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ORIGINAL ARTICLE

# Elevated liver stiffness without histological evidence of liver fibrosis in rural Ugandans

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## Abstract

Liver fibrosis may be assessed noninvasively with transient electrography (TE). Data on the performance of TE for detecting liver fibrosis in sub-Saharan Africa are limited. We evaluated the diagnostic accuracy of TE by performing liver biopsies on persons with liver fibrosis indicated by TE. We enrolled HIV-infected and HIV-uninfected participants with TE scores consistent with at least minimal disease (liver stiffness measurement [LSM]≥7.1 kPa). Biopsies were performed and staged using the Ishak scoring system. A concordant result was defined using accepted thresholds for significant fibrosis by TE (LSM ≥ 9.3 kPa) and liver biopsy (Ishak score ≥ 2). We used modified Poisson regression methods to quantify the univariate and adjusted prevalence risk ratios (PRR) of the association between covariates and the concordance status of TE and liver biopsy in defining the presence of liver fibrosis. Of 131 participants with valid liver biopsy and TE data, only 5 participants (3.8%) had Ishak score ≥ 2 of whom 4 had LSM ≥ 9.3 kPa (sensitivity = 80%); of the 126 (96.2%) with Ishak score < 2, 76 had LSM < 9.3 kPa (specificity = 61%). In multivariable analysis, discordance was associated with female gender (adjPRR = 1.80, 95%CI 1.1-2.9; $P$ = .019), herbal medicine use (adjPRR 1.64, 95% CI = 1.0-2.5; $P$ = .022), exposure to lake or river water (adjPRR 2.05, 95% CI = 1.1-3.7; $P$ = .016), and current smoking (adjPRR 1.72, 95%CI 1.0-2.9; $P$ = .045). These data suggest that TE among rural Ugandans has low specificity for detection of histologically confirmed liver fibrosis. Caution should be exercised when using this tool to confirm significant liver fibrosis.

KEYWORDS

fibrosis, HIV, liver biopsy, liver disease, transient electrography

**Abbreviations:** Anti-HBc, antibody to hepatitis B; APRI, aspartate amino-transferase to platelet ratio index; BMI, body mass index; CCA, circulating cathodic antigen; HBsAg, hepatitis B surface antigen; LSM, liver stiffness measurement; PRR, prevalence risk ratios; TE, transient electrography; WHR, waist-to-hip ratio.

## 1 | INTRODUCTION

In response to chronic injury and infection, the liver accumulates fibrosis that may progress to cirrhosis, liver failure and hepatocellular cancer with subsequent high mortality.[1-4] Liver fibrosis is predominantly caused by chronic viral hepatitis infection but alcohol consumption, fatty liver disease in the absence of alcohol use, environmental exposures and genetic disorders may also be contributing factors.[5] Emerging information in sub-Saharan Africa also suggests herbal medicinal use and exposure to large water bodies as associated with increased risk of liver fibrosis.[6,7] Although the data on the prevalence of liver fibrosis in the setting of HIV infection in sub-Saharan Africa are limited, the available literature suggests a prevalence of 3%-26%.[6,8-11]

Liver biopsy has historically been the reference standard for diagnosing and staging liver fibrosis, but has notable technical limitations including sampling error, high dependence on biopsy specimen length, and inter- and intra-observer variability.[12-14] Furthermore, liver biopsy is an invasive procedure with limited patient and provider acceptance and the expertise to perform and interpret the results is often unavailable in sub-Saharan Africa. In contrast, non-invasive staging of liver fibrosis using transient elastography (TE) is a quick, painless and safe procedure that has been established to accurately stage fibrosis in the setting of chronic viral hepatitis in North America, Europe and Asia.[15-18] TE uses ultrasound to measure the velocity of an elastic shear wave transmitted through and reflected from the liver.[19] Despite increasing use of this technology in the region,[10,11,20] there is emerging evidence of discordant results in the performance of TE for staging liver fibrosis when compared to liver biopsy.[17,21-24]

We previously used TE to evaluate the prevalence of liver fibrosis in a community-based cohort in Rakai, Uganda.[6] We observed a high prevalence of significant liver fibrosis based on TE that was significantly associated with HIV infection but without any other apparent aetiology such as chronic hepatitis B virus (HBV) infection or heavy alcohol use. In follow-up to these earlier findings, we evaluated the diagnostic accuracy of TE by performing liver biopsies on persons with liver fibrosis indicated by TE.

## 2 | METHODS

### 2.1 | Study design and participants

We enrolled HIV-infected and HIV-uninfected persons from our previous study conducted by the Rakai Health Sciences Program (RHSP) which examined the prevalence and risk factors for liver disease using TE.[6] Briefly, HIV-infected patients were recruited from five HIV clinics, while HIV-uninfected participants were recruited from the population-based Rakai Community Cohort Study (RCCS).[25] Persons who had a TE measurement ≥7.2 kPa previously were reassessed by TE and if follow-up examination indicated liver stiffness ≥7.2 kPa, participants were offered a liver biopsy to investigate the underlying cause of potential liver disease. Each participant underwent a structured interview focused on exposures potentially associated with liver disease.

