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Delayed access to different diagnostic tests and its impact on accurate diagnosis of HBV-related liver diseases in Burkina Faso

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LIST OF ACRONYMS

AASLD: American Association for the Study of Liver Diseases
ALT: Alanine Aminotransferase
APASL: Asian Pacific Association for the Study of the Liver
APRI: Aspartate Aminotransferase to Platelet Ratio Index
AST: Aspartate Aminotransferase
AUROC: Area Under the Receiver Operating Characteristic curve
CHB: Chronic Hepatitis B
CI: Confidence Interval
DNA: Deoxyribonucleic Acid
EASL: European Association for the Study of the Liver
FIB-4: Fibrosis-4
GP: General practitioner
HBsAg: Hepatitis B surface antigen
HBeAg: Hepatitis B envelope antigen
HBV: Hepatitis B Virus
HCC: Hepatocellular Carcinoma
HCV: Hepatitis C Virus
HEPSANET: Hepatitis B in Africa Collaborative Network
HIV: Human Immunodeficiency Virus
NPV: Negative Predictive Value
OR: Odds Ratio
PPV: Positive Predictive Value
Ref: Reference
TREAT- B: Treatment eligibility in Africa for the hepatitis B virus
WHO: World Health Organization

ABSTRACT

Title: Delayed access to different diagnostic tests and its impact on accurate diagnosis of HBV-related liver diseases in Burkina Faso.

Background: Hepatitis B virus (HBV) diagnostic tests are essential for evaluating the risk of progression to fibrosis and the decision for treatment eligibility. Access, particularly to HBV DNA and FibroScan, is often limited and delayed in resource-limited settings. While there are efforts to implement less expensive markers, their accuracy varies across different settings, and there is a lack of evidence on the impact of the delayed access on their accuracy.

Objectives: This study aims to (1) describe delays in completing prescribed HBV diagnostic tests, (2) evaluate how these delays impact the diagnostic accuracy of alternative fibrosis markers, namely APRI and FIB-4, and (3) assess the agreement level and accuracy of the different antiviral treatment eligibility criteria.

Methods: In this retrospective cohort study, medical records of all consecutive persons living with HBV (n=630) followed at Bogodogo University Hospital Centre from 2014 to 2022 were extracted. Individuals were prescribed five tests: HBV DNA, FibroScan, HBeAg, transaminases, and platelet count, and test dates and results were recorded upon receipt of test outcomes. Individuals less than 18 years old and those with HIV or HCV coinfections were excluded from this analysis. In the diagnostic accuracy analysis, FibroScan was used as the reference test for fibrosis staging, while the 2017 European Association for the Study of the Liver (EASL) was used as the reference standard for treatment eligibility decision.

Results: A substantial proportion of individuals were able to complete the essential tests required for treatment eligibility: 95.1% for ALT, 87.5% for HBV DNA, and 85.9% for FibroScan. Within 3 months of the first visit at Bogodogo, 57.6% completed HBV DNA testing, while 63.0% had a FibroScan. A testing delay of 1-month to 1-year was associated with increased odds of fibrosis diagnostic misclassification compared to a 3 days interval: OR: 2.28 (95% CI: 1.06- 5.10) using APRI and OR: 3.09 (95% CI: 1.20- 8.69) using FIB-4. In the presence of delays, agreement with EASL 2017 was lower for the 2024 WHO treatment guidelines (Cohen's kappa=0.36) compared to alternative treatment eligibility criteria that are based on accessible tests, namely TREAT B (0.66) and HEPSANET (0.67).

Conclusion: These findings highlight the negative impact of diagnostic delays on the accuracy of non-invasive fibrosis markers, and reinforces the prioritization of FibroScan for fibrosis staging. In settings where HBV DNA testing is unavailable, treatment decisions can rely on guidelines developed for resource-limited settings, TREAT B and HEPSANET.

Key Words: fibrosis, diagnostic delay, APRI, FIB-4, antiviral treatment eligibility

I. Introduction

1.1. Background

Hepatitis B is a viral liver infection by the hepatitis B virus (HBV) that can spread through blood, or bodily fluids. Globally, 254 million were living with HBV, and there were 1.2 million new infections in 2022, of which 63% are accounted for in the African Region.[1] Additionally, viral hepatitis-related deaths increased from 1.1 million deaths in 2019 to 1.3 million in 2022, with 83% of them from the HBV virus.[1] Burkina Faso, a country in the West African region, has a high HBV prevalence, estimated at 9.41% in the general population.[2] This high burden has significant implications on individuals, healthcare systems as well as the country's public health infrastructure.

In fact, while acute HBV can resolve on its own, it can also lead to chronic hepatitis B (CHB), which in turn can lead to serious liver complications including liver fibrosis, cirrhosis, hepatocellular carcinoma (HCC) and even death. Liver fibrosis refers to the accumulation of scar tissue in the liver due to repeated injury, which in this context is caused by the HBV, though other factors like alcoholic liver disease or hepatitis C virus can also lead to fibrosis. This scar tissue replaces the healthy tissue, and obstructs the blood flow to and from the liver and eventually impacts the liver's vital functions. Liver fibrosis presents different stages, and if detected early enough and treated, liver fibrosis can be reversed.[3] However, the absence of symptoms in the early stages, combined with insufficient routine monitoring, often leads to the progression of the disease to advanced fibrosis and cirrhosis in some individuals.

It has been estimated that the incidence rates of cirrhosis among persons with CHB range from 1.6-9.7% depending on the presence of active infection.[4] Cirrhosis indicates severe liver damage and end stage of chronic liver disease, and there are two types of cirrhosis: compensated and decompensated cirrhosis. In compensated cirrhosis, the liver remains functional, and individuals may not exhibit any noticeable symptoms. On the other side, individuals with decompensated cirrhosis experience a significant decline of their liver function, accompanied by the onset of clinical symptoms. These symptoms include ascites, which is the accumulation of fluid in the abdomen; jaundice, characterized by yellowing of the skin, eyes, and mucous membranes; variceal bleeding, which involves bleeding from enlarged veins in the gastrointestinal tract; and hepatic encephalopathy, a condition marked by confusion or disorientation due to liver-related brain dysfunction. Individuals with decompensated cirrhosis have lower survival rates compared to those with compensated cirrhosis.[5] Additionally, long-term cirrhosis can lead to HCC, which is the most common type of liver cancer. An annual incidence of HCC ranging from 10% to 17% has been estimated

among persons with CHB with cirrhosis,[4] and HCC can also occur in persons with CHB without cirrhosis.[6]

Due to the risk of progression to fibrosis and cirrhosis for persons with CHB, regular testing and continuous monitoring are essential to ensure timely risk stratification and appropriate treatment. While there is no cure for CHB, a lifelong antiviral therapy can help to suppress viral replication and maintain liver functions, however this therapy is often life-long and costly, and presents potential risk including neuropathy, myopathy, and kidney toxicity.[7] Treatment decision is based on the combination of diagnostic tests indicating viral replication, liver inflammation and fibrosis. Unfortunately, CHB diagnosis and treatment remains a challenge globally with only 13.4% of individuals diagnosed, and 2.6% receiving antiviral therapy.[1] In the African region, access to diagnosis and treatment is extremely limited with only 4.2% diagnosis coverage, and among those diagnosed, 5.5 % received treatment.[1] With the WHO's goal to eliminate viral hepatitis by 2030, ensuring widespread access to timely diagnosis and effective treatment—particularly in low- and middle-income countries with a high HBV burden—is crucial for achieving this target.

1.1.1. Fibrosis Diagnosis and Staging

HBV diagnosis and fibrosis staging relies on a range of clinical and biochemical markers. For the scope of this study, only a selected number of tests relevant to this study will be described in detail, and approximate costs of each test in Burkina Faso have been documented in APPENDIX A.

1. HBsAg

HBsAg (Hepatitis B surface antigen) is a protein found on the surface of the HBV virus. As an early indicator of infection, HBsAg is vital for confirming the presence of the virus and tracking its progression over time.

