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ORIGINAL ARTICLE

# The gamma-glutamyl transpeptidase to platelet ratio (GPR) predicts significant liver fibrosis and cirrhosis in patients with chronic HBV infection in West Africa

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## ABSTRACT

**Background** Simple and inexpensive non-invasive fibrosis tests are highly needed but have been poorly studied in sub-Saharan Africa.

**Methods** Using liver histology as a gold standard, we developed a novel index using routine laboratory tests to predict significant fibrosis in patients with chronic HBV infection in The Gambia, West Africa. We prospectively assessed the diagnostic accuracy of the novel index, Fibroscan, aspartate transaminase-to-platelet ratio index (APRI), and Fib-4 in Gambian patients with CHB (training set) and also in French and Senegalese CHB cohorts (validation sets).

**Results** Of 135 consecutive treatment-naïve patients with CHB who had liver biopsy, 39% had significant fibrosis (Metavir fibrosis stage  $\geq$ F2) and 15% had cirrhosis (F4). In multivariable analysis, gamma-glutamyl transpeptidase (GGT) and platelet count were independent predictors of significant fibrosis. Consequently, GGT-to-platelet ratio (GPR) was developed. In The Gambia, the area under the receiver operating characteristic curve (AUROC) of the GPR was significantly higher than that of APRI and Fib-4 to predict  $\geq$ F2,  $\geq$ F3 and F4. In Senegal, the AUROC of GPR was significantly better than Fib-4 and APRI for  $\geq$ F2 (0.73, 95% CI 0.59 to 0.86) and better than Fib-4 and Fibroscan for  $\geq$ F3 (0.93, 0.87 to 0.99). In France, the AUROC of GPR to diagnose  $\geq$ F2 (0.72, 95% CI 0.59 to 0.85) and F4 (0.87, 0.76 to 0.98) was equivalent to that of APRI and Fib-4.

**Conclusions** The GPR is a more accurate routine laboratory marker than APRI and Fib-4 to stage liver fibrosis in patients with CHB in West Africa. The GPR represents a simple and inexpensive alternative to liver biopsy and Fibroscan in sub-Saharan Africa.

## INTRODUCTION

HBV infection is highly endemic in sub-Saharan Africa (SSA)<sup>1</sup> and is the main cause of hepatocellular carcinoma (HCC), one of the most frequent cancers in Africa.<sup>2–3</sup> Immunisation is not sufficient to control the HBV epidemic in SSA.<sup>4</sup> To reduce the disease burden of HBV infection in SSA, it may be critical to identify HBV-infected subjects with significant liver disease and treat them with anti-viral therapy.

## Significance of this study

### What is already known on this subject?

- ▶ HBV infection is responsible for a significant burden of liver disease in sub-Saharan Africa (SSA).
- ▶ The lack of non-invasive and inexpensive fibrosis markers for the assessment of chronic HBV infection is one of the main barriers for care and treatment of people chronically infected with HBV in Africa.
- ▶ WHO recommends the use of aspartate transaminase-to-platelet ratio index (APRI) as a non-invasive test for cirrhosis in resource-limited settings, however, the evidence is limited in SSA where only two small studies have assessed the diagnostic performance of the non-invasive markers.
- ▶ A new simple and inexpensive marker of fibrosis in SSA is highly needed.

### What are the new findings?

- ▶ For identifying significant fibrosis and cirrhosis in West African HBV carriers, the new GPR (gamma-glutamyl transpeptidase (GGT)/platelets ratio) is a more accurate routine laboratory test than APRI and Fib-4 tests.
- ▶ Transient elastography (Fibroscan) has excellent diagnostic accuracy for predicting significant fibrosis and cirrhosis in patients infected with HBV in West African setting but the device remains expensive.
- ▶ The GPR represents an accurate and inexpensive alternative to liver biopsy and Fibroscan for SSA countries.

### How might it impact on clinical practice in the foreseeable future?

- ▶ WHO is calling for better strategies to fight against the global burden of viral hepatitis in particular in resource-poor settings. GGT and platelets are inexpensive biomarkers, easy to measure even in peripheral laboratories in SSA. Thus, GPR will be easy to use in routine clinical practice.

Fibrosis staging is an essential step in the clinical assessment of patients with chronic HBV (CHB) infection to identify those who require treatment. Liver biopsy (LB) is an invasive and expensive procedure that is very difficult to perform in routine practice in SSA. Thus, non-invasive methods to evaluate liver fibrosis are particularly needed in SSA.

Transient elastography (Fibroscan) is a point-of-care procedure that has been validated in patients with CHB infection in Western and Asian countries.<sup>5–10</sup> Simple biochemical markers such as the aspartate transaminase (AST)-to-platelet ratio index (APRI)<sup>11</sup> and the fibrosis-4 (Fib-4)<sup>12</sup> scores have the advantage of comprising only two or three inexpensive laboratory tests. In March 2015, WHO published its first guidelines on the management of CHB infection. They recommend the use of APRI as a non-invasive tool to detect liver cirrhosis and significant fibrosis in resource-limited settings.<sup>13</sup> However, these guidelines also underline a need for additional evidence from SSA. Indeed, only two small studies to date assessed the diagnostic performance of non-invasive markers of fibrosis in SSA.<sup>14 15</sup>

Within the framework of the PROLIFICA programme (Prevention of Liver Fibrosis and Cancer in Africa, funded by the European Commission FP7, P34114)<sup>16</sup> we identified a new simple laboratory index, the gamma-glutamyl transpeptidase (GGT)-to-platelet ratio (GPR), in a cohort of CHB carriers in The Gambia, West Africa and then assessed its diagnostic accuracy in two external validation sets from France<sup>17</sup> and Senegal.<sup>15</sup> We compared the diagnostic performance of GPR with that of Fibroscan, APRI and Fib-4 tests, using liver histology as a gold standard.