### 2.2 | Primary outcome

Performance characteristics of TE to diagnose liver fibrosis using liver biopsy as the gold standard.

### 2.3 | Transient elastography

Following formal training and certification from the manufacturer, two RHSP study nurses performed all transient elastography (TE) scans as recommended by the manufacturer (Fibroscan©, Echosens).[6] A conservative liver stiffness measurement (LSM) cut-off of ≥9.3 kPa was used to define significant fibrosis (equivalent to Metavir $F \geq 2$)[17] based on findings from a prior validation study in a community cohort of Americans of African descent with chronic HCV infection. According to the manufacturer's recommendations, persons with scans demonstrating high variability, defined as an interquartile range (IQR) greater than 30% of the median LSM value from an individual examination (IQR/LSM < 30%), were not considered valid and were excluded from the analysis.

### 2.4 | Laboratory assays

HIV-1 serology was determined by two HIV-1 enzyme immunoassays (EIAs): Vironostika HIV-1, BioMerieux; and Recombigen, Cambridge Biotech. EIA discordant results were confirmed by HIV-1 Western blots(GS HIV-1 Western Blot, Bio-Rad Laboratories, BioMerieux-Vitek). For HIV-infected participants, the most recent CD4 count (within 12 months) and CD4 count nadir were extracted from the RHSP HIV Care Program database. CD4 counts were measured by FACSCalibur flow cytometer (software version 1.4; Becton Dickinson). HBV surface antigen (HBsAg) and antibodies to HBV core (anti-HBc) status were determined using ELISA (ETI-EKB s Plus and ET-AB-COREK Plus, respectively; Diasorin). Evidence of schistosomiasis infection was tested using circulating cathodic antigen (CCA) and the Kato-Katz method. Liver enzymes, including aspartate aminotransferase (AST) and alanine aminotransferase (ALT), were tested using standard methods (COBAS CII; Roche). Hepatotoxicity was defined by ALT elevations and classified per AIDS Clinical Trials Group (ACTG) criteria.[26]

### 2.5 | Liver biopsy

Ultrasound-assisted liver biopsies were performed by one of two hepatologists using a 14-gauge needle to obtain at least a 15 mm core biopsy sample. Samples that were less than 5 mm in length