The HBsAg Test is used to diagnose both acute or chronic HBV infections. A result that remains positive for more than 6 months indicates chronic infection, while a negative result coupled with the presence of antibodies indicates a resolved infection or immunity due to vaccination.

2. HBV DNA

HBV DNA represents the HBV genetic material, and indicates the viral load in a patient's blood. The HBV DNA quantification test is often performed using polymerase chain reaction (PCR) technology which is highly sensitive. A high viral load indicates active viral replication,

while a low or undetectable viral load suggests either an inactive infection or effective suppression of the virus.

The HBV DNA quantification test is important to evaluate the risk of transmission, and the effectiveness of the antiviral treatment. However, due to costs and limited laboratory infrastructure, the test is often inaccessible in resource-limited settings.

3. HBeAg

HBeAg (Hepatitis B envelope antigen) is a protein found in the blood if there is active replication of the HBV virus, and can inform the infection phase of the patient. HBeAg-positive individuals have an increased risk of transmission, and the loss of HBeAg, accompanied by the presence of anti-HBe antibodies, marks the shift from the immune-active phase to the inactive carrier state.[8]

HBeAg test can also be used as a surrogate marker of the HBV DNA, in settings where HBV DNA is not accessible, but HBeAg negative individuals can still have high HBV DNA.[9]

4. Transaminases

There are two types of transaminases, Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST), that can help assess liver inflammation. While ALT is primarily found in the liver, AST can also be found in the heart, kidneys, brain, and other major tissues. Elevated levels of ALT and AST suggest liver injury, though they may result from non-hepatic conditions. These blood enzyme tests are less costly including in resource-limited settings.

5. Platelet count

Platelets are cell fragments that form clots and prevent or stop bleeding. In the context of HBV, platelet count can drop significantly in the progression to liver fibrosis and cirrhosis. As it is for transaminases, it can be used to assess liver damage in resource-limited settings. However, a reduced platelet count is not specific to HBV and may also arise from other causes such as bone marrow disorders or the effects of certain medications. Therefore, when evaluating HBV progression, platelet levels should be interpreted in conjunction with other clinical and laboratory markers.

6. Liver biopsy

Liver biopsy is considered as the gold standard to determine the severity of liver inflammation and fibrosis, but it is an invasive procedure, particularly for individuals with advanced liver damage.[10]Additionally, due to its high costs, it is often unavailable in resource-limited settings.

The Meta-analysis of Histological Data in Viral Hepatitis (METAVIR) score was developed to standardize the interpretation of liver biopsy results in individuals with chronic viral hepatitis, both HBV and HCV. The METAVIR score is important to monitor HBV progression as well as determine eligibility for treatment. It consists of two key components: the activity score that measures the degree of inflammation and the fibrosis score that measure the degree of liver scarring. Table 1 shows the different fibrosis stages as defined by the fibrosis score.

Due to the liver biopsy's costs and its invasive nature, there are efforts to increase accurate staging of liver disease in individuals living with CHB in low-income regions by shifting to more affordable and non-invasive tests.

Table 1: METAVIR scoring system[11]

Description	Fibrosis Level (Stage)	Severity of Fibrosis
No fibrosis/no scarring	F0	
Portal tract fibrosis without septa formation/ Minimal scarring	F1	Mild to moderate fibrosis
Portal tract fibrosis without infrequent/rare septa formation (Scarring around vessels within the liver)	F2	Significant fibrosis
Numerous septa, but no cirrhosis (Bridging fibrosis)	F3	Advanced fibrosis
Cirrhosis/Advanced scarring	F4	

7. FibroScan

FibroScan is a non-invasive method that uses vibration controlled transient elastography to assess the stiffness of the liver. The vibration waves are detected and examined to assess their speed as they move through liver tissue. This speed directly reflects the liver's stiffness — the stiffer the tissue, the faster the waves travel. High liver stiffness indicates advanced fibrosis and cirrhosis.

Although FibroScan offers a less invasive and highly accurate alternative[12,13]to liver biopsy for staging liver fibrosis and determining treatment eligibility, its relatively high cost-ranging from 38 to 46 euros per scan on average in Burkina Faso[14] -can limit timely access for

individuals. Moreover, its accuracy may be reduced in individuals with both chronic hepatitis B and non-alcoholic fatty liver disease.[15]

8. APRI

The Aspartate Aminotransferase to Platelet Ratio Index (APRI) has been used as an indirect marker of significant fibrosis (>F2) and cirrhosis (>F4) among individuals with CHB, and relies on two widely available biomedical tests—AST levels and platelet count.[16]However, the cutoffs recommended for staging both significant fibrosis and cirrhosis have changed over time, reflecting results from ongoing validation studies across different patient populations and liver disease aetiologies.

The 2015 WHO APRI cutoffs for staging significant fibrosis and cirrhosis had low sensitivity, which led to the exclusion of some persons with cirrhosis from treatment, prompting their revision in 2024. Moreover, the evidence used to develop the 2015 guidelines included only one study from sub-Saharan Africa.[17] The 2024 WHO cut off values were informed by new evidence,[18] however the accuracy of these new values has yet to be validated.

9. FIB-4

Fibrosis-4 (FIB-4) is another validated, less expensive biomarker that relies on four parameters: age, AST, ALT, and platelet count. It is commonly used to assess advanced liver fibrosis (>F3). Similar to the APRI score, various cutoffs have been validated across different patient populations.

Although FIB-4 is not included in the 2024 WHO guidelines—since it targets advanced rather than significant fibrosis—a meta-analysis that contributed to those guidelines have recommended specific cutoffs for identifying advanced fibrosis[18], and these were the same in 2015 WHO guidelines.

1.1.2. Treatment eligibility

The above tests and markers play a key role in risk stratification to assess eligibility for antiviral treatment.[19] In addition to the WHO treatment guidelines,[20,21] there are other treatment guidelines for CHB developed by major hepatology associations that have served as key references in both clinical practice and research. The European Association for the Study of the Liver (EASL) guidelines[22] have been widely used as a reference standard in validation studies for new treatment criteria and were last updated in 2017, reflecting modifications to the earlier 2012 version.[23] Similarly, the American Association for the Study of Liver Diseases (AASLD) has published comprehensive clinical practice guidelines for HBV, with the most

recent update released in 2018.[24] The Asian Pacific Association for the Study of the Liver (APASL) last released its evidence-based guidelines for the management of CHB in 2015.[25] In 2019, the Ministry of Health of Burkina Faso developed its national treatment eligibility recommendations[26], informed by the European criteria.

These clinical practice guidelines are largely based on diagnostic tools such as liver biopsy, FibroScan, and HBV DNA quantification test that are often expensive and not accessible in resource-limited settings like Burkina Faso. In response to these challenges, recent studies have focused on developing and validating simplified treatment eligibility criteria specifically tailored to the resource-limited context. One such tool is the Treatment eligibility in Africa for the hepatitis B virus(TREAT-B) score, which was developed using data from The Gambia and relies solely on ALT levels and HBeAg status.[27] Another important contribution is the Hepatitis B in Africa Collaborative Network (HEPSANET) score, which was developed and validated using data from eight different African countries.[28] These tools aim to improve access to antiviral treatment by offering practical alternatives for identifying individuals eligible for treatment in resource-limited settings. Details on the specific tests and thresholds used for each criterion are provided in APPENDIX B.

However, these new simplified treatment criteria, as well as the clinical practice guidelines, do not consider the reality that most persons with CHB in low- and middle-income countries may be unable to afford all the required tests at once, as these are often paid out-of-pocket. The resulting delays in completing each test may compromise the accuracy of diagnosis and hinder the timely initiation of antiviral treatment for CHB.

