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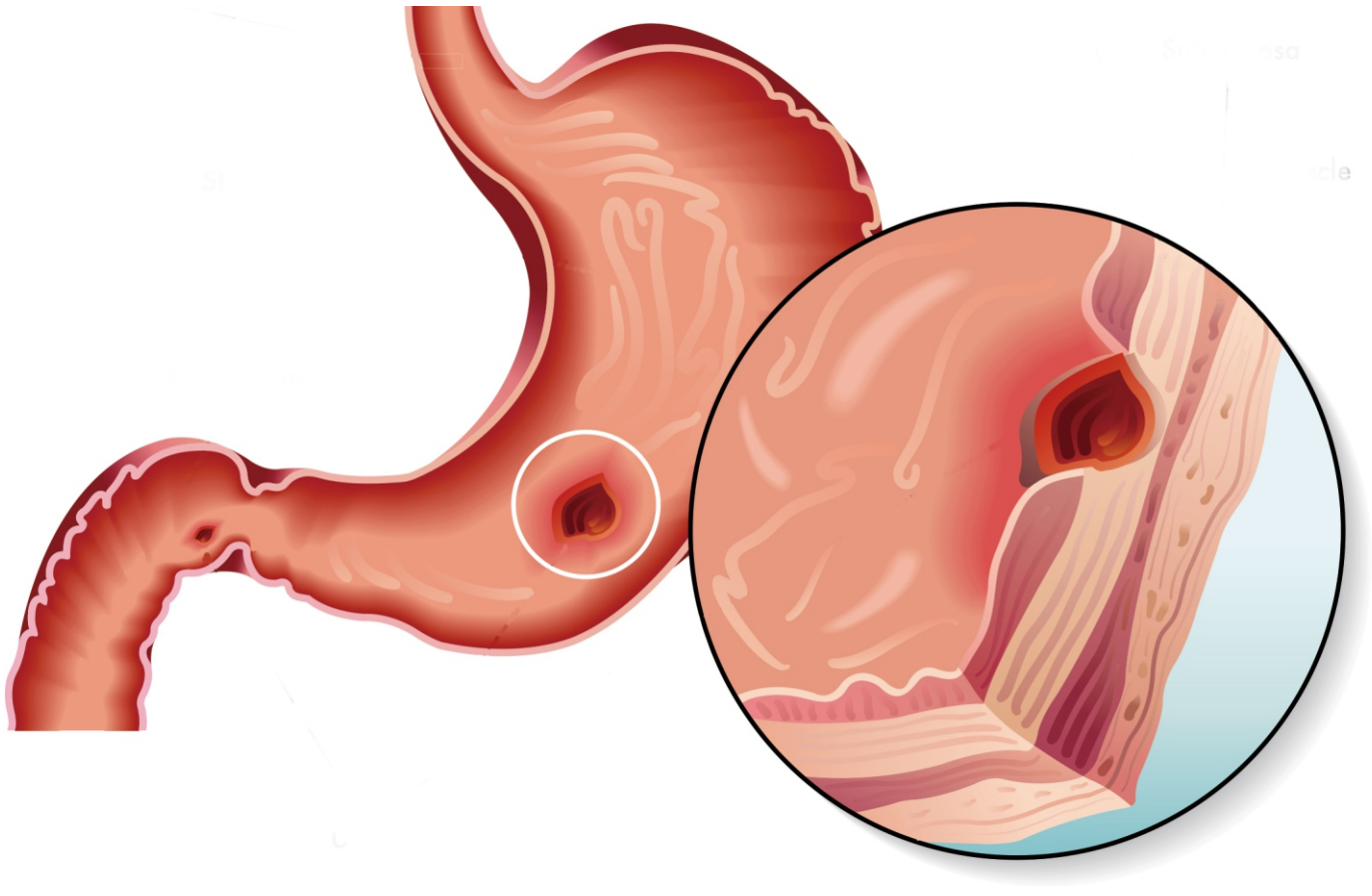

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Diagnosis of *Helicobacter pylori* Infection in Low Out-Outcome Country: Rapid Urease Test, Serological Test, versus Direct Microbiological Examination with Gram Stain

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Abstract

Introduction: *Helicobacter pylori* is a gram-negative bacillus responsible for numerous gastroduodenal pathologies, and this infection is a public health problem. The prevalence of infection with this bacterium remains high in countries with limited resources. Diagnosis relies mainly on numerous invasive and noninvasive methods. The aim of this work was to evaluate the different indirect diagnostic methods using bacterial cultures. **Methods:** We conducted a cross-sectional and analytical study from January to May 2022 in the gastroenterology departments of Douala General Hospital and Douala Military Hospital. All patients aged 18 years and older who were in the gastroenterology consultation and agreed to participate were included in our study. Sociodemographic, clinical, and paraclinical data were collected. Urease, liquid urea, and culture tests were performed from the specimens obtained by fibroscopy. Serological tests were performed on the blood sample. **Results:** 101 patients were included, 58 were female and 43 were male, for a sex ratio of 1.3. The mean age was 44.2 ± 16 years. The prevalence of infection was 90.5%, 44.1%, 40.6% and 21.8% for serology, direct microbiological examination, RUT (rapid urea test) and culture, respectively. Comparison of the different tests showed sensitivity and specificity of 67.1% and 64%, respectively, for RUT, 100% and 73.7%, respectively, for direct microbiological

examination, and 100% and 14.8%, respectively, for serology. The positive and negative predictive values were 39.5% and 100% for serology, 39% and 85% for RUT, and 55.6% and 100% for direct microbiological examination, respectively. **Conclusion:** The prevalence of *Helicobacter pylori* infection depends on the type of test used. Direct examination is more reliable than RUT and serology.