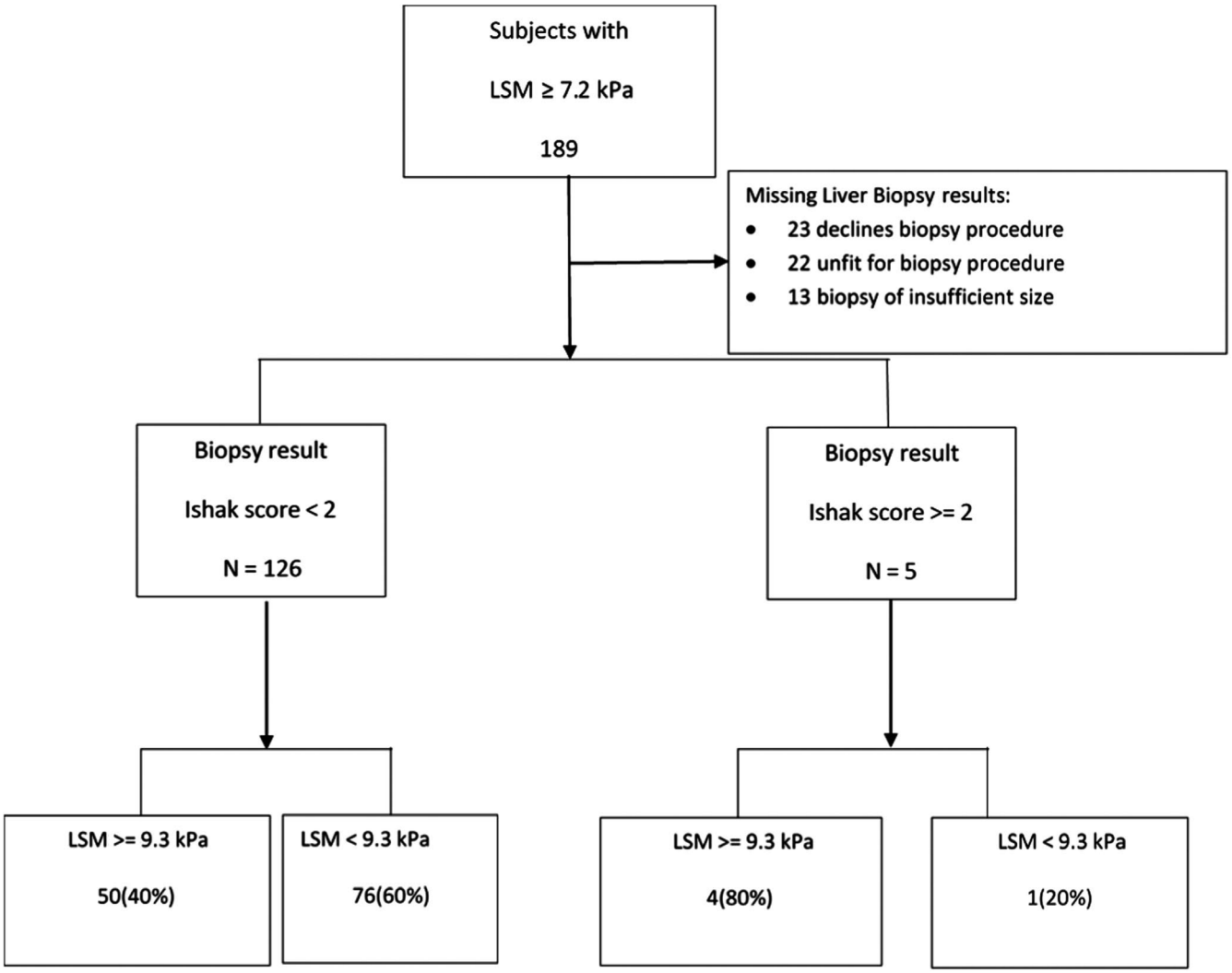


**FIGURE 1** Flow diagram of participants with a transient elastography score ≥ 7.2 kPa and subsequent liver biopsy staging

or those with fewer than five identifiable portal triads were rendered inadequate and were excluded. If an adequate sample was not obtained on the initial attempt, the biopsy was repeated a maximum of 3 times. Biopsies were fixed in 10% buffered formalin, embedded and shipped to the National Institutes of Health (NIH) for staging and grading. At the NIH, biopsies were accessioned in the SoftPath Laboratory Information System of the Laboratory of Pathology, National Cancer Institute (NCI), using the patient's unique study ID. The biopsies were handled using the standard operating procedures of the Laboratory of Pathology and cut into 4-micron sections. Sections from each biopsy were stained with haematoxylin and eosin, Masson trichrome, reticulin, periodic acid-Schiff with and without diastase pre-digestion, and iron and copper stains. Immunohistochemistry was used as necessary to enhance the diagnostic information. The biopsies were evaluated by a single experienced hepatopathologist (DK) who was blinded to TE results. Fibrosis was staged according to the Ishak scoring system[27]; hepatic steatosis, iron and other findings were recorded using an expanded scoring system used for drug-induced liver injury,[28] and hepatic iron was staged as none, mild and moderate to severe. Participants were provided with instructions and referrals for appropriate follow-up care once biopsy results were available.

## 2.6 | Statistical analysis

Baseline characteristics measured as continuous variables were summarized as medians with interquartile ranges and compared using t tests and the Wilcoxon-Mann-Whitney test, whereas categorical variables were summarized as proportions and compared using Pearson's chi-squared test. Due to substantial discordance between TE and biopsy classification, we sought to examine covariates associated with discordant results (ie high TE without significant fibrosis on biopsy; TE ≥ 9.3 and Ishak < 2) compared to concordant 'low' results (TE < 9.3 and Ishak < 2). Since the prevalence of discordance was greater than 10%, we used a modified Poisson regression model to quantify the univariate and multivariate prevalence risk ratios (PRR) with 95% confidence intervals (CI) of the association between covariates and the discordance outcome

**TABLE 1** Comparison of demographic, clinical and biopsy parameters by HIV status among participants without histological liver fibrosis

| Variable | HIV-infected N (%)/Median (IQR) | HIV-uninfected N (%)/Median (IQR) | *P*-value |
|---|---|---|---|
| | 97 (77%) | 29 (23%) | |
| Demographics | | | |
| Age | 40.5 (35-47) | 41.9 (39-52) | .139 |
| Female gender | 63 (65%) | 17 (59%) | .535 |
| Currently use alcohol | 36 (37%) | 14 (48%) | .281 |
| Currently smokes | 11 (11%) | 2 (7%) | .490 |
| Current herb use | 16 (16%) | 8 (28%) | .182 |
| Ever exposed to lake or river | 68 (70%) | 13 (45%) | .013 |
| Lifetime occupational fishing | 11 (11%) | 2 (7%) | .490 |
| Clinical parameters | | | |
| LSM | 9.0 (8-11) | 8.8 (8-9) | .598 |
| Ishak Score | | | |
| 0 | 83 (86%) | 26 (90%) | .572 |
| ≥1 | 14 (14%) | 3 (10%) | |
| Discordant classification[a] | 42 (43%) | 8 (28%) | .129 |
| Obese (BMI > 30) | 5 (5%) | 4 (14%) | .113 |
| WHR > 1.0 for males or 0.85 for females | 32 (33%) | 5 (17%) | .102 |
| AST level, IU/L | 23.9 (19-31) | 20.1 (17-25) | .063 |
| ALT level, IU/L | 12.5 (8-20) | 13.2 (8-18) | .832 |
| Platelet count, ×1000 cells/μL | 221.0 (180-270) | 239.0 (209-267) | .142 |
| Albumin level, g/dL | 4.6 (4-5) | 4.6 (4-5) | .562 |
| Urine CCA positive | 6 (6%) | 2 (7%) | .890 |
| Anti-HBc positive | 57 (59%) | 14 (48%) | .318 |
| HBsAg positive | 11 (11%) | 1 (3%) | .204 |
| Biopsy factors | | | |
| Biopsy specimen length (mm) | 9.0 (7-12) | 10.0 (7-15) | .372 |
| Any steatosis | 34 (35%) | 13 (45%) | .340 |
| Any hepatic iron | 46 (47%) | 7 (25%) | .034 |