1.2. Rationale

Timely access to HBV diagnostic tests remains a major challenge in resource-limited settings, despite their critical role in risk stratification and determining eligibility for antiviral treatment. After conducting a literature review, we found that while various treatment eligibility criteria have been developed for persons living with CHB, no study has addressed the challenging reality that most individuals in resource-limited settings do not have simultaneous access to the full range of diagnostic tests required to assess treatment eligibility.

Although tests used to calculate APRI and FIB-4 scores are non-invasive and less expensive, the diagnostic accuracy of the new WHO 2024 cutoffs for staging fibrosis has not been validated in sub-Saharan Africa. This study aims to evaluate the diagnostic accuracy of APRI and FIB-4 scores compared to FibroScan, the reference test, in Burkina Faso. Additionally, we explore whether these delays in completing these tests contribute to misclassification of fibrosis stage based on these accessible tests.

Furthermore, the 2024 WHO guidelines have expanded treatment eligibility criteria to include individuals with significant fibrosis while only those with cirrhosis were eligible for treatment under the 2015 WHO guidelines. These new guidelines have also lowered the viral load threshold from >20,000 IU/mL to >2,000 IU/mL, expanded treatment for all adults and adolescents (>12 years old), and included those with comorbidities such as diabetes, HIV or with family history of HCC or cirrhosis. In addition to the WHO guidelines, other treatment criteria present different criteria and threshold values. We aim as a secondary objective of this study to assess the level of agreement of these new WHO guidelines and other existing treatment criteria as well as their accuracy.

1.3. Main objectives

The general objective of the project is to study the impact of delayed access to diagnostic tests on accurate diagnosis of HBV-related liver diseases.

The specific objectives of the study were:

1. Describe delays in completing each diagnostic test.
2. Estimate the diagnostic accuracy of APRI and FIB-4's to stage fibrosis compared to FibroScan, the reference test.
3. Evaluate the impact of the time interval between APRI/FIB-4 tests and FibroScan on the discordance of their results in staging liver fibrosis.
4. Assess the agreement level and accuracy of the different treatment eligibility criteria compared to the reference standard, EASL 2017.

This study was conducted as part of my master's internship, a six-month placement in the Unit of Emerging Diseases Epidemiology, withing the Elimination of Viral Hepatitis Group, concluding at the end of July. As the primary data analyst on the project, I designed the statistical analysis plan and carried out necessary data cleaning and analysis under my professional advisor' supervision. Following the oral defence of the thesis, I will prepare the manuscript for submission to a peer-reviewed journal. In addition to this project, I contributed to a separate study within the hepatitis B group, serving as a reviewer for the screening and selection of articles for a systematic review on the prevalence of occult hepatitis B among children born from HBsAg positive mothers.

II. METHODS

2.1. Data and study design

The retrospective cohort study is based on a database of all consecutive individuals with CHB (n=630) followed at the hepato-gastro-enterology service of at the Bogodogo University Hospital Centre in Ouagadougou, Burkina Faso from 2014 to 2022.

As shown in Figure 1, the database includes both individuals who initially consulted general practitioners (GPs) outside Bogodogo University hospital and were subsequently referred to a hepato-gastroenterologist in Bogodogo for further testing and monitoring (Scenarios A to D), as well as those who had their first consultation directly at the university hospital (Scenarios E to G). During their initial visit at Bogodogo hospital, all individuals were prescribed the five tests required to assess eligibility for antiviral treatment: FibroScan, HBV viral load, transaminase, HBeAg and platelet count, unless any of these tests had been recently performed by a GP and the specialist deemed retesting unnecessary. For individuals unable to afford all tests simultaneously, they were encouraged to return with the results of any tests they were able to complete during follow-up. The tests results and their corresponding dates were documented in the individuals' medical records. This information was recorded to monitor disease progression and to determine treatment eligibility once all necessary tests had been completed. From 2014 to 2019, treatment eligibility at the hospital was assessed according to the EASL 2012 clinical practice guidelines. Since 2019, national treatment recommendations have been applied.

For the purpose of this study, the data was retrospectively extracted from physical medical records and entered into a spreadsheet. It includes data on the individuals' first hospital visit date, tests performed and corresponding dates, as well as personal information such as age, sex, family history of cirrhosis and HCC and presence of any clinical symptoms indicating decompensated cirrhosis. Individuals aged less than 18 years old and those with an HIV or HCV coinfection were excluded from this analysis.

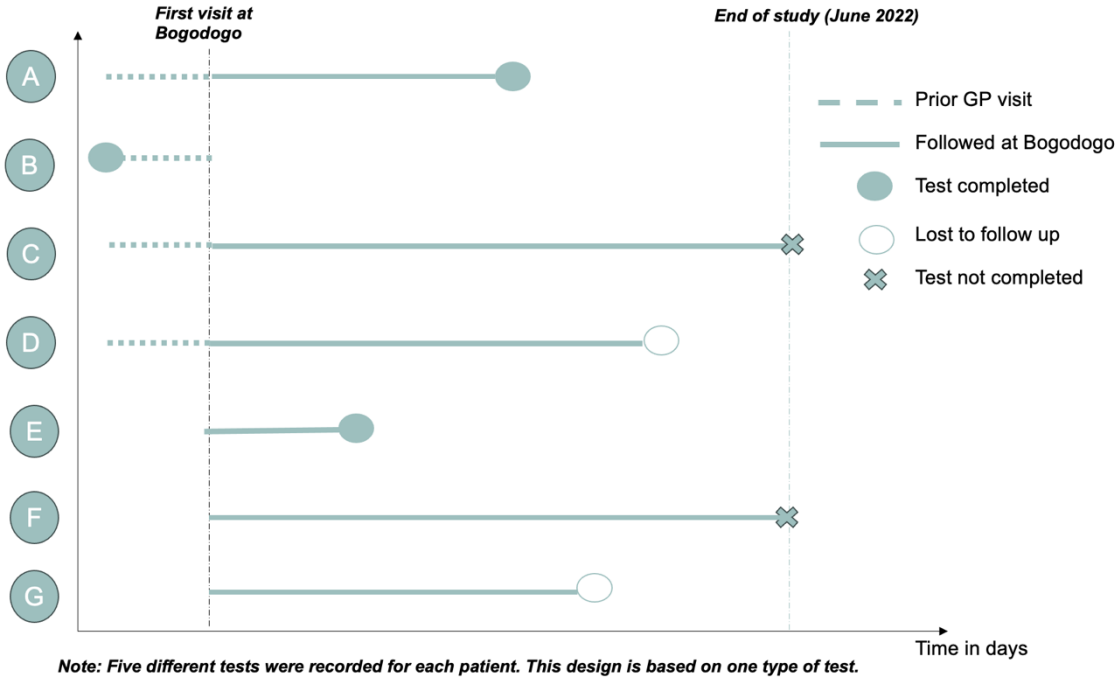

Figure 1: Timeline of participant enrolment and test completion scenarios.

2.2. Ethical considerations

This study is a secondary analysis of the data of all consecutive individuals followed at the hepato-gastroenterology service at Bogodogo University Hospital. Following informed consent, participants were invited to participate in the study at the time of consultation. The approval for access to the data was granted by the Health Research Ethics Committee in Burkina Faso (Reference number 2024-08-252). The dataset was anonymized to protect the privacy and confidentiality of individuals, and no personally identifiable information was accessible to the researchers.

2.3. Variables of interest

Table 2 provides a detailed summary of the eligibility assessment tests in the dataset. From the recorded test results, corresponding dates, and personal and clinical factors, secondary variables were constructed as described below.