## METHODS

### Patients

From November 2011 to November 2013, all consecutive patients with positive hepatitis B surface antigen test (Determine, Alere, USA)<sup>18</sup> enrolled in the PROLIFICA programme in The Gambia underwent a standardised clinical examination including Fibroscan, abdominal ultrasound and blood tests: haematology (Medonic SE-12613, Boule Medical AB, Sweden); biochemistry (VITROS 350 analyser, Ortho, USA); hepatitis B e antigen (ETI-EBK Plus, Diasorin, Italy); antibody against HCV (anti-HCV) (AxSYM, Abbott, USA); antibody against hepatitis D virus (ETI-AB-DELTAK-2, Diasorin, Italy); antibody against HIV (anti-HIV) (Genscreen ULTRA, Biorad, USA); and HBV DNA using a quantitative in-house PCR (detection limit: 50 IU/mL). The data were prospectively collected using a specific case report form. Patients with at least one of the criteria below were invited for LB in the absence of contraindications: transaminases (AST or alanine transaminase (ALT))  $\geq 40$  IU/L, liver stiffness measurement (LSM)  $\geq 6.5$  kPa, family history of cirrhosis or HCC in a first-degree relative, or HBV DNA  $\geq 2000$  IU/mL. Subjects with the following conditions were excluded from the study: co-infection with HCV, hepatitis D virus or HIV, prior or concurrent HBV antiviral therapy, focal hepatic lesion including HCC, concomitant tuberculosis, acute malaria, transaminases levels more than 10 times the upper limit of normal (ULN),<sup>19</sup> significant alcohol consumption ( $>20$  g/day), acute heart failure and pregnancy.

### Liver biopsy

Percutaneous LB was performed using ultrasound localisation and the Menghini technique after written informed consent. Liver samples were formalin-fixed and paraffin-embedded for histological analysis. Liver histology was interpreted by two liver pathologists in The Gambia and England. Liver samples with

less than three portal tracts were considered as poor quality and therefore excluded from the analysis. Both pathologists were blinded to the clinical information including the results of non-invasive tests. In case of discrepancies, slides were reviewed by a third highly experienced hepatopathologist (UK) who was blinded to the clinical information and the diagnosis of the other pathologists. The degree of liver activity and fibrosis were scored according to the Metavir system.<sup>20</sup>

### Transient elastography (Fibroscan)

All LSMs were performed fasting<sup>21</sup> on the same day of LB using a Fibroscan device (FS402, Echosens, France) by experienced operators according to the manufacturer's protocol.<sup>22</sup> The value of LSM was expressed in kilopascal (kPa) as the median of 10 successful acquisitions. Unreliable measurement was defined as IQR/LSM of  $>0.30$  when LSM is  $\geq 7.1$  kPa.<sup>23</sup>

### APRI and Fib-4 calculation

APRI and Fib-4 were calculated as (AST (IU/L)/its ULN)/platelet count ( $10^9/L$ ) $\times 100$ <sup>11</sup> and as (age (years) $\times$ AST (IU/L))/(platelets ( $10^9/L$ ) $\times$ (ALT (IU/L))<sup>1/2</sup>),<sup>12</sup> respectively. These markers were measured at enrolment, usually 1–2 weeks prior to the LB.

### Validation sets

Two external validation data sets of treatment-naïve mono-infected CHB carriers with viral load  $\geq 2000$  IU/mL and without excessive alcohol intake from France<sup>17</sup> and Senegal<sup>15</sup> were used to assess the performance of the novel index and other non-invasive markers. In the French cohort (n=138), only the patients with available data on GGT and platelet counts and a length of liver fragment  $>15$  mm were included in the current analysis (n=63). Fibroscan was not performed in this cohort. In Senegal, only the patients with LSM ranging between 7 kPa and 13 kPa underwent LB following the scientific committee's recommendation, and the original analysis was restricted to patients with normal ALT (n=69).<sup>15</sup> In the current analysis, we also included patients who had elevated ALT, and thus we analysed 80 Senegalese patients.

### Statistical analysis

The baseline characteristics of the three cohorts were compared using  $\chi^2$  test or Kruskal-Wallis test. In order to identify predictors of significant liver fibrosis ( $\geq F2$ ), univariable logistic regression was computed for the following variables: age, sex, AST, ALT, alkaline phosphatase, GGT, albumin, total bilirubin, platelet count and HBV DNA levels. All the continuous variables were transformed in logarithmic scale. Multiple logistic regression models were then fitted by including all the factors associated with the significant liver fibrosis in the univariable analyses (two-sided p value of  $<0.05$ ), and the final prediction model was selected using the backward stepwise procedures. A simplified formula was derived using the independent predictors of the final model.