Keywords

Helicobacter pylori, Diagnostic Tests, Sensitivity, Specificity, Positive and Negative Predictive Value

1. Introduction

Helicobacter pylori is a gram-negative bacillus, that is considered the most common bacterial infection [1] [2]. Transmission is mainly interpersonal via the fecal-oral, oro-oral or gastro-oral route, but also iatrogenic by gastric intubation [2] [3] [4]. Primary infection with *Helicobacter pylori* occurs mainly in childhood and is favored by promiscuity and low socioeconomic status [4]. In the long term, colonization with *Helicobacter pylori* can damage the gastric mucosa and cause various gastrointestinal diseases such as gastritis, peptic ulcer disease (PUD), and gastric cancer (adenocarcinoma and lymphoma) [3] [5]. The prevalence is estimated at 50% worldwide, with 70% - 80% of cases from resource-limited countries and 15% - 30% from developed countries [6] [7]. In Cameroon, the overall prevalence of *H. pylori* infection varies from 47.4% to 72.5% depending on the study [8] [9] [10] [11], which, is due to the different techniques used to diagnose *H. pylori* infection. Several invasive and non-invasive methods have been developed and validated for the diagnosis of *H. pylori* [7] [12] [13] [14]. Noninvasive methods include serology based on the search for IgG antibodies, stool antigen test, and the urea breath test [12]. Invasive methods require the performance of esogastroduonal endoscopy, in which biopsies are taken for analysis. These methods are the rapid urease test performed in the endoscopy room, cultures, molecular testing and histology [12]. Invasive tests require strict pre-analysis conditions for the preservation and transport of prior which are rarely or poorly applied in current practice [14]. In addition, the invasiveness of endoscopy strongly contributes to the use of noninvasive tests. Although the noninvasive tests are very sensitive and specific, they often require additional tests to confirm the diagnosis, as is the case with serology. In the African study to determine the prevalence of *H. pylori* infection, it was found that the diagnostic methods used varied from one series to another, contributing to a wide variation in the results obtained [8] [10] [11] [15]. Most gastroenterologists prefer the readily available rapid urease test, or the unfortunately very expensive pathological examination, as recommended for the diagnosis of *H. pylori* infection [13] [16]. Serology is often tested by other specialists or general practitioners. Microbiological test

with Gram stain although available and readily easy is very underutilized, because unknown to many gastroenterologists. The aim of the study was to compare tests commonly used for the diagnosis of *H. pylori* (serological test, rapid urea test) versus direct microbiological test; and thus determine sensitivity, specificity and predictive value.

2. Methods

2.1. Type of Study

We conducted a cross-sectional study over a 6-month period, from January 1 to June 30, 2022. The setting was 02 hospitals in Douala City, the general hospital and the military hospital, which have a digestive endoscopy service. All patients who were at least 18 years old and admitted to the digestive endoscopy service for esogastroduodenal endoscopy and agreed to participate in the study were included in the study. Patients with tumor-like lesions and those who had taken antibiotics (amoxicillin, clarithromycin, metronidazole, levofloxacin) and/or a proton pump inhibitor in the month before study entry were excluded. The University of Douala Human Research Ethics Committee approved the study, and all subjects provided written informed consent prior to participation.

2.2. Sampling Procedure

Each patient received an information sheet about the study from the principal investigator, supplemented by verbal explanations. After the patient gave verbal consent to participate in the study, he or she was presented with an informed consent form for signature. The investigator completed a prepared anonymous data collection form for each patient. Sociodemographic data (age, sex), medical history and concomitant diseases (hypertension, diabetes, HIV, *H. pylori* anterior wall infection), clinical symptoms of the patients (epigastralgia, dyspepsia, regurgitation, pyrosis, nausea, vomiting) were recorded. For blood collection, 3 ml of venous blood was drawn with a vacutainer needle and a dry tube for serological analysis. During esogastroduodenal endoscopy performed by the gastroenterologist, the following biopsies were taken for microbiological analysis: 02 at the antrum, 02 at the fundus, and 01 at the angle of the lesser curvature. These biopsies were used to perform the liquid urea stain and culture test. Two additional biopsies were taken from the antrum to perform a rapid urea test. During the procedure, biopsy specimens collected with biopsy forceps were placed in prelabeled sterile urine boxes (anonymity, sex, and age of patient) containing 3 ml of brain heart broth and 20% glycerol (transport medium). These samples were transported to the site in a cool box with dry ice. Samples were stored in the refrigerator (4˚C - 8˚C) for 24 hours or at −60˚C beyond 24 hours.

3. Analytical Steps

1) Serological test

Serological assay was performed with the antibodies directed against *H. pylori*

from the Diaspot kit using an enzymatic immunosorbent assay. Two drops of serum samples and one drop of buffer were added to the well of the cassette using a vertical dropper. After 10 minutes of migration, the positivity of the assay is determined by the presence of two bars.

2) Rapid urease test

Biopsies were placed in the well containing an acidic pH urea solution with a yellow color marker (phenolphthalein). The result was read 5 - 30 minutes after the biopsy was placed. The test was positive if the circumference of the disc turned pink. The intensity of the red coloration of the disc depends on the density of the population of *Helicobacter pylori* at the sampling site.

3) Direct microbiological examination and culture [17]

Once in the laboratory, the biopsies were immediately crushed with a pestle in a mortar that contained a few drops of heart-brain broth to facilitate the crushing. At the end of this phase, each grinder was subjected to two further treatments: direct microbiological examination and culture.

❖ **Direct microbiological examination with Gram stain**

A small amount of the breaker was applied to a clean blade and spread with circular movements. Drying of the blade at room temperature was followed by staining by the Gram method and finally observation of the dried smear in a drop of immersion oil microscopically with objective 100. Observation of a spiral-shaped, 2 - 4 µm long and 0.5 - 1 µm wide, pink (Gram) stained bacillus indicates the presence of *helicobacter pylori*.

The principle of Gram staining is based on the staining of the bacterial cell wall. It consists of four steps: Staining of the smear with gentian violet (30 seconds to 1 minute), etching with Lugol (30 seconds to 1 minute), staining of the slide with alcohol (30 seconds), and counterstaining with fuchsin (30 seconds to 1 minute).

❖ **Culture**

We added 47 g of Columbia agar (powder) to 1 l of distilled water and heated the mixture until it was fully boiled. The bottled mixture was then autoclaved for 15 min (to eliminate bacteria that could not be killed at high temperatures). At a temperature of 45˚C, 10% of the human blood added to the mixture was homogenized. Finally, the obtained mixture was mixed with an OXOID brand preparation containing vancomycin (10 mg/l), trimethoprim (05 mg/l), cefsulodin (10 mg/l) and amphotericin B (10 mg/l), and then poured into Petri dishes. A small amount of the biopsy homogenate was sprinkled on the culture medium and then incubated at 37˚C in the absence of oxygen for a maximum of 10 days. The incubated culture dishes were examined every 24 hours. Only after the 10th day of incubation, when no suspicious colonies were visible, was the culture considered sterile. The isolated suspicious colonies (small colonies about 0.5 to 1 mm in diameter, translucent, shiny, and nonhemolytic) were subjected to morphological and biochemical identification tests.

Morphological identification: was performed by the Gram control, which

consists in performing a Gram stain on the suspicious colonies spread on a slide (see direct microbiological examination above).

Biochemical identification: consists of performing a catalase test, an oxidase test and a urea indole test.