Table 2: Summary table of HBV-related tests included in the dataset

Test	What it measures	Units
Transaminase (AST & ALT)	Enzymes released into the blood when liver cells are damaged, and elevated levels suggest liver	U/L (Units per Liter)

	inflammation or injury. AST is found in multiple tissues; ALT is more liver-specific.	
Platelet count	Number of platelets in the blood; low count is associated with liver cirrhosis.	x10^9/L (billion per liter)
HBV viral load	Amount of viral load in the blood; indicates how actively the virus is replicating.	IU/mL (International Units per mL)
FibroScan	Measures liver stiffness using transient elastography.	kPa (kilopascals)
HBeAg	Presence of this antigen indicates active HBV replication and high infectivity.	Positive or Negative result

2.3.1. APRI score

The APRI score was calculated based on two blood markers in the dataset: AST levels, platelet count, and the AST Upper Limit of Normal (ULN) (which is considered as 40 U/L conventionally) according to the formula below:

$$APRI = \frac{AST}{AST\ ULN * platelet\ count} * 100$$

A high APRI score indicates greater liver damage, indicating liver cirrhosis, and Table 3 indicates the cutoffs recommended in the WHO 2015 and WHO 2024 guidelines for staging liver fibrosis and cirrhosis.

Table 3: Recommended APRI cutoffs to stage fibrosis and cirrhosis in the WHO guidelines

	WHO 2015	WHO 2024
Significant fibrosis(>F2)	>1.5	>0.5
Cirrhosis (>F4)	>2	>1

2.3.2. FIB-4 score

The FIB-4 score was calculated based on age, AST & ALT levels as well as platelet count according to the formula below. This score is used to stage advanced fibrosis (>F3), and the low cutoff of 1.45 from the 2015 WHO guidelines was applied in this analysis, since they were not changed in the WHO 2024.

$$FIB-4 = \frac{Age\ (in\ years)\ *\ AST}{Platelet\ count \times \sqrt{ALT}}$$

2.3.3. FibroScan

The FibroScan results were recorded in the dataset as the median of 10 readings, and all of them were valid. No further calculation was computed. The FibroScan cutoffs for staging liver fibrosis and cirrhosis were derived from a recent meta-analysis, with values of 7.0 kPa and 12.5kPa for the diagnosis of significant fibrosis and cirrhosis respectively.[18]

2.3.4. Delay between APRI/FIB-4 and FibroScan

APRI and FIB-4 scores rely on two distinct tests: transaminase (ALT and AST obtained at the same time) and platelet count, while FibroScan is a single test. The delay between APRI/FIB-4 and FibroScan was defined as the sum of the time intervals between FibroScan and each of the APRI/FIB-4 components—transaminase and platelet count.

2.3.5. Treatment Eligibility based on different criteria

For each of the guidelines (EASL 2017, EASL 2012, WHO 2015, WHO 2024, AASLD 2018, APASL 2015, National recommendations, TREAT-B, HEPSANET), a variable was created indicating eligibility for treatment (1), non-eligibility (0) and inconclusive (NA) in the case of missing test results. This eligibility status was determined using published treatment criteria, with details of each guideline provided in APPENDIX B.

2.4. Statistical Analysis

Prior to analysis, data cleaning was conducted to address issues arising from manual entry of the dataset. This process included variable labelling and recoding, data formatting as well as data quality checks. In cases where test dates were incorrectly entered, corrections were made when accurate information was available; otherwise, those dates were treated as missing. For test results lacking corresponding dates, a conservative approach was applied by imputing the most recent date on which the patient underwent any other test. Complete case analysis was conducted for each objective, and detailed information on restriction for each objective can be found in APPENDIX C.

The following analyses were performed: i) description of the population ii) describing time to testing across different types of tests with Kaplan-Meier analysis iii) diagnostic accuracy iv) logistic regression analyses iv) Cohen's kappa agreement

All statistical analyses were performed using Rstudio 4.3.2.

2.4.1. **Descriptive Analyses**

Variables of interest including demographic characteristics such as age and gender, clinical information as well as test uptakes proportions, and tests completion time were summarized. Categorical variables were reported as counts and proportions, while medians and interquartile ranges (IQR) were reported for continuous variables.

Additionally, a summary table of the treatment eligibility status according to the various treatment criteria was presented.

2.4.2. **Kaplan-Meier survival analysis**

To describe delays in completing each diagnostic test, the probability of completion for each test over 3 years was estimated using Kaplan Meier Analysis. Since some individuals had undergone a number of tests during their visit with a GP outside of Bogodogo, the types and number of tests prescribed by the GP varied depending on the GP's practices and test availability. To minimize this variability in the sample, this analysis was restricted to individuals who had their first visit in Bogodogo as all of them were instructed to complete the five tests during their first visit (Scenarios E, F and G in Figure 1). Furthermore, for individuals who had previously visited a GP outside Bogodogo hospital, only the dates of their test results were available, while the dates of their initial GP visit were unknown.

The event of interest was defined as test completion. The five tests: transaminase, viral load, platelet count, FibroScan, and HbeAg were analysed separately. Participants who were lost to follow up within 3 years of follow up were censored, and their last known test date was used as their date of last known status. The packages survival and survminer in R were used for this analysis.

2.4.3. **Diagnostic accuracy**

To compare the APRI and FIB-4 scores—our index tests—with FibroScan as the reference test, we assessed the diagnostic accuracy of APRI for staging significant fibrosis (>F2) and cirrhosis (>F4), and of FIB-4 for advanced fibrosis (>F3). This analysis was restricted to those with tests results as shown in APPENDIX C. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) as well as their 95% confidence intervals (CI) were reported. The area under the receiver operating characteristic curve (AUROC) was also computed, and the Delong's method was used to compute its 95% CI. Additionally, the optimal cutoff for APRI and FIB-4 in this dataset was determined using Youden's index that

maximises the sum of the sensitivity and specificity.[29] The epiR package was used for diagnostic accuracy analysis, and pROC package was used to estimate the AUROC.

2.4.4. **Logistic regression analyses**

To evaluate the impact of the delay between APRI/FIB-4 tests and FibroScan on discrepancies in fibrosis staging results, a logistic regression analysis was conducted. The independent variable was the time interval between the tests, defined as the sum of the time intervals (in days) between FibroScan and each of the two blood tests required to calculate APRI and FIB-4: transaminase (AST and ALT) and platelet count. As the assumption of log-linearity was not satisfied, the time interval variable was categorized based on clinically relevant thresholds.

The outcome was defined as discordance in fibrosis staging between alternative markers, using the 2024 WHO cutoffs for APRI, and FibroScan. Discordance referred to differences in fibrosis stages using APRI (F0–1 vs. F2–3 vs F4) and using FIB-4 (F0–2 vs. F3–4) compared to FibroScan. A value of 0 was assigned for no discordance in staging decisions, and a value of 1 was given when the decisions on the fibrosis stage differed. The crude model was adjusted for potential confounders available in the dataset, including age, sex, and family history of hepatocellular carcinoma (HCC).

Crude model:

$log\ \frac{P}{1-P} = \beta_0 + \beta_1\ time\ interval$ where P is the probability of discordance between APRI/FIB-4 and FibroScan in staging fibrosis.

Adjusted model:

$log\ \frac{P}{1-P} = \beta_0 + \beta_1\ Time\ interval + \beta_2\ Age + \beta_3\ Sex +$

$\beta_4\ Family\ history\ of\ Cirrhosis$ where P is the probability of discordance between APRI/FIB-4 and FibroScan in staging fibrosis.

To further understand the impact of the time delay on diagnostic accuracy of accessible markers, we estimated the sensitivity, specificity and AUROC stratified by each time delay category. AUROC across time delay categories were compared using Delong's test. This additional analysis focused significant and advanced fibrosis given that the number of individuals with cirrhosis in the sample was limited.

2.4.5. **Cohen's kappa agreement**

To assess the agreement level among different treatment criteria, Cohen's kappa (κ) was used through pairwise comparisons. Study participants with missing decision on treatment eligibility for any of the guidelines (WHO 2015, WHO 2024, EASL 2012, EASL 2017, APASL 2015, AASLD 2018, National recommendations, HEPSANET, TREATB) were excluded from this analysis (APPENDIX C).

