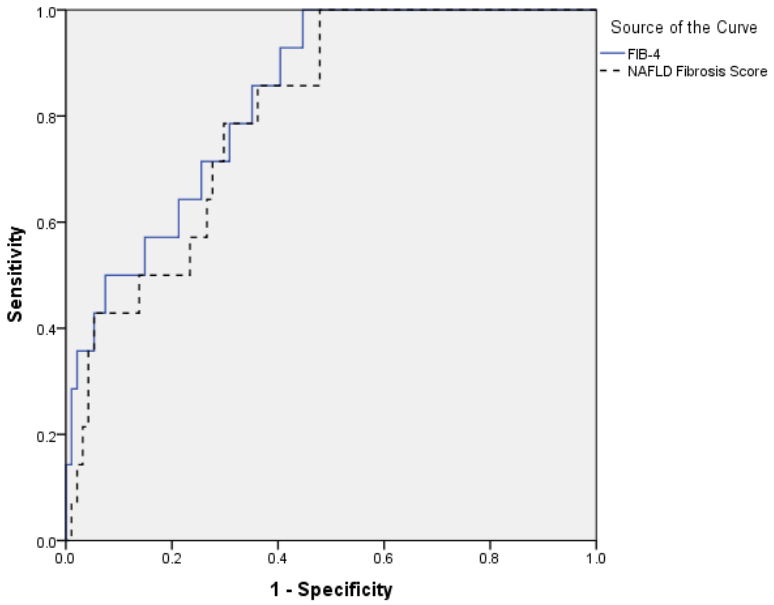


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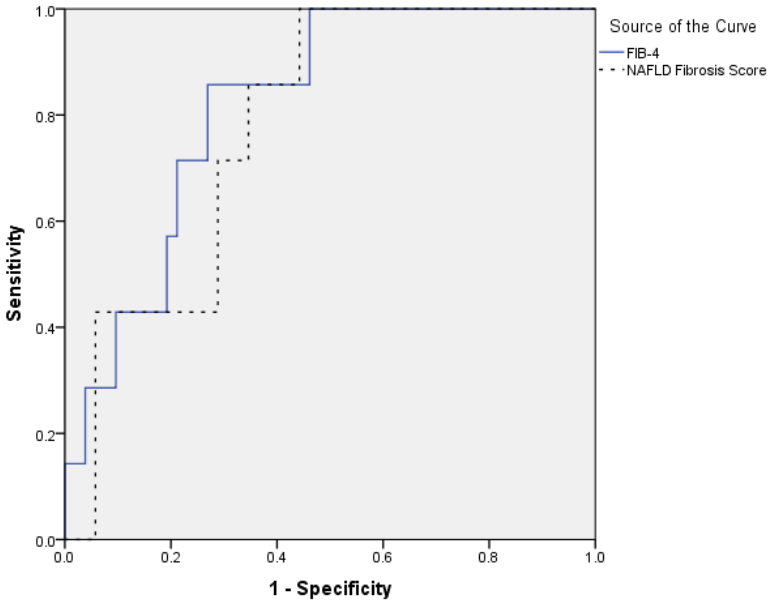


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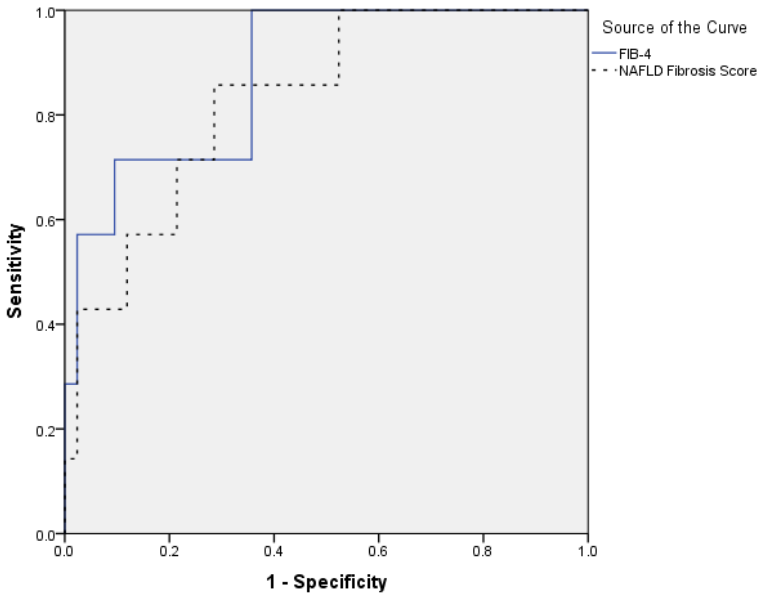
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