The diagnostic accuracy of established fibrosis markers (Fibroscan, APRI and Fib-4) and the new index (GPR) was estimated by using the receiver operating characteristic (ROC) curve. Optimal cut-offs for LSM and GPR were selected to maximise the sum of sensitivity and specificity. For APRI and Fib-4, predefined cut-offs were used (0.5 and 1.5 for APRI to distinguish F0–1 and F2–4, 1.0 and 2.0 for APRI to distinguish F0–3 and F4,<sup>11</sup> and 1.45 and 3.25 for Fib-4 to distinguish F0–2 and F3–4<sup>12</sup>). The sensitivity, specificity, positive and negative predictive values and the area under the ROC curve (AUROC) of each non-invasive test for significant fibrosis ( $F\geq 2$ ), extensive

fibrosis (F $\geq$ 3) and cirrhosis (F4) were obtained by comparing patients of F2–4 with F0–1, F3–4 with F0–2, and F4 with F0–3, respectively. AUROC was compared between non-invasive tests for each fibrosis stage. In order to assess the associations of liver fibrosis stages, activity grade and presence of steatosis with a score of each non-invasive marker, a linear regression was modelled. The effects of these factors were mutually adjusted in a multivariable analysis. Finally, the GPR was applied to the two validation data sets and AUROC was obtained. All the analyses were performed using STATA V.13.0 (Stata Corporation, College Station, Texas). This study was reported in accordance with the Standards for Reporting of Diagnostic Accuracy (STARD).<sup>24</sup>

## RESULTS

### Training set in The Gambia

#### Study population

Between November 2011 and November 2013, 1042 subjects tested positive for hepatitis B surface antigen were prospectively enrolled in the Prevention of Liver Fibrosis and Cancer in Africa (PROLIFICA) programme in The Gambia. Figure 1 summarises the flow diagram of the study population. After exclusion of patients with HCC (n=113) or other diagnoses (n=9), 225 (24.5%) met the study criteria for LB and were invited. Sixty-eight (30.2%) refused the procedure. The clinical and biological characteristics of subjects who refused the procedure were not statistically different from those who accepted the procedure (data not shown). Eleven procedures were cancelled on the day of LB (figure 1). Thus, 146 LBs were performed. Of them, 11 were excluded from the analysis (reasons are indicated in figure 1). Thus, 135 patients were included in the final analysis. All patients had reliable LSM values using the criteria proposed by Boursier *et al.*<sup>23</sup> Indication for LB was as follows: elevated transaminases alone (9, 6.7%), elevated LSM alone (39, 28.8%), elevated viral load alone (7, 5.1%), family history of cirrhosis or HCC alone (5, 3.7%), and combination of two or more of these (75, 55.5%). Table 1 summarises the main characteristics of the study patients.

### Histopathology

No complication was observed after LB. Fifty-three (39%) patients had fibrosis  $\geq$ F2 and 20 (15%) had cirrhosis. A third of patients (37; 27%) had liver activity A2/A3 and the vast majority of the patients had no liver steatosis (table 1). Both pathologists were initially in agreement for 94 (69.6%) specimens. For the 41 specimens showing discrepancies, a final diagnosis was reached by a third histopathologist (figure 1).

### The GPR predicts significant liver fibrosis

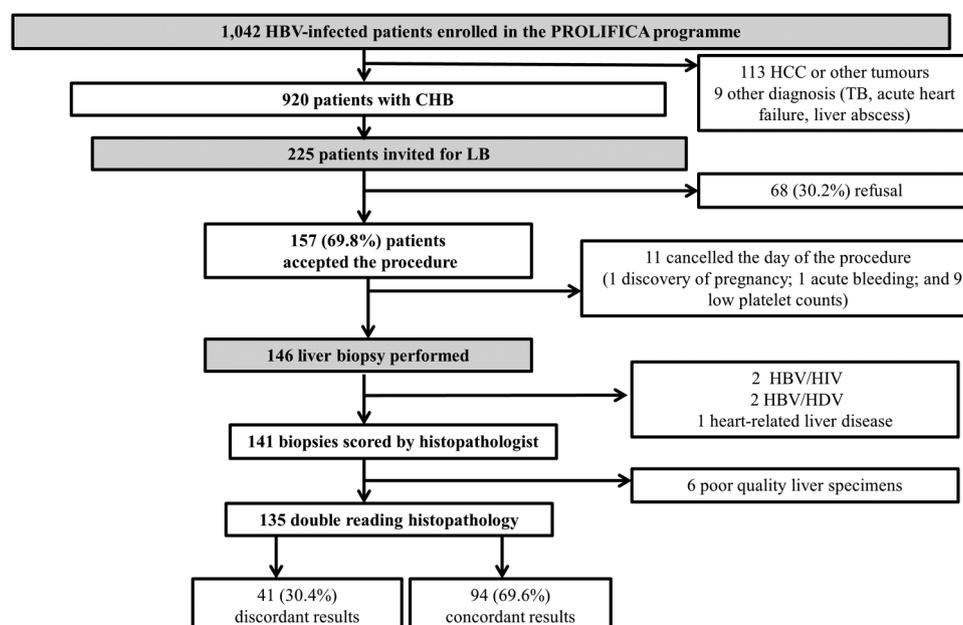
The presence of significant liver fibrosis ( $\geq$ F2) was associated with male sex, AST, ALT, GGT, albumin, platelet count and HBV DNA levels in univariable analyses (table 2). Subsequent multivariable analysis using backward stepwise procedures identified GGT and platelet count as independent predictors of significant fibrosis.