Cohen's Kappa[30] was used to quantify the level of agreement between two treatment guidelines, while accounting for the proportion of agreement that could occur by chance. Cohen's Kappa is obtained through this formula:

$$\kappa = \frac{P_o - P_e}{1 - P_e}$$

where P_o is the observed agreement between treatment criteria and P_e the hypothetical probability of agreement by chance.

A Cohen's kappa value above 0.8 indicates almost perfect agreement; 0.6-0.8, substantial agreement; 0.4-0.6, moderate agreement; 0.2-0.4, fair agreement, and 0-0.2, slight agreement. The package irr in R was used for this analysis.

Following the agreement analysis, diagnostic accuracy of treatment criteria of interest, namely WHO 2015, WHO2024, HEPSANET and TREAT-B was carried out to compare to the reference standard, EASL 2017. EASL 2017 was used as a reference standard as it is based on all three aspects necessary to determine treatment eligibility: viral replication (HBV DNA), liver inflammation (ALT), and liver fibrosis (FibroScan). Additionally, this guideline has served as a reference guideline in a recent similar study in sub-Saharan Africa.[28] In this analysis, sensitivity, specificity, PPV, NPV as well as AUROC were estimated, and Delong's test was used for AUROC comparison.

III. Results

3.1. Descriptive analyses

As shown in Figure 2, a total of 609 individuals were included in the analysis after excluding persons with an HIV or HCV coinfection (n=10) and those aged less than 18 (n=11). The median age was 34 years and male individuals comprised 57% of the sample as presented in Table 4. In terms of clinical characteristics, 3.9% reported a family history of cirrhosis or HCC, while only 1.1% exhibited symptoms of decompensated cirrhosis such as jaundice, ascites, or variceal bleeding. 69.0% of the individuals were initially seen by a GP before their referral, and

had undergone at least one of the eligibility assessment tests before presenting to Bogodogo University Hospital. At the end of the follow up period in 2022, the transaminase test was the most frequently performed (95.1%), followed by the HBeAg test (89.0%), HBV viral load (87.8%), FibroScan (85.9%), and finally, platelet count (64.7%). Both the median AST and ALT levels were below the upper limit of normal (ULN) levels. 67 individuals (18%) were HbeAg positive, and the median APRI and FIB-4 scores were 0.31(IQR: 0.21-0.48), and 0.84 (IQR: 0.62-1.35) respectively. At the end of follow up, over half of the individuals (54%) completed all five recommended tests, with a median time interval of 400 days (IQR: 34-746). Meanwhile, 50 individuals (8.2%) had two tests or less, including 24 individuals who did not complete any of the tests.

The Kaplan Meier analysis results indicating the test completion probability over 3 years of follow up of each recommended test for those with a first visit in Bogodogo University Hospital are presented in Figure 3 and Table 5. Within three months of follow-up, 86.3% had completed transaminase testing; 63.0% had undergone a FibroScan, and 57.6% of individuals had completed the HBV viral load test. By the end of 1 year of follow up, 92.5% had completed the transaminase test, and over three-quarters of the sample had undergone the HBeAg (77.2%), FibroScan (76.0%), and HBV viral load (76.1%), but only 51.1% had completed the platelet count test. As shown in Figure 3, test completion continued to increase between 1 and 3 years of follow-up, with an increase ranging from 4.8% to 22.2% for the different tests. There was no statistically significant difference in test completion within 3 months between those who had their first visit at Bogodogo and those who saw a GP first outside of Bogodogo (APPENDIX D).

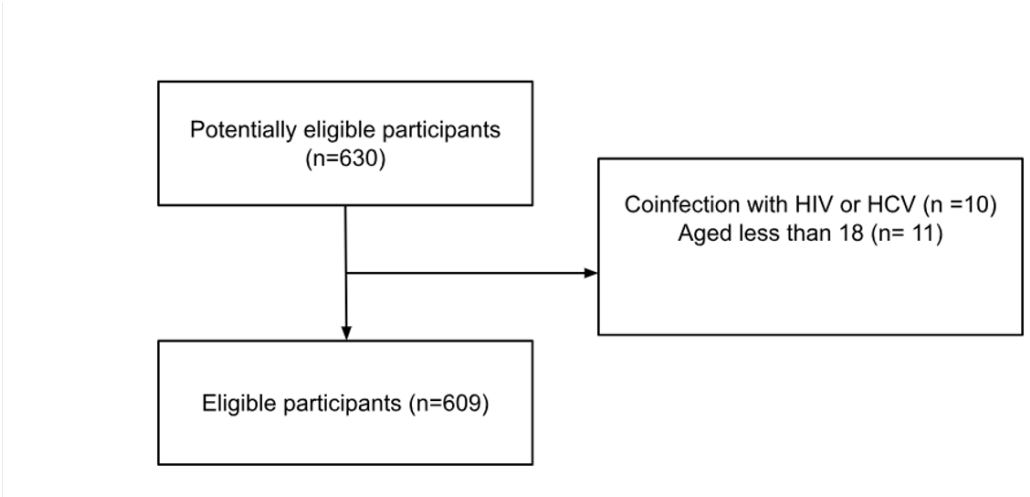

Figure 2: Flowchart of exclusion criteria for the study population

Table 4: Characteristics of overall study sample (n=609). Reported statistics are n (%) or median (IQR), as appropriate.

Variable	N = 609
Age	34 (28-44)
Sex	
Female	264 (43.3%)
Male	345 (56.7%)
Family History of cirrhosis or HCC	
Yes	24 (3.9%)
No	585(96.1%)
Decompensated cirrhosis	
Yes	7 (1.1%)
No	602(98.9%)
First visit location	
Bogodogo	162(26.6%)
Elsewhere (with GP before referral)	420(69.0%)
Missing	27(4.4%)
Transaminase test	
Yes	579 (95.1%)
No	30 (4.9%)
AST levels (IU/L)	24.6 (20.0-32.0)
Missing	43
ALT levels (IU/L)	21.0 (15.9-33.0)
Missing	46
Platelet count test	
Yes	388 (63.7%)
No	221 (36.3%)
Platelet count (*10^9/L)	221 (174.2-268.0)
Missing	223
FibroScan test	
Yes	517 (85.9%)
No	92 (15.1%)

FibroScan-TE results (kPa)	6.0 (4.9-7.6)
Missing	105
HBV DNA test	
Yes	535 (87.8%)
No	74 (12.2%)
Viral Load (HBV DNA copies)	480.0 (24.0-5,674)
Missing	75
HBeAg test	
Yes	542 (89.0%)
No	67 (11.0%)
HBeAg results	
Negative	454 (74.5%)
Positive	58 (9.5%)
Missing	97(15.9%)
Number of tests completed	
All 5 tests	330(54.2%)
4 tests	184(30.2%)
3 tests	45(7.4%)
2 tests	14(2.3%)
1 test	12(2.0%)
No test	24(3.9%)
Days to completion of all five tests	400(34.0-746.0)
APRI score	0.31 (0.21-0.48)
Missing	233
FIB-4 score	0.87 (0.62-1.35)
Missing	236