Abbreviations: Anti-HBc, antibody to hepatitis B; BMI, body mass index; HBsAg, hepatitis B surface antigen; LSM, liver stiffness measurement; TB, tuberculosis; WHR, waist-to-hip ratio.

[a]Discordant results represent conservative liver stiffness measurement (LSM) cut-off of ≥9.3 and Metavir *F* Ishak score < 2.

variable representing agreement status between TE and liver biopsy to diagnose liver fibrosis.[29] Covariates evaluated as potential confounders included the following: age, gender, alcohol consumption, smoking, traditional herbal medicinal use, occupational fishing, exposure to Lake Victoria or river water, and clinical parameters including body mass index (BMI), waist-to-hip ratio (WHR), AST, ALT, albumin levels, platelet count, HIV status, anti-HBc status, HBsAg status and urine circulating cathodic antigen (CCA) status (as a marker of active *Schistosomiasis* infection). Liver biopsy factors included histological measures described above and biopsy specimen length. Factors with univariate associations at a *P*-value < .10 and biologically plausible confounders (age, gender and HIV status)[6,30] were also included in adjusted models. Statistical significance was considered *P*-value ≤ .05. In addition to comparing TE levels to biopsy, we also assessed for correlation between TE and noninvasive liver fibrosis measures including the aspartate amino-transferase to platelet ratio index (APRI), hepatic iron, periostin and the FIB-4 index.[31,32] All statistical analyses were performed using STATA 14 (StataCorp Lp).

The study was approved by IRBs of the National Institute of Allergy and Infectious Diseases, Johns Hopkins Medical Institutions, Western IRB (Olympia), the Uganda Virus Research Institute Research and Ethics Committee, and the Uganda National Council for Science and Technology. All participants provided written informed consent. This study is registered on clinicaltrials.gov (#NCT00782158).

## 3 | RESULTS

Of 189 participants with LSM ≥ 7.2 kPa, 131 (69%) had a reliable liver biopsy result following the exclusion of 23 prospective participants who declined liver biopsy, 22 were deemed medically unfit to undergo biopsy mainly due to low platelets or haemoglobin levels, and 13 were found to have biopsies of insufficient size or quality to provide accurate diagnostic information (had <5 mm length or <5 portal triads). The median age of participants was 40.9 years (IQR = 35.2-47.3), the majority were female [62.6% (82/131)] and HIV infected [77.1% (01/131)]. Overall, the median length of biopsy samples extracted was 9 mm (IQR = 7-12 mm); 17% of biopsies were at least 15 mm in length. Of the eligible participants, 5 (4%) participants had an Ishak fibrosis score ≥ 2 (significant fibrosis) of whom 4 had an LSM ≥ 9.3 kPa, and the other 126 (96%) had an Ishak score < 2 (insignificant fibrosis) of whom 76 (60%) had LSM < 9.3 kPa (Figure 1). Among those with insignificant fibrosis, 97 (77%) were HIV infected and they were comparable to the HIV uninfected in demographic, clinical and biopsy characteristics (Table 1).

The biopsies showed generally mild changes with little inflammation or fibrosis. Out of 131 cases, 29 (22%) had no inflammation at all and only 22 (17%) had total inflammation scores of 4 or greater (generally considered the lower threshold for 'mild' inflammation). Not surprisingly, only 23 (18%) had an inflammatory pattern suggesting chronic hepatitis. Steatosis, which is a common finding in North American HIV-infected patients, was significant (>5% steatosis) in only 12 (9%) cases, with the rest showing only minimal steatosis or none at all. None of the biopsies showed cholestatic changes or steatohepatitis, although 4 had non-necrotizing granulomas. Five cases