Box plots of GGT and platelet count in relation to the Metavir fibrosis stage are presented in figure 2A, B. While GGT values had a positive correlation with Metavir score (Spearman's correlation coefficient  $r=0.48$ ,  $p<0.0001$ ), platelet count was negatively correlated ( $r=-0.33$ ,  $p=0.0001$ ). In order to improve the prediction of significant fibrosis using these variables, a novel index called the GPR was derived as  $\text{GGT}/\text{ULN of GGT}/\text{platelet count (10}^9/\text{L)} \times 100$ .

There was a statistically significant positive correlation between GPR and Metavir fibrosis stage (figure 2C), with a higher correlation coefficient than GGT or platelet count alone ( $r=0.53$ ,  $P<0.0001$ ). AUROC for predicting significant fibrosis was higher with GPR (0.80, 95% CI 0.72 to 0.88) than using GGT alone (0.77, 95% CI 0.68 to 0.85,  $p=0.07$ ) or platelet count alone (0.70, 95% CI 0.61 to 0.79,  $p=0.02$ ).

### Comparisons of AUROC between GPR and other established non-invasive markers

In The Gambian cohort, the GPR and established non-invasive markers (LSM, APRI and Fib-4) increased with increasing liver fibrosis stage (figure 2D–F). For the prediction of significant fibrosis ( $\geq$ F2), AUROC of GPR (0.80, 95% CI 0.72 to 0.88)



**Figure 1** Flow diagram of the study population and reasons for exclusion, from November 2011 to November 2013, The Gambia. CHB, chronic HBV; HCC, hepatocellular carcinoma; HDV, hepatitis D virus; LB, liver biopsy; TB, tuberculosis.

**Table 1** Baseline characteristics of the study population in training set (The Gambia) and in validation sets (France and Senegal)

Variables	The Gambia (n=135)	France (n=63)	Senegal (n=80)	p Value
Male sex, n	105 (78%)	41 (65%)	67 (84%)	0.03
Median age (years) (IQR)	34 (29–39)	38 (29–49)	28 (24–31)	<0.001
Median BMI (kg/m <sup>2</sup> ) (IQR)	21.6 (19.9–24.8)	Not reported	20.9 (18.9–24.0)	0.1
Median fasting LSM value (kPa)	7.5 (6.5–9.2)	Not reported	8.8 (7.8–10.2)	<0.001
Median IQR of LSM (kPa)	1.3 (0.9–1.8)	Not reported	1.4 (1.1–2.6)	0.006
Median ALT (IU/L) (IQR)	30 (23–49)	61 (39–125)	39 (30–53)	<0.001
Median AST (IU/L) (IQR)	36 (29–48)	36 (28–66)	41 (31–53)	0.4
Median GGT (IU/L) (IQR)	36 (24–59)	32 (18–52)	26 (22–42)	0.002
Median total bilirubin (IU/L) (IQR)	11 (9–15)	11 (8–14)	12 (9–14)	0.4
Median platelet counts (10 <sup>9</sup> /L) (IQR)	177 (136–226)	171 (143–210)	179 (156–210)	0.5
Negative HBeAg, n	129 (96%)	28 (60%)	66 (83%)	<0.001
Median HBV DNA level (log <sub>10</sub> IU/mL) (IQR)	2.6 (1.9–4.5)	5.7 (4.4–7.1)	3.9 (3.5–5.2)	<0.001
Median APRI (IQR)	0.52 (0.37–0.82)	0.56 (0.31–1.01)	0.55 (0.41–0.80)	0.7
Median Fib-4 (IQR)	1.30 (0.86–1.84)	1.09 (0.75–1.69)	0.96 (0.74–1.32)	<0.001
Median size of liver biopsy (mm) (IQR)	11 (8–12)	20 (17–25)	28 (22–32)	<0.001
Metavir fibrosis stage (F0/F1/F2/F3/F4)	28 (21%)/54 (40%)/10 (7%)/ 23 (17%)/20 (15%)	6 (10%)/27 (43%)/15 (24%)/ 11 (17%)/4 (6%)	18 (23%)/36 (45%)/17 (21%)/ 9 (11%)/0 (0%)	<0.001
Metavir activity grade (A0/A1/A2/A3)	30 (22%)/68 (50%)/23 (17%)/14 (10%)	Not reported	42 (53%)/29 (37%)/8 (10%)/0 (0%)	<0.001
Steatosis (none/mild/moderate/severe)	125 (93%)/6 (4%)/4 (3%)/0 (0%)	30 (64%)/15 (32%)/2 (4%)/0 (0%)	73 (91%)/7 (9%)/0 (0%)/0 (0%)	<0.001

ALT, alanine transaminase; APRI, (AST)-to-platelet ratio index; AST, aspartate transaminase; BMI, body mass index; HBeAg, hepatitis B e antigen; GGT, gamma-glutamyl transpeptidase; LSM, liver stiffness measurement.

was significantly higher than that of APRI (0.66, 95% CI 0.57 to 0.76,  $p < 0.001$ ) and Fib-4 (0.66, 95% CI 0.56 to 0.76,  $p = 0.003$ ), but not higher than LSM (0.85, 95% CI 0.78 to 0.91,  $p = 0.2$ ) (table 3). For predicting significant fibrosis the optimal cut-off value of GPR was 0.32.