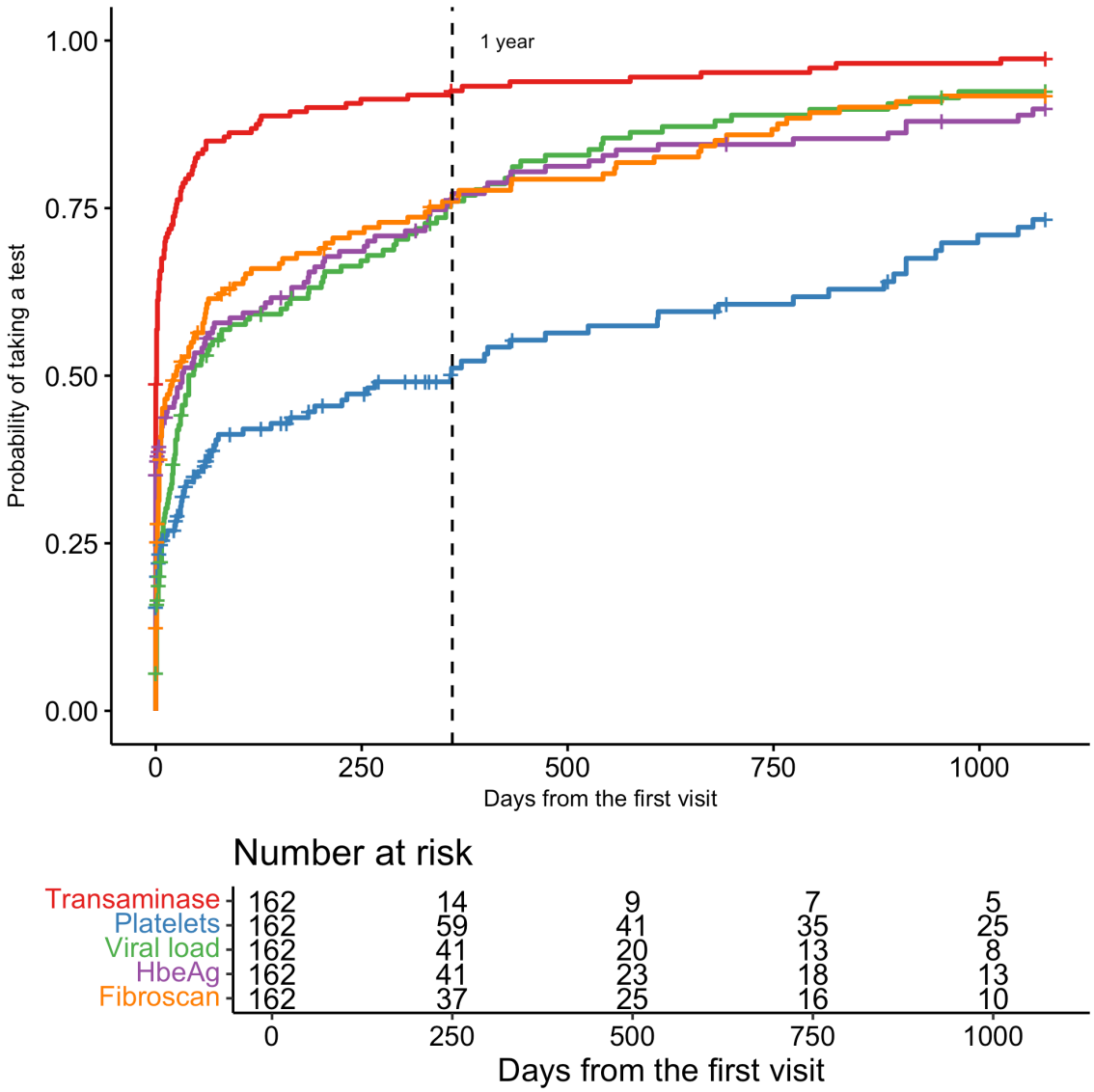

Figure 3: Time to testing over 3 years of follow up at Bogodogo University hospital (n=162)

Table 5: Test completion probability over three years of follow up from the Kaplan Meier Results (n=162)

	1 month	3 months	6 months	1 year	3 years
Transaminase	77.5%	86.3%	89.4%	92.5%	97.3%
HBeAg	49.0%	58.8%	64.0%	77.2%	89.8%
FibroScan	52.1%	63.0%	68.3%	76.0%	91.7%
Viral Load	44.1%	57.6%	61.5%	76.1%	92.4%
Platelets	30.5%	41.2%	43.7%	51.1%	73.3%

3.2. Fibrosis diagnosis

3.2.1. Diagnostic accuracy of APRI and the impact of delay

Data on APRI and FibroScan results were available for 342 individuals, i.e., 56.2% of the total sample (APPENDIX C). As shown in Table 6, for the WHO 2015 and the WHO 2024 cutoffs, APRI's sensitivity to stage significant fibrosis (>F2) compared to FibroScan, the reference test was low: 16.7% (19/114 [95%CI:10.3%-24.8%]) and 39.5% (45/114[95%CI:30.4%-49.1%]) respectively, while its specificity was high 99.6% (227/228 [95%CI: 97.6%-100%]) and 85.5% (195/228 [95%CI: 80.3%-89.8%]) respectively. The AUROC for staging significant fibrosis with APRI score was 67.3% [95%CI: 61.1%-73.5%], indicating limited ability to stage significant fibrosis. The optimal cutoff of 0.531 determined using Youden's index also resulted in a low sensitivity of 39.5% and high specificity of 88.6% (Figure 4).

The sensitivity of APRI cutoffs for diagnosing cirrhosis was 27.6% (8/29; [95% CI: 12.7%–47.2%]) using the WHO 2015 cutoff and 62.1% (18/29; [95% CI: 42.3%–79.3%]) using the WHO 2024 cutoff. Specificity was 99.7% (312/313; [95% CI: 97.6%–100%]) and 97.1% (304/313; [95% CI: 80.3%–89.8%]) for the 2015 and 2024 cutoffs respectively. The AUROC for detecting cirrhosis with APRI was 86.0% [95% CI: 77.6%–94.4%], indicating a good ability to diagnose cirrhosis.

A time interval of 1 month to 1 year between APRI and FibroScan was significantly associated with 134% (OR 2.34 [95% CI: 1.10-5.17]) higher odds of discordance in fibrosis staging compared to a 3-day interval, and 128% (OR 2.28 [95% CI: 1.06-5.10]) higher odds in the adjusted model (Table 7). As shown in Table 8, a time interval of 1 month to 1 year, compared to a time interval of less than 3 days, led to lower sensitivity (33.3% vs 61.1%) and specificity (80.9% vs 90.3%) in staging significant fibrosis with APRI, as well as significantly lower AUROC (p=0.022).

Table 6: Diagnostic accuracy of APRI score compared to FibroScan to stage significant fibrosis and cirrhosis (n=342)

	SIGNIFICANT FIBROSIS (F2)		CIRRHOSIS (F4)	
Performance measure	WHO 2015 (APRI>1.5)	WHO 2024 (APRI>0.5)	WHO 2015 (APRI>2)	WHO 2024 (APRI>1)
Sensitivity	0.167 [0.103-0.248]	0.395 [0.304-0.491]	0.276 [0.127-0.472]	0.621 [0.423-0.793]

Specificity	0.996 [0.976-1]	0.855 [0.803-0.898]	0.997 [0.982-1]	0.971 [0.946-0.987]
PPV	0.95 [0.751-0.999]	0.577 [0.460-0.688]	0.889 [0.518-0.997]	0.667 [0.46-0.835]
NPV	0.705 [0.652-0.754]	0.739 [0.681-0.791]	0.937 [0.905-0.961]	0.965 [0.938-0.982]
AUROC	0.673 [0.611-0.735]		0.860 [0.776,0.944]	