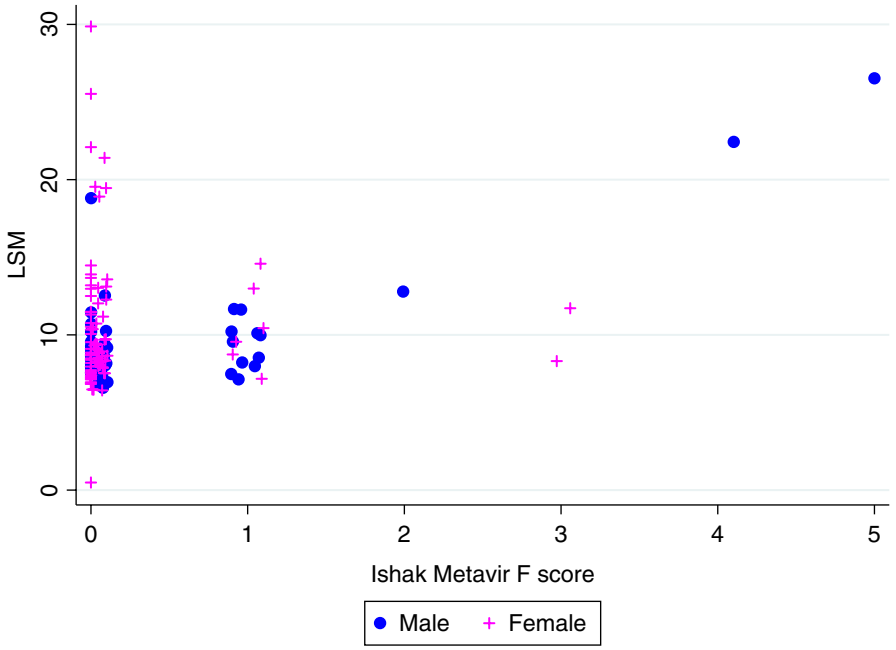


**FIGURE 2** Scatterplot of liver stiffness measurement (LSM) scores obtained with transient elasography (Fibroscan) and Ishak fibrosis Metavir *F* score staging, stratified by gender