For predicting cirrhosis, AUROC of GPR (0.83, 95% CI 0.72 to 0.94) was significantly better than that of APRI (0.70, 95% CI 0.55 to 0.86,  $p = 0.03$ ) and Fib-4 (0.73, 95% CI 0.58 to 0.87,  $p = 0.03$ ) although the AUROC of GPR was significantly inferior to LSM (0.98, 95% CI 0.97 to 1.00,  $p = 0.003$ ) (table 3). The optimal cut-off value of GPR for cirrhosis was 0.56.

The AUROC of each marker did not change substantially when the analysis was restricted to the subjects with liver specimen  $\geq 12$  mm (data not shown). Among fibrosis stage, activity grade and steatosis, fibrosis was the only parameter significantly associated with GPR ( $p < 0.001$ ), LSM ( $p < 0.001$ ), APRI

( $p = 0.008$ ) and Fib-4 scores ( $p = 0.006$ ) in multiple linear regression.

#### Validation set in Senegal

In the Senegalese cohort (n=80), 18 (23%) were F0, 36 (45.0%) were F1, 17 (21%) were F2 and 9 (11%) were F3 based on the liver histology results (table 1). None had cirrhosis since the study protocol did not allow LB in patients with suspected cirrhosis.

For predicting significant fibrosis, the AUROC of GPR (0.73, 95% CI 0.59 to 0.86) was higher than that of Fib-4 (0.57, 95% CI 0.42 to 0.71,  $p = 0.04$ ) and APRI (0.62, 95% CI 0.48 to 0.76,  $p = 0.05$ ). For predicting extensive fibrosis, the AUROC of GPR (0.93, 95% CI 0.87 to 0.99) was significantly better than that of Fib-4 (0.71, 95% CI 0.53 to 0.89,  $p = 0.02$ ) and Fibroscan (0.71, 95% CI 0.53 to 0.89,  $p = 0.04$ ). There was no significant difference between GPR and APRI (0.89, 95% CI 0.80 to 0.98,  $p = 0.3$ ). Using the optimal cut-off value determined in the Gambian training set (0.32), the sensitivity and specificity of GPR to predict severe fibrosis was 89% and 80%, respectively (table 3).

#### Validation set in France

In the French cohort (n=63), fibrosis stage according to histopathology was as follows: 6 (10%) in F0, 27 (43%) in F1, 15 (24%) in F2, 11 (17%) in F3 and 4 (6%) in F4 (table 1).

The performance of GPR was as good as APRI and Fib-4: AUROC at 0.72 (95% CI 0.59 to 0.85) to predict significant fibrosis, 0.76 (95% CI 0.62 to 0.90) for extensive fibrosis and 0.87 (95% CI 0.76 to 0.98) for cirrhosis (table 3). Applying the optimal cut-off (0.56) determined in the training data set, the sensitivity and specificity of GPR to predict cirrhosis were 100% and 68%, respectively (table 3).

#### DISCUSSION

Assessing the severity of CHB infection is one of the main barriers for offering care and treatment to CHB carriers in SSA. In

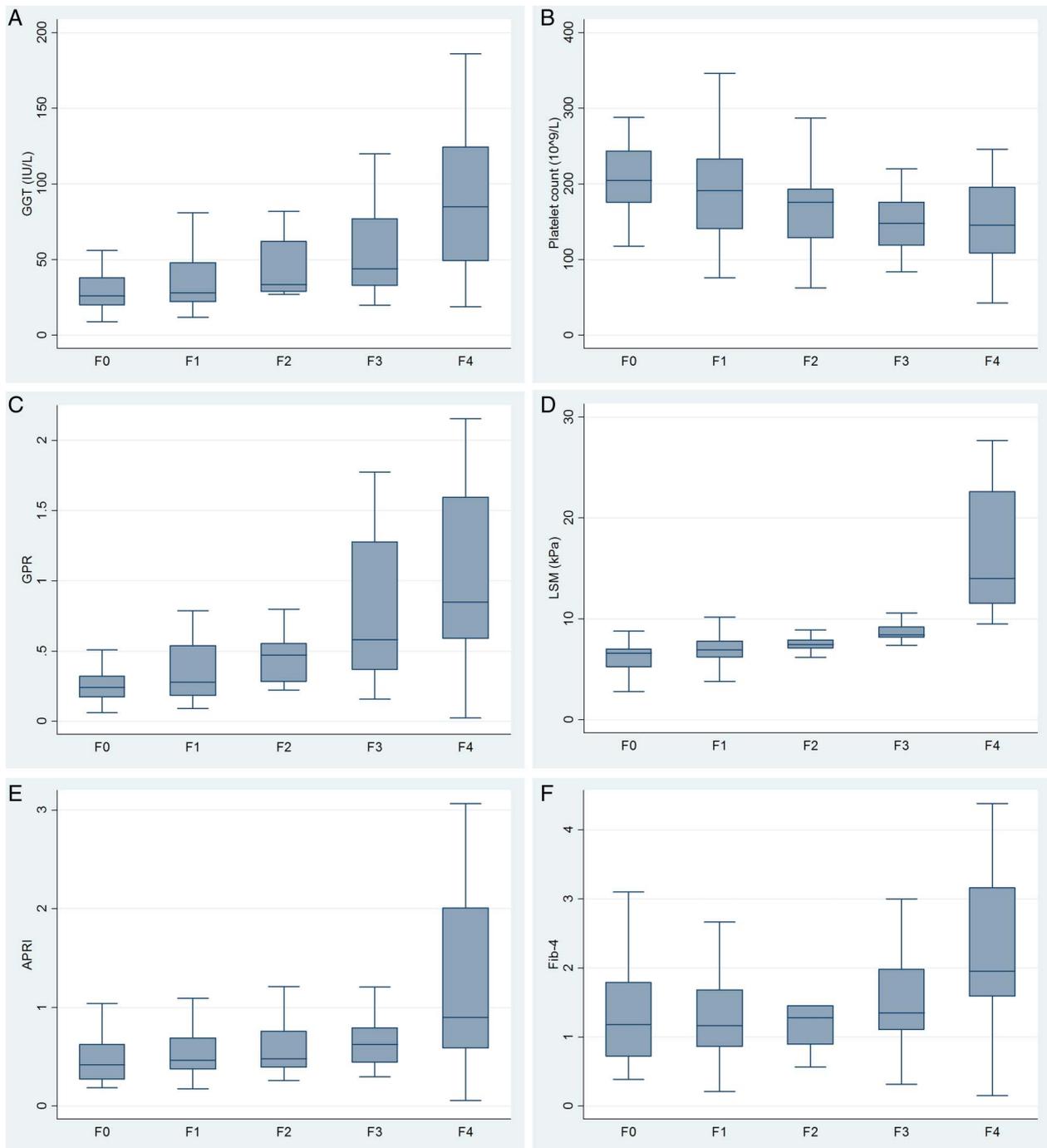
**Table 2** Crude analysis for factors associated with the presence of significant fibrosis (F2–4) in the training set, in The Gambia