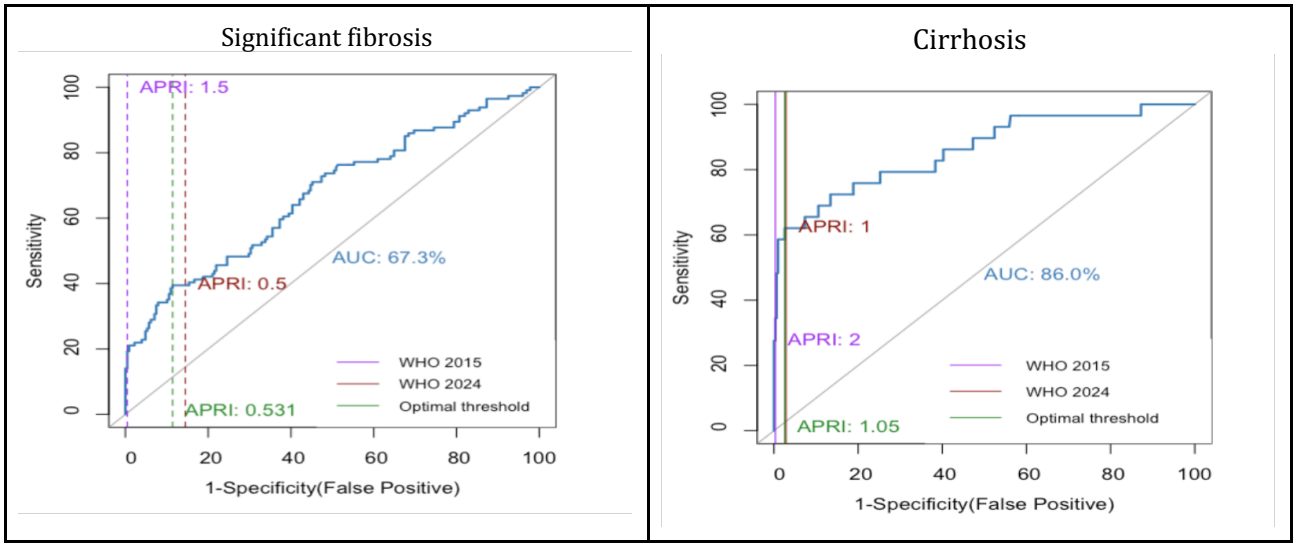

Figure 4: AUROC curve for APRI score to detect significant fibrosis and cirrhosis showing accuracy associated with different cutoffs (WHO 2015, WHO 2024 and Youden Index optimal cutoff)

Table 7: Impact of delay between APRI and FibroScan on the discordance of their significant fibrosis staging results

	Discordance/ Total	%	Crude model OR (95% CI)	p-value	Adjusted model** OR (95% CI)	p-value
Within 3 days	13/49	20.4%	1.00	0.037*	1.00	0.003*
3 days-1month	13/51	23.5 %	0.95 [0.39-2.33]		0.94[0.38-2.36]	
1month-1 year	38/83	39.8%	2.34 [1.10-5.17]		2.28[1.06-5.10]	
>1 year	49/159	29.6%	1.23 [0.61-2.60]		1.25[0.61-2.66]	

**Likelihood ratio test: p-value<0.05* **Adjusted for age, sex and family history of HCC or cirrhosis

Table 8: Sensitivity and specificity of APRI>0.5 to predict significant fibrosis (>F2)

	Significant fibrosis (>F2)			
	Sensitivity	*Specificity*	*AUROC*	*p-value**
All (n=342)	0.395 [0.304- 0.491]	0.855 [0.803- 0.898]	0.673 [0.611-0.735]	
Within 3 days *(n=49)*	*0.611 [0.357-0.827]*	*0.903 [0.742- 0.980]*	0.757 [0.630-0.885]	Ref
3 days-1month *(n=51)*	*0.455 [0.167- 0.766]*	*0.850 [0.702- 0.943]*	0.652 [0.489-0.816]	0.325
1month-1 year *(n=83)*	*0.333 [0.186-0.510]*	*0.809 [0.667-0.909]*	0.570 [0.474-0.668]	0.025
>1 year *(n=159)*	*0.347 [0.217- 0.496]*	*0.864 [0.785-0.922]*	0.605 [0.531-0.680]	0.047

***AUROC comparison with Delong's test**

3.2.2. **Diagnostic accuracy of FIB-4 and the impact of delay**

FIB-4 scores and FibroScan results were available for 339 individuals (APPENDIX C). FIB-4 low cutoff of 1.45 demonstrated a sensitivity of 59.5% (25/42; 95% CI: 43.3%–74.4%) and a specificity of 84.2% (250/297; 95% CI: 79.5%–88.1%) (Table 8). The AUROC was 80.1% (95% CI: 72.3%–88.0%), suggesting a modest ability to detect advanced fibrosis. The optimal cutoff value was 2.16, yielding a low sensitivity of 52.4% and a high specificity of 96.0% (Figure 5).

A time interval of 1 month to 1 year between FIB-4 tests and FibroScan was significantly associated with 209% (OR 3.09 [95% CI: 1.20-8.69]) higher odds of discordance compared to a time interval of 3 days in the adjusted model (Table 9). This time interval of 1 month to 1 year was also led to lower diagnostic accuracy of FIB-4 compared to a time interval of 3 days with a sensitivity of 38.5% vs 75.0% and a specificity of 75.4% vs 86.1% as shown in table. The AUROC was also significantly lower (p=0.024).

Table 9: Diagnosis accuracy of FIB-4 score compared to FibroScan to stage advanced fibrosis(n=339)

	ADVANCED Fibrosis (F3)
Performance measure	WHO 2015 & WHO 2024 (FIB-4>1.45)
Sensitivity	0.595 [0.433-0.744]
Specificity	0.842 [0.795-0.881]
PPV	0.347 [0.239-0.469]
NPV	0.900 [0.86-0.932]
AUROC	0.801[0.723-0.880]

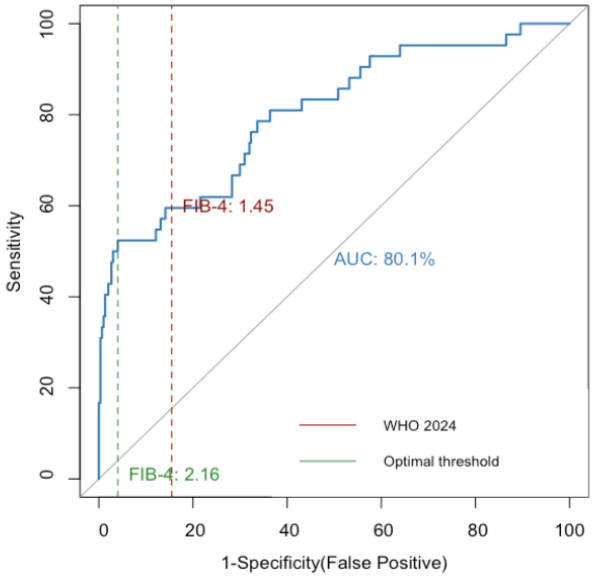

Figure 5: AUROC curve for FIB-4 score to detect advanced fibrosis showing accuracy associated with different cutoffs (WHO 2024 and Youden Index optimal cutoff)

Table 10: Impact of delay between FIB-4 and and FibroScan on the discordance of their advanced fibrosis staging results

			Crude model	p-value	Adjusted model**	p-value
	Discordance/ Total	%	OR [95% CI]		OR [95% CI]	
Within 3 days	8/48	16.7%	1.00	0.009*	1.00	<0.001*
3 days-1month	4/50	8.0 %	0.43 [0.11-1.49]		0.58 [0.14-2.13]	
1month-1 year	25/82	30.5%	2.19 [0.93-5.65]		3.09 [1.20-8.69]	
>1 year	27/159	17.0%	1.02 [0.45-2.57]		1.44 [0.59-3.89]	

**Likelihood ratio test: p-value<0.05* **Adjusted for age, sex and family history of HCC or cirrhosis

Table 11: Sensitivity and specificity of FIB-4>1.45 to predict advanced fibrosis

	Advanced fibrosis (>F3)			
	Sensitivity	*Specificity*	*AUROC*	*p-value**
All (n=339)	*0.595 [0.433-0.744]*	0.842 [0.795-0.881]	0.673 [0.611-0.735]	
Within 3 days *(n=48)*	*0.750 [0.428-0.945]*	*0.861 [0.705-0.953]*	0.806 [0.665-0.946]	Ref
3 days-1month *(n=50)*	*1.000 [0.398-1.000]*	*0.913 [0.792-0.976]*	0.957 [0.915-0.998]	0.047
1month-1 year *(n=82)*	*0.385 [0.139- 0.684]*	*0.754 [0.635- 0.849]*	0.569 [0.422-0.716]	0.024
>1 year *(n=159)*	*0.538 [0.251-0.808]*	*0.856 [0.789- 0.909]*	0.697 [0.553-0.841]	0.2932

***AUROC comparison with with Delong's test for independent samples**

3.3. Treatment eligibility

Based on the 2017 EASL treatment guidelines, 12.8% of individuals qualified for antiviral treatment; 75.9% did not meet the criteria, and eligibility could not be determined for 11.3% due to missing diagnostic tests, as shown in Table 12. The 2012 EASL guidelines only indicates a 1.8% increase in the proportion of eligible individuals. The 2024 WHO guidelines led to fewer inconclusive cases (4.4%), but a substantially higher proportion of individuals (42%) were eligible for treatment. In comparison, the WHO 2015 guidelines identified only 10.0% of individuals for treatment eligibility, which is closer to the 2017 EASL guidelines, the reference standard.