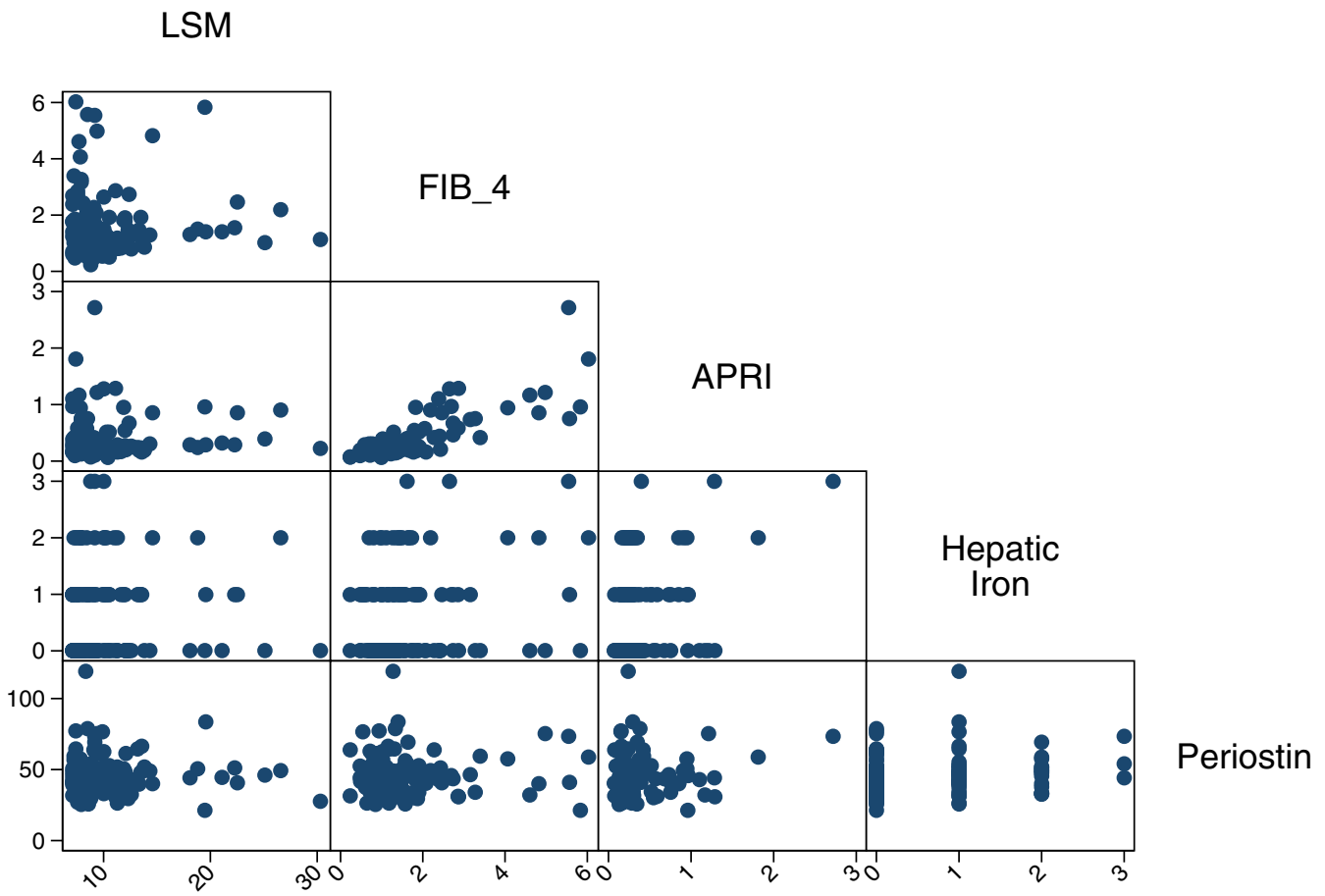


**FIGURE 3** Correlation matrix of liver stiffness measurement (LSM), FIB-4 score (FIB_4), aspartate amino-transferase to platelet ratio index (APRI), hepatic iron and periostin

(4%) had evidence of parasite eggs, morphologically suggestive of schistosome eggs, and 8 (6%) had pigment within Kupffer cells consistent with malarial pigment.

Overall, there was no observed correlation between the LSM score and histological biopsy staging ($r^2$ = 0.33; *P*-value < .001), Figure 2. However, we observed strong correlation among males

**TABLE 2** Factors associated with significant liver stiffness (LSM ≥ 9.3kPa) among participants without histological evidence of liver fibrosis

| | Univariate | | Multivariate | |
|---|---|---|---|---|
| **Characteristics** | **PRR (95% CI)** | ***P*-values** | **PRR (95% CI)** | ***P*-values** |
| Demographics | | | | |
| Age | 0.99 (1.0-1.0) | 0.593 | 1.00 (1.0-1.0) | .772 |
| Female sex | 1.34 (0.8-2.2) | 0.236 | 1.80 (1.1-2.9) | .019 |
| Currently uses alcohol | 1.29 (0.8-2.0) | 0.237 | | |
| Currently smokes | 1.66 (1.0-2.7) | 0.045 | 1.72 (1.0-2.9) | .045 |
| Current herb use | 1.49 (1.0-2.3) | 0.081 | 1.64 (1.1-2.5) | .022 |
| Lifetime occupational fishing | 1.42 (0.8-2.5) | 0.223 | | |
| Ever exposed to lake or river | 1.97 (1.1-3.5) | 0.018 | 2.05 (1.1-3.7) | .016 |
| Clinical parameters | | | | |
| HIV status | 1.57 (0.8-3.0) | 0.164 | 1.36 (0.7-2.6) | .334 |
| Obese (BMI > 30) | 0.83 (0.3-2.2) | 0.701 | | |
| WHR > 1.0 for males or > 0.85 for females | 1.60 (1.1-2.4) | 0.027 | | |
| AST level, IU/L | 1.00 (1.0-1.0) | 0.663 | | |
| ALT level, IU/L | 0.99 (1.0-1.0) | 0.585 | | |
| Platelet count, ×1000 cells/μL | 1.00 (1.0-1.0) | 0.766 | | |
| Albumin level, g/dL | 0.97 (0.6-1.4) | 0.878 | | |
| Urine CCA positive | 1.13 (0.8-1.6) | 0.505 | | |
| Anti-HBc positive | 0.99 (0.6-1.5) | 0.949 | | |
| HbsAg positive | 0.61 (0.2-1.7) | 0.331 | | |
| Biopsy factors | | | | |
| Biopsy specimen length | 0.98 (0.9-1.0) | 0.386 | | |
| Any steatosis | 0.95 (0.6-1.5) | 0.808 | | |
| Any hepatic iron | 1.25 (0.8-1.9) | 0.300 | | |

Abbreviations: Anti-HBc, antibody to hepatitis B; BMI, body mass index; HBsAg, hepatitis B surface antigen; TB, tuberculosis; WHR, waist-to-hip ratio.

($r^2$ = 0.78, *P*-value < .001) but no correlation among females ($r^2$ = −0.005, *P*-value = .960). In a scatterplot analysis, LSM levels were not strongly correlated with serologic measures of liver disease (AST, ALT, APRI or FIB-4), Figure 3. When lower cut-offs for detection of liver fibrosis were used, APRI and FIB-4 had comparable sensitivity and specificity of detecting fibrosis compared to the fibroscan (Table S2). LSM levels were not associated with histologic features of hepatic steatosis and/or iron or copper deposition. Self-reported alcohol intake or histologic evidence of alcohol abuse also was not associated with high liver stiffness. Neither was there a correlation with *Schistosomiasis* infection as determined by urine CCA, the Kato-Katz method or by liver histology (Table S1).