Factors*	No or mild fibrosis n=82	Significant fibrosis or more n=53	Crude p value†
Age (years)	36±10	35±11	0.7
Male sex, n (%)	58 (71)	47 (89)	0.02
AST (IU/L)	39±22	62±61	0.01
ALT (IU/L)	37±25	64±79	0.007
ALP (IU/L)	97±44	112±69	0.1
GGT (IU/L)	35±21	72±55	<0.001
Albumin (g/L)	43±4	41±11	0.003
Total bilirubin (IU/L)	12±5	17±21	0.1
Platelets (10 <sup>9</sup> /L)	201±67	185±183	0.007
HBV DNA (log <sub>10</sub> IU/mL)	6.9±7.8	8.5±9.3	0.01

\*Continuous variables are presented as mean±SD.

† p Values were obtained using logarithmic transformed values except for sex.

ALT, alanine transaminase; ALP, alkaline phosphatase; AST, aspartate transaminase; GGT, gamma-glutamyl transpeptidase.



**Figure 2** Box plots of gamma-glutamyl transpeptidase (GGT) (A), platelet count (B), GGT to platelet ratio (GPR) (C), liver stiffness measurement (LSM) (D), aspartate transaminase-to-platelet ratio index (APRI) (E), and Fib-4 (F) according to the Metavir fibrosis stage in the Gambian cohort.

most resource-limited settings LB is impractical and Fibroscan is rarely available.

Within the framework of the PROLIFICA programme in The Gambia,<sup>16</sup> we developed a new simple and inexpensive biomarker index, the GPR, to identify HBV-infected subjects with significant fibrosis or cirrhosis in SSA. The AUROC of GPR to predict fibrosis and cirrhosis was generally high throughout the Gambian cohort and two independent validation data sets (from Senegal and France).

As previously reported in patients with chronic hepatitis C<sup>25 26</sup> and B<sup>6 27</sup> from Western countries, Fibroscan performed better than biochemical markers in the Gambian cohort with excellent diagnostic accuracy for predicting significant fibrosis

and cirrhosis. In our study in The Gambia, LSMs were performed fasting<sup>21</sup> on the same day of the LB in contrast to other studies which performed Fibroscan within 1 month, 6 months<sup>6 15</sup> or even 12 months of the LB.<sup>14</sup> Our best Fibroscan cut-offs for significant fibrosis (7.9 kPa) and cirrhosis (9.5 kPa) were close to those proposed in Western studies.<sup>6 28</sup> Among those included in the current analysis in The Gambia, none had unreliable LSM. Indeed, in our study population obesity (body mass index (BMI)  $\geq 30$  kg/m<sup>2</sup>), which is often associated with unreliable measurement, was rarely observed (4.4%, 6/135). In addition, because LSM was one of the criteria for the LB indication, patients with invalid measurement who did not meet other criteria for biopsy were excluded.

**Table 3** Diagnostic performances of GPR, Fibroscan, APRI and Fib-4 in the training set (The Gambia) and in validation sets (France and Senegal)\*