- **Catalase test** is performed by placing a drop of hydrogen peroxide on a slide that previously contained a colony of isolated bacteria. Positivity of the test is indicated by the appearance of gas bubbles on the slide.
- **Oxidase test** consists of contacting a suspected colony of *Helicobacter pylori* with an oxidase disc. The positive reaction is indicated by a purple coloration of the disc.
- **Urea-indole test** is performed by placing a suspect colony in an Eppendorf tube containing a small amount of indole urea. The color change from yellow to pink after 24 hours indicates that the test is positive.

Statistical analysis

Data were analyzed using SPSS version 26.0 software. The dichotomized data were used to calculate sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). Values are reported with a 95% confidence interval. Each assay was tested against a culture as a gold standard.

4. Results

We included 101 patients with gastroduodenal symptoms consecutively admitted to the endoscopy department. As shown in Table 1, the mean age was 44.2 ± 16 years, with a median age of 44 years. We had 58 women and 43 men, corresponding to a sex ratio of 1.3. Hypertension was detected in 13 patients (12.9%) and diabetes in 5 patients (4.9%). Regarding lifestyle, 88.1% (n = 89) consumed spices, 54.5% (n = 55) consumed alcohol, and 7.9% (n = 8) smoked (Table 1). Oesopgastroduodenal endoscopy revealed lesions in 73.3% of patients (n = 74). The three main endoscopic lesions were erythematous antral gastropathy (57.4%), bulbar ulcer (14.9%), and pangastropathy (13.9%) (Table 1). The prevalence of *Helicobacter pylori* infection was 90.5% for the serological test, 57.4% for the liquid urea test, 44.1% for direct microbiological examination, 40.6% for rapid urease test, and 21.8% for culture. Table 2 shows that the sensitivity and specificity of the different diagnostic tests are 64% (IC 95% 52.5 - 73.6) and 67.1% (IC 95%: 48.6 - 78.5), respectively, for the rapid urease test, 100% (IC 95%: 95.6 - 100) and 73.7% (IC 95% 68.7 - 88.4), respectively, for the direct microbiological examination, and 100% (IC 95% 98 - 100) and 14.8% (IC 95% 8.3 - 17.9), respectively, for serology. The positive predictive values were 39.5% (IC 95% 12.7 - 83.2) for serology, 39% (IC 95% 41.6 - 78.4) for the rapid urease test, and 55.6% (IC 95% 52.3 - 77.9) for the direct microbiological examination (Table 2). As can be seen in Table 2, the negative predictive values were 100% (IC 95% 98 - 100) for serology, 85% (IC 95% 83.4 - 92.7) for the rapid urease test, 100% (IC 95% 97 - 100) for the direct microbiological examination.

Table 1. Population of study.

	Frequence (%)	Mean (SD)
Age (years)		44.2 (16)
Sex		
Men	48 (47.5)	
Women	53 (52.5)	
Comorbidities and lifestyle		
Hypertension	13 (12.9)	
Diabetes	5 (4.9)	
Alcohol	55 (54.5)	
Tobacco	8 (7.9)	
Spicy food	89 (88.1)	
Clinical presentation		
Epigastralgia	76 (75.3)	
GERD	60 (59.4)	
Dyspepsia	48 (47.5)	
Loss of weight	45 (44.6)	
Nausea	41 (40.6)	
Endoscopic features		
Normal	27 (26.7)	
Erythematous antral gastritis	58 (57.4)	
Bulbar ulcer	15 (14.9)	
Pangastritis	14 (13.9)	

Table 2. Sensitivy, specifity and predictives Values of RUT, serology and direct micorbiological examination according to culture.

	Sensitivity (IC 95%)	Specifity (IC 95%)	PPV (IC 95%)	NPV (IC 95%)
RUT	64% (52.5 - 73.6)	67.1% (48.6 - 78.5)	39% (41.6 - 78.4)	85% (83.4 - 92.7)
Direct examination	100% (95.6 - 100)	73.7% (68.7 - 88.4)	55.6% (52.3 - 77.9)	100% (97 - 100)
Serology	100% (98 - 100)	14.8% (8.3 - 17.9)	39.5% (12.7 - 83.2)	100% (98 - 100)

PPN: Positive Predictive Value, NPV: Negative Predictive Value.

5. Discussion

The prevalence of *H. pylori* infection depended on the diagnostic test used. For

the rapid urease test, the direct microbiological test, the prevalences found were comparable to those found in various studies in Cameroon as well as in other countries in sub-Saharan Africa [8] [10] [11] [18] [19]. There are a few studies in Cameroon with higher prevalences, but the type of diagnostic tests used and the target population must be considered [9]. The high prevalence found for the serological test is probably related to the fact that it is based on the search for Ig G antibodies [20]. It is therefore difficult to associate it with active infection. Regarding culture, the low prevalence, which contrasts strongly with available data on prevalence in Africa [18] and Cameroon, is related to technical constraints [17]. Indeed, the conditions for performing culture are often difficult to implement in current practice.

Although culture for the diagnosis of *H. pylori* infection has technical limitations and a low prevalence in this series, we wanted to use it as a gold standard for the comparison of diagnostic methods because it has a good specificity with a correct sensitivity in literature [16] [21].

The sensitivity of serology was good as reported in the literature as was that of direct microbiological testing. However, the sensitivity of the rapid urease test (RUT) was lower than that reported in various studies. Specificity was low with serology, Ig G can be found even after eradication of the bacteria [16]. Direct microbiological examination also has a good specificity compared to the rapid urease test. As for the predictive values, the direct microbiological test and the serological test had a good negative predictive value, which was significantly higher than the rapid urease test. All the tests we used had a low positive predictive value. The values were less than 60%. The direct microbiological examination had the better positive predictive values.

The results obtained with the rapid urease test are in contradiction with those described in many studies, although we performed biopsies in the antrum [21] [22]. Redeen *et al.* showed results over 90% for sensitivity, specifity, and predictive values for RUT when biospies were performed in the antrum [22]. The reliability of the kits and the time required to read the results could be critical factors in the perfomance of the rapid urease test. Van Horn *et al.* showed better sensitivity and specificity when the kit was checked 24 hours later [23]. However, the rapid urease test remains the better option for diagnostic testing because of its lower cost and immediate availability of results, which allow rapid treatment of the patient.

The results obtained with the direct microbiological examination with Gram stain open the door to its use in common practice, as Oyedeji *et al.* had mentioned in Nigeria [24]. He had found a higher prevalence in the use direct examination with Gram staining compared to culture and breath test. It could be an alternative to culture or even to histology, whose implementation conditions and the often high costs are a brake.

The main limitations of the studies were the lack of sufficient comparative data on the reliability of the direct microbiological examination with Gram stain, especially with the pathological test and the stool antigen test.