We further sought to identify factors associated with discordance by focusing on characteristics of individuals with high liver stiffness without significant liver fibrosis (termed 'discordant'; LSM ≥ 9.3 and Ishak < 2; N = 50) compared to those with concordant low liver stiffness and biopsy results (termed 'concordant'; LSM < 9.3 and Ishak < 2; N = 76). In a multivariable analysis (Table 2), discordant classification of liver fibrosis was independently associated with female gender (adjPRR = 1.80, 95% CI 1.1-2.9; *P* = .019), herbal medicine use (adjPRR 1.64, 95% CI = 1.0-2.5; *P* = .022), exposure to lake or river water (adjPRR 2.05, 95% CI = 1.1-3.7; *P* = .016), and current smoking (adjPRR 1.72, 95% CI 1.0-2.9; *P* = .045). Age, alcohol use, occupational fishing, body mass index, HIV status and biopsy factors were not significantly associated with increased liver stiffness. To further understand the underlying gender differences in discordant classification, we performed descriptive gender comparisons and found that males were more likely to report alcohol use (63% vs 26%), smoking (22% vs 4%), occupational fishing (28% vs 0%) and exposure to Lake Victoria or rivers (83% vs 54%) (Table 3).There was no significant difference in discordance when we compared samples with ≥15 mm vs <15 mm biopsies vs TE (Table S3).

**TABLE 3** Comparison of demographic, clinical and biopsy parameters by gender among participants without histological liver fibrosis

| Variable | Female N (%)/ Median (IQR) | Male N (%)/ Median (IQR) | *P*-value |
|---|---|---|---|
| Overall | 80 (63%) | 46 (37%) | |
| Demographics | | | |
| Age | 40.5 (36-47) | 41.6 (34-47) | .139 |
| Positive HIV status | 63 (79%) | 34 (74%) | .535 |
| Currently use alcohol | 21 (26%) | 29 (63%) | <.001 |
| Currently smokes | 3 (4%) | 10 (22%) | .001 |
| Current herb use | 16 (20%) | 8 (17%) | .720 |
| Lifetime occupational fishing | 0 (0%) | 13 (28%) | <.001 |
| Ever exposed to lake or river | 43 (54%) | 38 (83%) | .001 |
| Clinical parameters | | | |
| LSM | 9.1 (8-12) | 8.5 (8-10) | .074 |
| LSM > 9.3 kPa | 35 (44%) | 15 (33%) | .218 |
| Obese (BMI > 30) | 9 (11%) | 0 (0%) | .018 |
| WHR > 1.0 for males or >0.85 for females | 36 (45%) | 1 (2%) | <.001 |
| AST level, IU/L | 21.8 (18-26) | 26.1 (19-36) | .047 |
| ALT level, IU/L | 11.6 (7-18) | 15.1 (9-23) | .267 |
| Platelet count, ×1000 cells/μL | 234.5 (191-281) | 221.0 (164-255) | .329 |
| Albumin level, g/dL | 4.6 (4-5) | 4.6 (4-5) | .711 |
| Urine CCA positive | 1 (1%) | 7 (15%) | .002 |
| Anti-HBc positive | 46 (57%) | 25 (54%) | .731 |
| HbsAg positive | 7 (9%) | 5 (11%) | .696 |
| Biopsy factors | | | |
| Biopsy specimen length (mm) | 8.5 (7-12) | 9.0 (7-13) | .864 |
| Any steatosis | 28 (35%) | 19 (41%) | .481 |
| Any hepatic iron | 23 (29%) | 30 (67%) | <.001 |

Abbreviations: Anti-HBc, antibody to hepatitis B; BMI, body mass index; HBsAg, hepatitis B surface antigen; LSM, liver stiffness measurement; TB, tuberculosis; WHR, waist-to-hip ratio.

## 4 | DISCUSSION

In this rural African, mostly HIV-infected cohort with a low prevalence of viral hepatitis, we found marked discordance between liver fibrosis staging by elastography compared to liver biopsy, albeit the adequacy of biopsy samples for evaluating fibrosis was limited. Only a minority of participants with elevated liver stiffness were confirmed to have histologic evidence of liver fibrosis. Further, elevated liver stiffness without histological fibrosis did not appear to be explained by other liver processes as assessed by histological, clinical or biochemical abnormalities measured including liver inflammation, steatosis, iron, exposure to alcohol or *Schistosomiasis*. These findings suggest that caution should be used in interpreting TE in populations without chronic viral hepatitis.