	The Gambia (n=135)			France (n=63)†			Senegal (n=80)‡		
	F0-1 vs F2-4	F0-2 vs F3-4	F0-3 vs F4	F0-1 vs F2-4	F0-2 vs F3-4	F0-3 vs F4	F0-1 vs F2-4	F0-2 vs F3-4	
<b>GPR</b>									
AUROC (95% CI)	<b>0.80 (0.72 to 0.88)</b>	<b>0.81 (0.72 to 0.89)</b>	<b>0.83 (0.72 to 0.94)</b>	<b>0.72 (0.59 to 0.85)</b>	<b>0.76 (0.62 to 0.90)</b>	<b>0.87 (0.76 to 0.98)</b>	<b>0.73 (0.59 to 0.86)</b>	<b>0.93 (0.87 to 0.99)</b>	
Cut-off values§	0.32	0.32	0.56	0.32	0.32	0.56	0.32	0.32	
Sensitivity/specificity (%)	83/69	86/64	85/76	73/49	80/44	100/68	50/83	89/80	
Correctly classified (%)	74	71	77	60	52	70	73	81	
PPV/NPV (%)	63/86	53/91	39/97	56/67	31/88	17/100	59/78	36/98	
Positive/negative LR	2.7/0.3	2.4/0.2	3.5/0.2	1.4/0.6	1.4/0.5	3.1/0.0	3.0/0.6	4.5/0.1	
<b>Fibroscan</b>									
AUROC (95% CI)	<b>0.85 (0.78 to 0.91)</b>	<b>0.89 (0.82 to 0.95)</b>	<b>0.98 (0.97 to 1.00)</b>				<b>0.61 (0.48 to 0.74)</b>	<b>0.71 (0.53 to 0.89)</b>	
Cut-off values§	7.9	8.2	9.5				7.9	8.2	
Sensitivity/specificity (%)	81/81	93/82	100/89				81/30	89/41	
Correctly classified (%)	81	85	90				46	46	
PPV/NPV (%)	73/87	70/96	61/100				36/76	16/97	
Positive/negative LR	4.2/0.2	5.0/0.1	8.9/0				1.1/0.6	1.5/0.3	
<b>APRI</b>									
AUROC (95% CI)	<b>0.66 (0.57 to 0.76)</b>	<b>0.68 (0.58 to 0.78)</b>	<b>0.70 (0.55 to 0.86)</b>	<b>0.77 (0.65 to 0.89)</b>	<b>0.83 (0.72 to 0.93)</b>	<b>0.78 (0.57 to 0.98)</b>	<b>0.62 (0.48 to 0.76)</b>	<b>0.89 (0.80 to 0.98)</b>	
Cut-off values¶	0.5    1.5		1.0    2.0	0.5    1.5		1.0    2.0	0.5    1.5		
Sensitivity/specificity (%)	64/64    9/98		40/87    25/99	77/67    20/91		50/78    25/92	42/70    0/96		
Correctly classified (%)	64    62		80    88	71    57		76    87	61    65		
PPV/NPV (%)	54/73    71/62		35/89    75/87	68/76    67/56		13/96    17/95	41/72    0/67		
Positive/negative LR	1.8/0.6    3.8/0.9		3.0/0.7    28/0.8	2.3/0.4    2.2/0.9		2.3/0.6    3.0/0.8	1.4/0.8    0.0/1.0		
Indeterminate results (%)	42		13	40		14	31		
<b>Fib-4</b>									
AUROC (95% CI)	<b>0.66 (0.56 to 0.76)</b>	<b>0.68 (0.57 to 0.78)</b>	<b>0.73 (0.58 to 0.87)</b>	<b>0.75 (0.62 to 0.88)</b>	<b>0.86 (0.77 to 0.95)</b>	<b>0.83 (0.69 to 0.98)</b>	<b>0.57 (0.42 to 0.71)</b>	<b>0.71 (0.53 to 0.89)</b>	
Cut-off values**		1.45    3.25			1.45    3.25		1.45    3.25		
Sensitivity/specificity (%)		63/68    14/98			73/73    27/96		44/83    0/100		
Correctly classified (%)		66    71			73    79		79    89		
PPV/NPV (%)		48/79    75/70			46/90    67/81		25/92    NA/89		
Positive/negative LR		1.9/0.5    6.3/0.9			2.7/0.4    6.4/0.8		2.6/0.7    1.0/NA		
Indeterminate results (%)		36			29		20		
<b>Comparison of AUROC</b>									
GPR and Fibroscan	p=0.2	p=0.02	p=0.003	N/A	N/A	N/A	p=0.2	p=0.04	
GPR and APRI	p<0.001	p=0.002	p=0.03	p=0.4	p=0.2	p=0.3	p=0.05	P=0.3	
GPR and Fib-4	p=0.003	p=0.01	p=0.03	p=0.6	p=0.1	p=0.6	p=0.04	P=0.02	

Continued

Table 3 Continued

	The Gambia (n=135)			France (n=63)†			Senegal (n=80)‡		
	F0-1 vs F2-4	F0-2 vs F3-4	F0-3 vs F4	F0-1 vs F2-4	F0-2 vs F3-4	F0-3 vs F4	F0-1 vs F2-4	F0-2 vs F3-4	F0-3 vs F4
Fibroscan and APRI	p<0.001	p<0.001	p<0.001	N/A	N/A	N/A	p=0.9	p=0.1	
Fibroscan and Fib-4	p<0.001	p<0.001	p<0.001	N/A	N/A	N/A	p=0.6	p=0.9	
APRI and Fib-4	p=0.9	p=0.9	p=0.6	p=0.6	p=0.6	p=0.6	p=0.4	p=0.03	

\*The cut-off values for GGT were 58 in The Gambia, 45 in men and 30 in women in France, and 73 in men and 43 in women in Senegal. The cut-off values for AST were 46 in men and 25 in women in The Gambia, 45 in men and 40 in women in France, and 59 in men and 36 in women in Senegal.

†In France, Fibroscan was not performed.