6. Conclusion

The prevalence of *H. pylori* infection depends on the type of diagnostic tests used. The prevalences detected with the urease test and the direct microbiological examination are similar to those reported in the literature for Cameroon. The direct microbiological examination showed good results in terms of sensitivity and specificity, as well as a good predictive value. It could be an alternative to pathological examination, which is more costly. The rapid urease test has lower sensitivity and specificity than those found in the different studies, but it still has a good negative predictive value. It remains a more reliable test than the serological test

Author's Contrbutions

Data collection and analysis: Winnie Bekolo and Ilinga Kelly; Writing and corrections: Winnie Bekolo; Corrections: Sepo Sepo David, Nsenga Njapa Guy Roger Ndjitoyap Antonin, Kowo Mathurin, Eloumou Baganka Servais, Noah Noah Dominique, Ankouane Andoulo Firmin, Njoya Oudou; Sudy design: Eloumou Baganka Servais, Ankouane Andoulo Firmin, Eboumbou Carole.

Conflicts of Interest

The authors state that they have no conflict of interest.

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Abbreviations

H. pylori: *Helicobacter pylori*
RUT: Rapid Urea Test
NPV: Negative Predictive Value
PPV: Positive Predictive Value

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Evaluation of Non-Invasive Markers of Liver Fibrosis in Chronic Hepatitis B Patients in a Sub-Saharan African Setting: Transient Elastography versus APRI, FIB4, GTT/Platelet Scores

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Abstract

Background: Non-invasive markers which use routine laboratory tests are less expensive and highly needed to assess and stage liver fibrosis in chronic hepatitis B patients in Sub-Saharan Africa. We aimed at evaluating liver fibrosis, using the Aspartate aminotransferase to Platelet Ratio Index (APRI), Fibrosis Index Based on 4 factors (FIB4), and Gamma-glutamyl transpeptidase to Platelet Ratio (GPR) in chronic hepatitis B patients with transient elastography as the reference so as to choose an alternative to transient elastography. **Method:** We carried out a cross-sectional study using the records of patients who attended the Douala General Hospital and Marie O Polyclinic Douala from 2012 to 2017. Non-invasive tests were compared with Transient Elastography. The Spearman coefficient was used to determine correlation. The sensitivity, specificity, positive predictive values and negative predictive values were used to get the optimal cut-off values. The diagnostic accuracy was estimated by calculating the area under the Receiver Operating Characteristic Curve (ROC). $P < 0.05$ was considered statistically significant. **Results:** Of the 243 patient records studied, the median age or interquartile range

(IQR) was 35 (29 - 42) years with a male predominance of 73.7%. More than 60% of the study population had normal transaminases. Significant fibrosis was found in 88 (36.2%) patients and 32 (13.7%) patients had cirrhosis. APRI had the best cut-off values and highest area under the ROC Curve, for significant fibrosis and cirrhosis with 0.55 (0.823 95% CI [0.769 - 0.869], P < 0.001) and 0.65 (0.84 95% CI [0.788 - 0.884], P < 0.005) respectively. **Conclusion:** APRI, had the best diagnostic properties to detect liver fibrosis and cirrhosis in patients with Chronic Hepatitis B in Douala. The cut-off values are 0.55 and 0.65 for significant fibrosis and cirrhosis respectively.

Keywords

Chronic Hepatitis B, Liver Fibrosis, Non-Invasive Tests, Cross Sectional, Douala

1. Introduction

Hepatitis B virus (HBV) infection poses a major burden to health worldwide [1]. Out of every three persons in the world, at least one has been infected and lives with the virus [2]. About 240 million people are chronically infected worldwide of whom 30% - 45% will develop cirrhosis and/or hepatocellular carcinoma (HCC) [3]. It is estimated that 780,000 people die annually from HBV infection; 650,000 are attributed to chronic HBV [2] [3]. In Sub-Saharan Africa and East Asia, at least 5% of the adult population is chronically infected [4]. The prevalence of HBV in Cameroon is about 11.2% with more than 8% chronically infected [5].

Liver fibrosis which results in the distortion of the hepatic architecture and is a precursor of cirrhosis is the most common complication of chronic Hepatitis B [6] [7]. Severe fibrosis and cirrhosis can also increase the incidence and mortality of HCC [8]. Early detection and staging of liver fibrosis are important to determine prognosis and prioritize treatment, thus reducing or preventing its progression to cirrhosis and/or hepatocellular carcinoma [9]. Liver biopsy has been traditionally considered the gold standard for assessing the degree of liver fibrosis [10]. However, its high cost, invasiveness, sampling errors, patient discomfort, and risk of complications leading to poor patient compliance, as well as a need for expert histological interpretation raise questions regarding its suitability for use in low-income countries [4] [11] [12].

Several non-invasive markers have been validated since 2001 [13]. Transient Elastography a non-invasive alternative to liver biopsy has shown excellent diagnostic accuracy for predicting significant fibrosis and cirrhosis [14] [15]. Unfortunately, the unavailability, procedures involved in the performance and the cost of Transient Elastography make its use limited in our setting.

The World Health Organization (WHO) has recommended the use of non-invasive tests based on simple and available laboratory methods, in the assess-

ment of liver fibrosis [16]. The recent WHO HBV guidelines recommend APRI as the preferred non-invasive test to assess liver fibrosis in resource-limited settings [16]. However, APRI and other Non-Invasive Tests like FIB4 and GPR have been evaluated with good diagnostic accuracy [9]. Unfortunately, data is still sparse in our setting. The main objective of this study was to evaluate liver fibrosis, using APRI, FIB4 and GPR scores in chronic Hepatitis B patients with Transient Elastography as the reference to choose an alternative to Transient Elastography. We hope to provide data that will assist in the early detection and staging of fibrosis, the timely intervention and follow-up of the patients thus reducing the burden of HBV infection.

2. Methodology

2.1. Study Design and Setting

We conducted a 5 year (2012-2017) cross-sectional study at the Douala General Hospital (DGH) and Polyclinique Marie O (PMO) Douala from 1st January to 31st March 2018. The DGH is a tertiary referral health facility serving the entire country and neighboring countries with a capacity of 320 beds. It has a treatment center for hepatitis and also serves as a teaching hospital. PMO is a private clinic in Douala where the management of hepatitis B is carried out as in the DGH. Its particularity is that, it has a Transient Elastography (fibroscan®) machine unlike the DGH and most hospitals in Douala.