Transient electrography has been successfully validated against liver biopsy as an effective, noninvasive tool for staging liver fibrosis in persons with chronic liver disease due to chronic hepatitis B and C with similar performance characteristics in persons with and without HIV infection.[16,17,33-37] There are more limited data regarding TE performance for staging fibrosis in liver disease of other aetiologies, including alcohol-related liver disease,[17,38] nonalcoholic steatohepatitis,[39] primary biliary cirrhosis[40] and autoimmune hepatitis.[41-43] Notably, in addition to mild variation between study populations generally related to the underlying prevalence of fibrosis/cirrhosis, the optimal cut-points for defining fibrosis stages appear related to the aetiology of the underlying liver disease.[23,44-48] A technical review of a specific cut-points for defining fibrosis stages was reported in HCV infection but the evidence was considered low or very low, for HBV and fatty liver disease, respectively, due to inconsistency and imprecision including high heterogeneity, with wide range of liver stiffness cut-offs, and overlapping confidence intervals for rates of true positive, false negative, true negative and false positive.[49] Thus, one plausible explanation for our findings is that in persons without viral hepatitis, nonviral factors lead to elevated liver stiffness out of proportion to fibrosis.

Transient electrography has been suggested to have superior performance characteristics for staging liver fibrosis in the setting of chronic viral hepatitis compared to several noninvasive serum indices used to stage liver fibrosis (eg APRI and FIB4).[32] These serum indices have been used more sparingly in nonviral hepatitis populations, but data from Europe and North America suggest reasonable performance characteristics despite several limitations.

In our study population, we did not find substantial correlation of LSM levels with either APRI or FIB4 scores. Furthermore, the sensitivity, specificity and per cent discordance for APRI and FIB4 in defining fibrosis were comparable (OR comparably as poor as) to histology and TE. This discordance in noninvasive fibrosis measures compared to histology raises two distinct potential explanations. First, the liver biopsy could be inaccurately classifying patients with actual fibrosis as no fibrosis.[14] Second, there may be unique characteristics among this population that could falsely elevate liver stiffness measures on elastography.

Besides invasiveness and cost, the limitations of liver biopsy for staging fibrosis have been well documented, including the small sampling frame relative to liver size (as regional heterogeneity in fibrosis exists) and notably decreased sensitivity with smaller biopsy length.[13,14] The advantages of TE (eg increased sampling frame) directly address the sources of measurement error in biopsies. There are almost certainly participants in our study that have liver fibrosis

that were misclassified as no fibrosis by the biopsy. Overall median biopsy lengths of 9 mm permitted only limited assessments of portal tract which most likely contributed to misclassification bias. There was, however, no significant difference in discordance when we compared biopsy samples with length of ≥15 mm vs <15 mm biopsies. The markedly low prevalence of histological fibrosis among this subset of persons that should have been enriched for fibrosis suggests that the false-positive classification by TE also likely occurred.

Increases in liver stiffness in the absence of fibrosis have been reported in relation to obesity, elevated transaminases and to histologic findings of steatosis and hepatic iron.[17,22,23] Transient elevations in liver stiffness have also been noted in the hours following ingestion of fatty foods.[50] To address these limitations, we measured multiple histologic, clinical, ultrasound and laboratory measures which might explain these discordant results. In an analysis of discordance, we identified a number of factors associated with increased stiffness without fibrosis including: female gender, herbal medicine use, fresh water exposure and cigarette smoking. It remains unclear whether these factors could be causative (eg herbal medicines might cause liver injury and increase liver stiffness disproportionate to fibrosis) or whether these associations are simple confounders (eg if women were more likely to eat a fatty meal prior to TE examination). The differences in body habitus between men and women (BMI of 21.8 vs 23.6; WHR of 0.9 vs 0.8, respectively) in our study population may partly explain the marked difference in discordance observed (Table S1).

A strength of this study was that it enrolled a relatively large cohort of African patients who underwent liver biopsy. However, the modest biopsy lengths may have limited our ability to reliably identify histological fibrosis given that a substantial number of our biopsies were <15 mm in length; however, we expect minimal impact on findings because each sample was carefully examined by a pathological expert for meaningful histological determination. Other study limitations included the cut-off used to assess for liver stiffness measurements which was validated in a population of patients of African descent with chronic HCV infection and therefore may have been less generalizable to our population of patients in sub-Saharan Africa. However, this was a validated cut-point and was more conservative than more widely applied cut-points used in Europe and North America. It is difficult to assess whether the low prevalence of histological fibrosis in small numbers of misclassified participants contributed to low performance characteristics of TE in this population.

Our findings of significant discordant results between TE and liver biopsy in people without histological evidence of liver fibrosis emphasize the need for caution in applying TE to populations without viral hepatitis coinfection and should spark further investigation into appropriate cut-offs of TE for staging nonviral hepatitis liver disease and to developing practical algorithms to maximize performance.[51] Further validation studies should ensure adequate biopsy length, focus on development of disease-specific TE cut-offs and consider risk adjustment of cut-offs based on factors associated with discordance (eg gender and BMI).[46] Our results are particularly relevant to research and clinical practice in sub-Saharan African and resource-limited settings where non-invasive methods of staging liver fibrosis, including TE, are more likely to be preferred safe and potentially more valid alternatives to liver biopsy.[20]

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## CONFLICT OF INTEREST

There are no known competing interests.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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