‡In Senegal, there was no patient with cirrhosis (F4).

§Cut-off values for GPR and Fibroscan were determined to maximise the sum of sensitivity and specificity in the training data set.

¶Predetermined cut-off values of APRI were used (0.5 and 1.5 to distinguish significant fibrosis, and 1.0 and 2.0 to distinguish cirrhosis).

\*\*Predetermined cut-off values of Fib-4 were used (1.45 and 3.25 to distinguish extensive fibrosis).

APRI, AST-to-platelet ratio index AST; aspartate transaminase; AUROC, area under the receiver operating characteristic curve; GGT, gamma-glutamyl transpeptidase; GPR, gamma-glutamyl transpeptidase to platelet ratio; LR, likelihood ratio; NA, not applicable; NPV, negative predictive value; PPV, positive predictive value.

Despite its high diagnostic performance, the Fibroscan device is still expensive (€34 000 for the portable machine) and requires annual maintenance (€5000). Moreover, in Africa, when available, the machine is often only accessible in the main hospitals in the capital city or within the private sectors. In SSA countries, where antiviral therapy using tenofovir has now become available at generic cost and decentralisation of health-care provision is recommended,<sup>29</sup> there is an urgent need for simple and inexpensive alternative methods for identifying patients with CHB who need treatment. Consequently, the recent WHO guidelines<sup>13</sup> ranked APRI as a preferred non-invasive test in resource-limited settings, rather than Fibroscan.

However, the accuracy of APRI observed in SSA (including our study) is not acceptable. The AUROC of APRI to predict significant fibrosis was 0.61 (95% CI 0.46 to 0.76) in Burkina Faso,<sup>14</sup> 0.62 (0.48 to 0.76) in Senegal and 0.66 (0.57 to 0.76) in The Gambia, and for the prediction of cirrhosis it was 0.50 (0.32 to 0.68) in Burkina Faso and 0.70 (0.55 to 0.86) in The Gambia. A similar low performance of Fib-4 has been constantly observed in the Burkinabe study<sup>14</sup> and ours in The Gambia and Senegal. WHO guidelines<sup>13</sup> also recommend a single cut-off of the APRI score (2.0) to diagnose cirrhosis in resource-limited countries. Whether this cut-off can be applied for SSA countries is highly questionable because of the difference in natural history of CHB infection; in Asia CHB carriers frequently experience active hepatitis with elevated transaminase levels<sup>30</sup> while in SSA the vast majority are in inactive phase with normal transaminases.<sup>31</sup> By applying the WHO cut-off of APRI, the sensitivity for the diagnosis of cirrhosis in the Gambian cohort was only 25%. This implies that 75% of patients with cirrhosis will be erroneously categorised as patients without cirrhosis. In contrast the sensitivity of GPR to diagnose cirrhosis in The Gambia was 85% and 89% in Senegal to diagnose extensive fibrosis. In patients with CHB, GGT has been previously identified as an independent variable of significant fibrosis<sup>32-34</sup> and GPR was also suggested as a potential biomarker of fibrosis in a Turkish cohort.<sup>33</sup>

Our study in The Gambia also confirmed the difficulty of performing LB (a third of refusal) and sample preparations in SSA. We reported 30.4% of interobserver discrepancy and we faced several barriers obtaining good quality liver specimens mainly due to difficulties in fixing and cutting liver specimen in the local warm environment where softening of paraffin renders the cutting difficult.

Our study in The Gambia has limitations. First, for ethical and logistical reasons, the indication of LB depended on the results of index tests under the investigation (AST, ALT and LSM), and we could not invite all our HBV-infected participants for LB. As a result, the patients included in the current analysis are not representative of the general population with CHB infection in The Gambia. This might have caused verification bias resulting in overestimated sensitivities and underestimated specificities of these markers.<sup>35</sup> Nevertheless, the GPR was free from this bias because in absence of contraindications including platelets below 50,000/mm<sup>3</sup>, patients were invited for LB irrespective of their GGT and platelet levels. Second, we acknowledge that our liver specimens were relatively small and that only a small proportion of our biopsies were over 15 mm, which is the current recommendation.<sup>36</sup> However, in a subgroup of Gambian patients with a length of over 12 mm, AUROC of the non-invasive tests did not improve. Third, we excluded conditions that may be associated with elevated GGT (excessive alcohol consumption and obesity) and low platelet count (alcohol, HIV and malaria). Consequently, high diagnostic accuracy of the GPR may not be generalisable in patients with such conditions.

In summary, for the identification of CHB carriers with significant fibrosis or cirrhosis, non-invasive markers that depend on transaminase levels (APRI and Fib-4) are not accurate enough to be used in routine practice in SSA. Our study suggests that GPR may be a simple, accurate and inexpensive alternative to LB and Fibroscan in resource-constrained African settings. The GPR deserves to be further validated in other African and non-African populations.

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**Contributors** All authors contributed to this study at different levels. All authors read and approved the final version. ML and YS designed the study and wrote the paper. ML and RN were locally responsible for the clinical aspects of the study. MK, JL and RG were in charge of the histopathological analysis. PS prepared the liver specimens with the support of MK and MTa; MV and PSM were in charge of the Senegalese cohort. VM was responsible for the French cohort, conceived the GPR and revised the manuscript. MTH revised the design of the study and the manuscript. YS was responsible for the statistical analysis.

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