2.2. Data Collection

Patients were recruited from among those followed up at the aforementioned clinic (PMO and DGH), in whom chronic hepatitis B had been confirmed by the persistence of HB surface antigen for more than six months. Their records from 2012-2017 were reviewed. The study period was from 1st January to 31st March 2018. Included in the study were chronic hepatitis B patients with results of transient elastography, AST, ALT and platelets counts in their files. Excluded were patients with other well-defined liver diseases (e.g. Alcoholic liver disease, non-alcoholic fatty liver disease, acute liver failure, Wilson's disease), HCC and other cancers, co-infections with HIV and HCV, decompensated liver cirrhosis, pregnant women, patients on immunosuppressive therapy and HBV treatment. A data collection form was used to enter 1) Sociodemographic data; age, gender, occupation, 2) Clinical characteristics; comorbidities, risk factors (factors considered susceptible to favor hepatitis B contamination through the parenteral route; these included previous surgery, dental care and scarification, as these practices in our context are often done with non-sterile instruments), clinical presentation at time of diagnosis, 3) Laboratory investigations; general laboratory investigations, liver enzymes and functions, viral markers, virologic activity and Transient elastography results. The clinical, laboratory and TE data were all collected at a single point in time upon presentation.

BMI was classified according to literature: undernourished as less than 18, normal between 18 and 25, overweight between 25 and 30, and obesity as greater

than 30.

These laboratory investigations were done in the fully functional laboratories of DGH and PMO with the same protocols. Baseline diagnosis of hepatitis B in the patients included in the study was based on the results of HBs testing: first with rapid DETERMINE testing, then confirmed with ELISA.

HBV DNA quantification was done using COBAST TaqMan HBV test with high pure extraction (Roche Diagnostics) on the patient's plasma as per the manufacturer's protocol. This is a real-time PCR assay based on a dual-labeled hybridization probe targeting the pre-core and core regions.

2.3. Assessment and Classification of Liver Fibrosis

Liver fibrosis was assessed by Fibrotest, Fibrometer and Transient Elastography. Fibrotest and Fibrometer are not done in Cameroon. Transient elastography is done in PMO by the same operator. This is done using a Fibroscan® device (FS 502, Echosens) on a fasting patient for at least 2 hours. The median of 10 - 15 readings was employed and results were expressed in kilopascals (kPa). These results were then correlated with the METAVIR scoring system with Significant Fibrosis (METAVIR score ≥ F2) = 7.2 kPa and Cirrhosis (METAVIR F4) = 11 kPa [17] [18] [19].

Transient elastography is a technique that helps determine two physical parameters within the liver: its stiffness expressed in kilopascals (kPa) and the ultrasonic attenuation expressed in decibels per meter (dB/m). The results obtained correspond to the median value of 10 validated measurements ranging from 2.5 to 75 kPa. A value less than 7 kPa was considered as non-significant fibrosis, where a value ranging between 7 and 15 kPa defined significant fibrosis, and a value greater or equal to 15 kPa defined cirrhosis. We considered the interquartile range (IQR) which assesses the variability of validated measures (which should be lower than 30% of the median). Both the M and the XL probes were used.

For the APRI score the following formula and cut-off values according to the literature are (AST [IU/L]/ULN of AST)/platelet count (10^9/L) × 100, with significant fibrosis considered as a score greater than or equal to 1.5 and cirrhosis considered as a score greater than or equal to 2 [20].

For the FIB4 score the following formula and cut-off values according to the literature are (age [years] × AST [IU/L])/(platelet count [10^9/L] × (ALT [IU/L]) 1/2), with significant fibrosis considered as a score greater than or equal to 1.45 and cirrhosis considered as a score greater than or equal to 3.25 [9].

For the GPR score the following formula and cut off values according to the literature are (GGT (IU/L)/ULN of GGT)/platelet count (10^9/L) × 100, with significant fibrosis considered as a score greater than or equal to 0.32 and cirrhosis considered as a score greater than or equal to 0.56 [14].

2.4. Statistical Analysis

Data was entered in CsPro (Census and Survey Processing System) and analyzed using SPSS Version 23.0 and Medical statistical software version 14. Microsoft

Excel 2016 was used to draw figures.

The scores of the non-invasive tests were calculated from their respective formulae and correlated with the METAVIR score (significant fibrosis METAVIR ≥ F2 and Cirrhosis F4). Frequencies and percentages were computed for categorical variables. Mean (or Median) and standard deviation (or Interquartile range) were computed for continuous variables. Cross tables were drawn to determine the test characteristics (sensitivity, specificity, PPV, NPV, positive and negative likelihood ratios) for APRI, FIB4 and GPR.

The correlations between Transient Elastography scores and non-invasive scores (APRI, FIB4, GPR) were analyzed using the Spearman test.

The sensitivity, specificity, and positive and negative predictive values were used to get the optimal cut-off values of the non-invasive markers for significant fibrosis and cirrhosis.

The diagnostic accuracy of the non-invasive markers was estimated by calculating the Area under the Receiver Operating Characteristic Curve (AUROC). Threshold values were chosen to optimize specificity for the diagnosis of significant fibrosis and cirrhosis.

Statistical significance was set at a P-value < 0.05.

3. Results

3.1. Study Population

A total of 1827 patient records with CHB were reviewed. After excluding patients with co-infections, signs of decompensated cirrhosis, HCC and incomplete files, 243 patient files were studied. Figure 1 summarizes the flow chart of the study population.

3.2. Baseline Characteristics of the Study Population

The median (IQR) age of the study population was 35 (29 - 42) years with the majority being 30 - 40 years old. There was a male predominance of 73.7% (179/243). The majority of the patients 53.3% were employed. 2.1% of the patients were diabetic, 32.5% were overweight and 12.7% were obese. The most recorded risk factor was scarification (36.6%). More than 60% of the patients had normal transaminases. Significant fibrosis and cirrhosis were detected in 88 (36.2%) and 32 (13.7%) of the patients respectively, as shown in Table 1 which summarizes the baseline characteristics of the patients.

3.3. Correlation of APRI, FIB4 and GPR with Transient Elastography

The medians of APRI, FIB4 and GPR were shown to increase with stages of fibrosis Figures 2(a)-(c). There was a positive, moderate and statistically significant correlation between APRI and FIB4 with TE. ($r = 0.592$; $P < 0.001$, $r = 0.503$; $P < 0.001$) respectively. There was a positive but weak correlation between GPR and TE ($r = 0.391$; $P < 0.001$), as shown in Figures 3(a)-(c).

Table 1. Baseline characteristics of the study population.

Characteristics	Value
Median (IQR) age	35 (29 - 42)
Age groups	**N (%)**
<30	69 (28.4)
[30 - 40[	99 (40.7)
[40 - 50[	46 (18.9)
[50 - 60[	22 (9.1)
[60 - 70[	4 (1.7)
≥70	3 (1.3)
Gender	**N (%)**
Male	179 (73.8)
Female	64 (26.2)
Diabetes N (%)	**5 (2.1)**
BMI	**N (%)**
Undernourished	3 (1.2)
Normal	86 (35.5)
Overweight	79 (32.5)
Obese	31 (12.7)
Occupation	**N (%)**
Unemployed	43 (17.7)
Employed	130 (53.5)
Self employed	70 (28.8)
Mode of payment	**N (%)**
Cash	173 (71.2)
Insurance	70 (28.8)
Risk factors	**N (%)**
Scarification	89 (36.6)
Dental care	43 (17.7)
Previous surgery	31 (12.8)
Laboratory Characteristics	**N (%)**
Platelets (g/l)	
<150	65 (26.7)
≥150	178 (73.3)
ALT (IU/l)	
<40	147 (60.5)
≥40	96 (39.5)

Continued

AST (IU/l)	
<40	152 (62.6)
≥40	91 (37.4)
GTT (IU/l)	
<61	127 (52.3)
≥61	104 (42.8)
HBeAg negative	225 (92.6)
HBV DNA (IU/l)	
<2000	151 (62.1)
2000 - 20,000	56 (23.1)
>20,000	36 (14.8)
QHBsAg	
<100	4 (1.6)
100 - 1000	27 (11.1)
>1000	159 (65.4)

AST: Aspartate aminotransterase, ALT: Alanine aminotransferase, HBV DNA: Hepatitis B virus deoxy ribonucleic acid. Results are presented as counts (percentage) or otherwise stated.

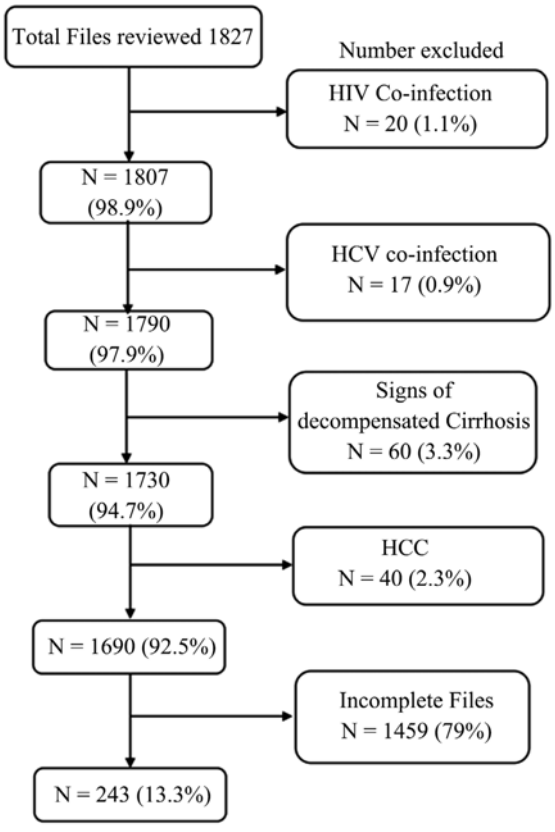
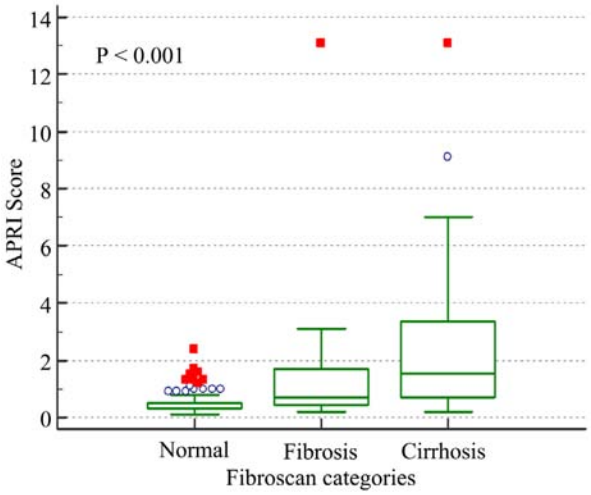
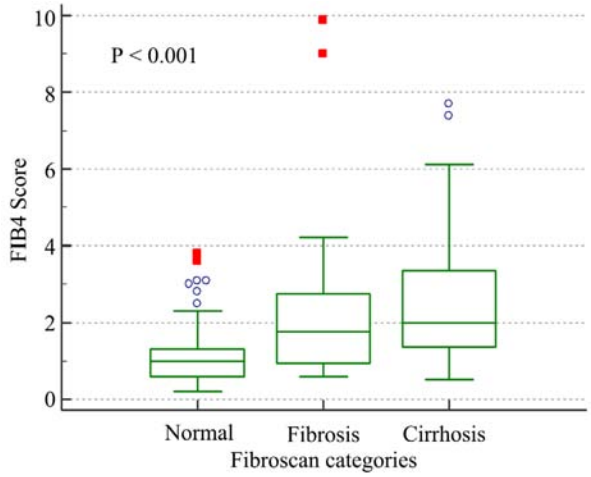


Figure 1. Flow chart of study population.



(a)



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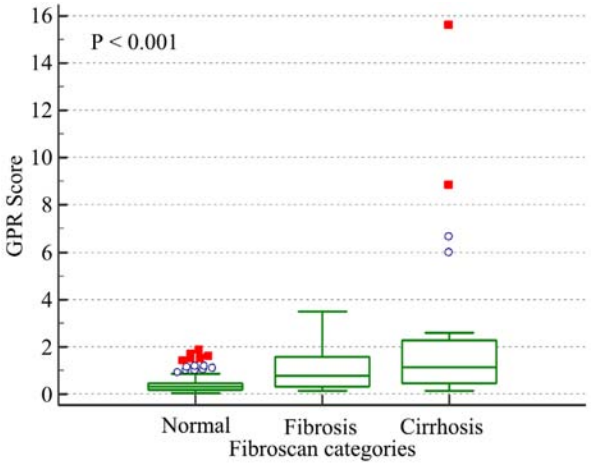
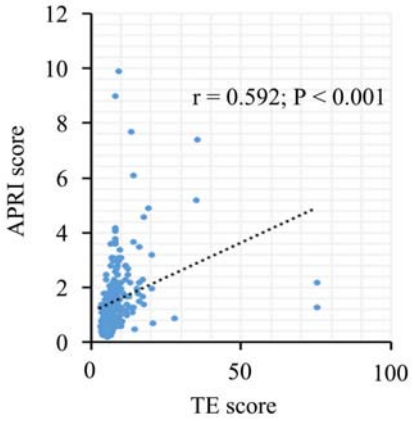
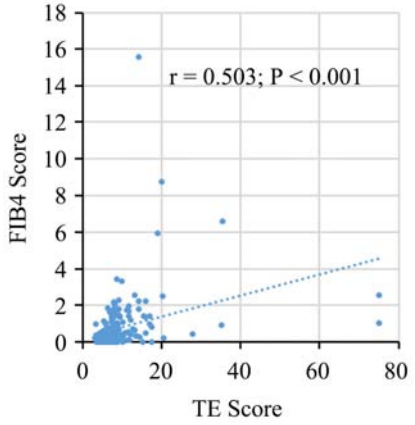


(c)

Figure 2. (a)-(c) Box plots of APRI, FIB4 and GPR respectively compared to the degree of fibrosis. The top and bottom of the whiskers represent the minimum and maximum values respectively. The top and bottom of the boxes represent the first and third quartiles and the horizontal lines across the boxes represent the median values.



(a)



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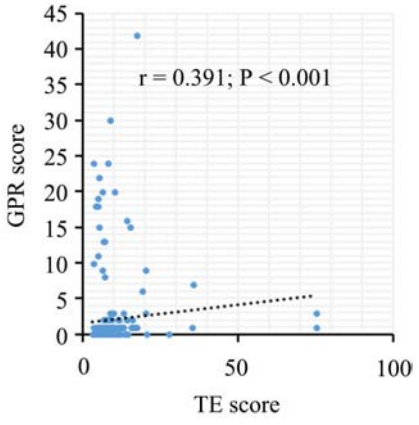


(c)

Figure 3. (a)-(c) Correlation between Transient Elastography and APRI, FIB4 and GPR respectively.

3.4. Classification of the Degree of Fibrosis by Non-Invasive Markers

Significant fibrosis detected by Transient Elastography, APRI, FIB4 and GPR was 36.2%, 14.8%, 35.4% and 67.1% respectively. Meanwhile, these methods detected cirrhosis, at 13.2%, 9.1%, 7%, and 33.8% respectively (Table 2).

Table 2. Classification of the degree of fibrosis by non-invasive markers.

Criteria	Score	Cut off	Count (%)
Significant Fibrosis (Metavir ≥ F2)	TE	≥7.2	88 (36.2)
	APRI	≥1.5	36 (14.8)
	FIB4	≥1.45	86 (35.4)
	GPR	≥0.32	155 (67.1)
Cirrhosis (MetavirF4)	TE	≥11	32 (13.2)
	APRI	≥2	22 (9.1)
	FIB4	≥3.25	17 (7)
	GPR	≥0.56	78 (33.8)

TE: Transient Elastography, APRI: Aspartate aminotransferase to platelet ratio index, FIB4: Fibrosis based on 4 factors, GPR: Gammaglutamyl transpeptidase to platelet ratio index.

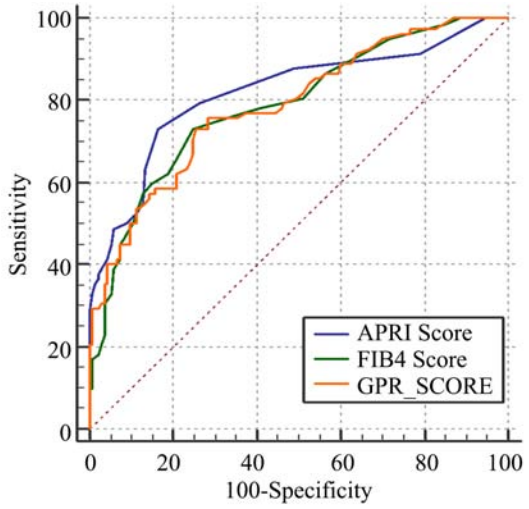
3.5. Diagnostic Performance of Non-Invasive Tests at Cut off Values Based on the Area under the Receiver Operating Characteristic Curve (AUROC)

Based on ROC analysis we obtained optimal cut-off values to diagnose significant fibrosis and cirrhosis. Figure 4(a), shows the AUROC of APRI (0.823), FIB4 (0.785) and GPR (0.787) for significant fibrosis and APRI (0.84), FIB4 (0.761) and GPR (0.780) for cirrhosis (Figure 4(b)).

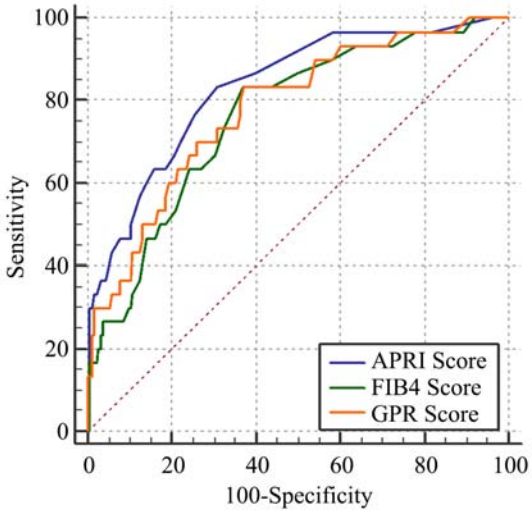
Table 3 presents the optimal cut-off values of APRI, FIB4 and GPR. For significant fibrosis we had as cut-offs 0.55, 1.25, 0.44 and for cirrhosis; 0.65, 1.35, 0.46 for APRI, FIB4 and GPR respectively.

4. Discussion

The assessment of the degree of liver fibrosis by using APRI, FIB4 and GPR and the progression of CHB is important in determining the treatment strategy and prognosis of CHB patients [21] [22]. We aimed at evaluating liver fibrosis using APRI, FIB4 and GPR scores with Transient Elastography as the reference in CHB patients, to choose a cheaper alternative to Transient Elastography. From our study, we observed that 92.6% of the study population was HBeAg negative. More than 60% had normal transaminases and 62.1% had HBV DNA < 2000 IU/l. Transient Elastography detected 36.2% of the study population with significant fibrosis and 13.2% with cirrhosis. APRI, FIB4 and GPR detected 14.8%, 35.4% and 67.1% with significant fibrosis and 9.1%, 7%, and 33.8% with cirrhosis respectively. APRI, FIB4, and GPR scores were shown to increase with fibrosis stages There was a positive and statistically significant correlation between Transient Elastography and all the non-invasive markers; APRI ($r = 0.592$; $P < 0.001$), FIB4 ($r = 0.503$; $P < 0.001$) and GPR ($r = 0.391$; $P < 0.001$). For the diagnostic performance of APRI, FIB4 and GPR for significant fibrosis and cirrhosis, APRI had the best diagnostic properties with corresponding cut-off values and



(a)



(b)

Figure 4. (a) and (b) AUROC of APRI, FIB4 and GPR to detect (a) significant fibrosis and (b) cirrhosis.

Table 3. Optimal cut off values and characteristics based on our ROC analysis.

Score	Criteria	Optimal Cut off	Sensitivity	specificity	PPV	NPV	AUC	95% CI
APRI	Significant Fibrosis	0.55	73.86	80	50.66	49.99	0.823	0.769 - 0.869
	Cirrhosis	0.65	78.12	74.41	31.64	95.73	0.84	0.788 - 0.884
FIB4	Significant Fibrosis	1.25	73.86	70.97	53.68	47.01	0.785	0.723 - 0.835
	Cirrhosis	1.35	84.37	60.66	60.82	39.58	0.761	0.702 - 0.813
GPR	Significant Fibrosis	0.44	73.17	74.44	52.24	48.40	0.787	0.726 - 0.84
	Cirrhosis	0.46	83.33	62.70	59.72	40.95	0.780	0.719 - 0.834

APRI: Aspartate aminotransferase to platelet ratio index, FIB4: Fibrosis based on 4 factors, GPR: Gammaglutamyl transpeptidase to platelet ratio index, PPV: positive predictive value, NPV: negative predictive value, AUC: Area under the curve, CI: Confidence interval.

AUROC of 0.55 (0.823 95% CI [0.769 - 0.869] P < 0.001) for significant fibrosis and 0.65 (0.84 95% CI [0.788 - 0.884], P < 0.005) for cirrhosis.

The best diagnostic properties of APRI in our studies could be compared with those in studies from Ethiopia [9], Brazil [23], and China [24] with high AUROC of APRI (0.79 - 0.86), which are slightly better than those from Europe, Australia and Asia [25] with the summary AUROC of APRI being 0.74 for significant fibrosis and 0.73 for cirrhosis. However, contrary to our findings, African studies from Burkina-Faso [26], Gambia and Senegal [14] have reported lower values between 0.50 - 0.66. This difference could be attributed to the fact that these studies employed strict exclusion criteria, excluding patients with excessive alcohol consumption, Hepatitis D virus infection who were otherwise included in our study.

Despite the highest AUROC of APRI in our study, the sensitivities were very low. Using the WHO cut-off values, APRI detected 14.8% and 9.1% of patients with significant fibrosis and cirrhosis. In clinical practice, this implies 85.2% of patients with significant fibrosis, who should commence treatment in order to avoid progressive liver disease will go unnoticed and 90.9% will be erroneously labeled as non-cirrhotic and not receive appropriate treatment and follow-up. This is consistent with the low sensitivities recorded in Ethiopia [5]; 10% for significant fibrosis and cirrhosis, Senegal and Gambia [13]; 0% for significant fibrosis in Senegal, and 9% in Gambia with 25% for cirrhosis. This raises questions as to whether the WHO thresholds will need to be modified in Africa given the fact that most of the populations in Africa are inactive carriers of Hepatitis B with normal transaminases.

With these low sensitivities, we sought to determine new cut-off values with better sensitivities and specificities of APRI, FIB4 and GPR in CHB patients. Based on our ROC analysis, the optimal cut-offs were; 0.55 and 0.65 for APRI, 1.25 and 1.35 for FIB4, 0.44 and 0.46 for GPR, for diagnosing significant fibrosis and cirrhosis respectively. These values were all lower than the proposed thresholds.

When the new cut-offs were used, APRI had a higher sensitivity (73.86% vs 14.8%) and relatively lower specificity (80% vs 98.1%) for significant fibrosis and a sensitivity of 78.12% vs 9.1% and specificity of 74.41% vs 95.73% for cirrhosis. FIB4 had a sensitivity of 73.86% vs 36.6% and a specificity of 70.97% vs 78.71% for significant Fibrosis and cirrhosis; a sensitivity of 84.37% vs 7% and specificity of 60.66 vs 95.73%. GPR provided 73.17% vs 67.1% sensitivity and 74.44% vs 47.37% specificity for significant fibrosis and 83.33% vs 33.8% sensitivity and 62.70% vs 69.19% specificity for cirrhosis.

From the above, it is worth noting that the sensitivities are better with our new cut-off values with relatively low specificities compared to the WHO thresholds. In clinical practice therefore, the cut-offs with high specificities (fewer false positive results) could be used to diagnose persons with significant fibrosis and cirrhosis and the cut-offs with high sensitivity (fewer false negative results)

could be used to rule out or screen the presence of significant fibrosis and cirrhosis.

This study had the following limitations: We used Transient elastography as the reference instead of liver biopsy which is the usual gold standard. It was a retrospective study susceptible to missing data. The study was carried out in one town in Cameroon which may not be representative of the entire population. Thus, a larger sample, the prospective and multicentered study will be necessary to validate the new cut-offs of APRI, FIB4 and GPR. That notwithstanding, this is one of the very few studies carried out in sub-Saharan Africa on the evaluation of non-invasive markers of liver fibrosis in CHB that has proposed cut-off values from the WHO traditional cut-off values.

5. Conclusion

APRI had the best diagnostic properties to detect liver fibrosis and cirrhosis in patients with CHB attending a hospital in Douala. This study supports the call of the WHO on the use of APRI in low-income settings based on its simplicity, availability in primary care, affordability and highest AUROC in our study. However, the WHO cut-offs might be higher. Our proposed cut-offs will need further validation for the use in Sub-Saharan Africa.

Acknowledgements

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Ethics Approval and Consent to Participate

We conducted this study in strict compliance with the fundamental principles of medical research:

- The principle of the interest and benefit of research
- The principle of research safety
- Confidentiality.

Informed Consent

Informed consent was obtained from all subjects and/or their legal guardian(s).

In this study all methods were carried out in accordance with relevant guidelines and regulations

Availability of Data and Materials

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Author Contribution

SAFBE, TWBN, ANN and CKS conceived the study. SAFBE, TWBN, ANN,

GGAG, GRDS, CKS, AM and DNN collected the data. SAFBE, TWBN, ANN and HNL analyzed the data and drafted the manuscript. SAFBE, TWBN, ANN, DNN, FAA, CT, and HNL proofread and corrected the manuscript. All authors agreed with the final manuscript to be submitted for publication.

The study was approved by the institutional review board of the Faculty of Health Sciences of the University of Buea-Cameroon 2018/140/UB/SG/IRB/FHS and the Douala General Hospital No 017AR/MISANTE/HGD/DM/01/18.

Conflicts of Interest

Not applicable.

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