Among individuals with a treatment decision across all guidelines (n=320), one-on-one Cohen's kappa analysis (Table 13) shows that the WHO 2015, TREAT-B, and HEPSANET criteria had the highest levels of agreement with the reference guideline, EASL 2017, with kappa values of 0.60, 0.66, and 0.67, respectively—indicating stronger agreement in determining treatment eligibility. In contrast, the 2024 WHO guidelines showed the lowest agreement with EASL 2017, with a kappa value of 0.36.

Further analysis of diagnostic accuracy showed that TREAT-B and HEPSANET demonstrate higher diagnostic accuracy. TREAT-B achieved a sensitivity of 91.2% (62/68 [95% CI: 81.8%–96.7%]) and a specificity of 85.3% (215/252[95% CI: 80.3%–89.4%]), while HEPSANET showed a sensitivity of 80.9% (55/68[95% CI: 69.5%–89.4%]) and a specificity of 90.5% (228/252[95% CI: 86.2%–93.8%]) (Table 14). In contrast, the WHO 2015 guideline exhibited lower sensitivity at 52.9% (36/68[95% CI: 40.4%–65.2%]) but high specificity at 98.0% (247/252[95% CI: 95.4%–99.4%]). On the other hand, the WHO 2024 guideline had high sensitivity at 95.6% (65/68[95% CI: 87.6%–99.1%]), but low specificity at 59.5% (150/252[95% CI: 53.2%–65.6%]).

Table 12: Eligibility according to each treatment criteria (n=609)

Guidelines	Eligible	Ineligible	Inconclusive
EASL 2017	78 (12.8%)	462 (75.9%)	69 (11.3%)
EASL 2012	89 (14.6%)	447 (73.4%)	73 (12%)
WHO 2024	256 (42%)	326 (53.5%)	27 (4.4%)
WHO 2015	61 (10.0%)	508 (83.4%)	40 (6.6%)
APASL 2015	71 (11.7%)	487 (80.0%)	51 (8.3%)
AASLD 2018	99 (16.3%)	415 (68.1%)	95 (15.6%)
National Recommendations 2019	195 (32.0%)	298(48.9%)	116(19.1%)
TREAT-B	140 (23%)	381 (62.6%)	88 (14.4%)
HEPSANET	99 (16.3%)	290 (47.6%)	220 (36.1%)

Table 13: Cohen's Kappa Agreement Between Treatment Eligibility Criteria (n=320)

	EASL 2017	EASL 2012	WHO 2024	WHO 2015	APASL 2015	AASLD 2018	National 2019	TREAT-B	HEPSANET
EASL 2017	**1**	**0.58**	**0.36**	**0.6**	**0.56**	**0.57**	**0.53**	**0.66**	**0.67**
EASL 2012	0.58	1	0.4	0.55	0.75	0.96	0.53	0.36	0.35

WHO 2024	0.36	0.4	1	0.24	0.31	0.4	0.67	0.41	0.4
WHO 2015	0.6	0.55	0.24	1	0.58	0.55	0.29	0.39	0.42
APASL 2015	0.56	0.75	0.31	0.58	1	0.77	0.41	0.43	0.47
AASLD 2018	0.57	0.96	0.4	0.55	0.77	1	0.51	0.4	0.39
National 2019	0.53	0.51	0.65	0.3	0.41	0.49	1	0.38	0.35
TREAT-B	0.66	0.36	0.41	0.39	0.43	0.4	0.38	1	0.77
HEPSANET	0.67	0.35	0.4	0.42	0.47	0.39	0.35	0.77	1

Table 14: Diagnostic accuracy of different treatment criteria score compared to EASL 2017 as the reference standard (n=320

Performance measure	TREAT-B	HEPSANET	WHO 2015	WHO 2024
Sensitivity	0.912 [0.818; 0.967]	0.809 [0.695; 0.894]	0.529 [0.404; 0.652]	0.956 [0.876; 0.991]
Specificity	0.853 [0.803; 0.894]	0.905 [0.862; 0.938]	0.98 [0.954; 0.994]	0.595 [0.532; 0.656]
PPV	0.626 [0.523; 0.721]	0.696 [0.582; 0.795]	0.878 [0.738; 0.959]	0.389 [0.315; 0.468]
NPV	0.973 [0.942; 0.99]	0.946 [0.91; 0.971]	0.885 [0.842; 0.92]	0.98 [0.944; 0.996]

AUROC	0.882 [0.842;0.923]	0.857 [0.806;0.907]	0.755 [0.694;0.815]	0.771 [0.736;0.815]
p-value	Ref	0.267*	Ref	0.540**

*p-value for AUROC comparison between TREAT-B and HEPSANET (DeLong's test)
** p-value for AUROC comparison between 2015 WHO and 2024 WHO (DeLong's test)

IV. DISCUSSION

This is the first study to our knowledge to examine delays in HBV diagnostic access and how these delays impact accurate diagnosis of liver fibrosis, highlighting the real-life diagnostic challenges in resource-limited settings. This study revealed important key findings that can inform care management and guide treatment eligibility decisions for patients living with CHB.

First, although HBV diagnostic costs are out-of-pocket expenses for patients in Bogodogo University hospital, a substantial proportion of individuals were able to complete the essential tests required for treatment eligibility: 95.1% for ALT, 87.5% for HBV DNA, and 85.9% for FibroScan. However, more than half of the population experience long delays of over a year in completing the prescribed tests, which delays treatment eligibility decision. Secondly, among those who had their initial visit in Bogodogo University Hospital, 57.6% of individuals were able to undergo the HBV DNA quantification test within the first 3 months, which is a costly yet essential test in determining treatment eligibility. Notably, relying on less expensive biomedical markers such as APRI and FIB-4 to stage fibrosis resulted in low sensitivity to stage fibrosis compared to FibroScan, the reference test, leading to missed fibrosis diagnoses despite the new WHO 2024 cutoffs. A delay of 1 month to 1 year in getting these tests significantly contributes to this diagnostic misclassification, leading to lower sensitivity and specificity compared to tests taken conducted within 3 days. Additionally, this study also indicates that the 2015 WHO guidelines lead to under-treatment, while the revised 2024 guidelines result in over-treatment. To improve treatment eligibility decision-making, criteria based on expensive tests such as HBV DNA and FibroScan can be replaced with more accessible alternatives: the TREAT-B and HEPSANET scores. These two criteria, specifically developed for use in resource-limited settings, demonstrated greater diagnostic accuracy in this study compared to EASL 2017.

The affordability of HBV DNA quantification test remains a significant challenge in HBV endemic areas with limited resources,[31] and there have been efforts to replace it with other affordable alternatives.[32] Our study demonstrates that individuals face significant delays in accessing other essential tests needed to monitor disease progression and inform treatment decisions, underscoring challenges in timely care delivery. FibroScan, for instance, has been shown to be a good proxy to stage liver fibrosis, but its accessibility in resource-limited settings remains a challenge due to its cost. A study on barriers to linkage to care for patients living with chronic HBV infection in Burkina Faso[33] also identified patient's ability to pay for testing as one of the main barriers.

While the use of non-invasive tests to stage fibrosis are recommended in the WHO treatment guidelines, [20,21] a meta-analysis focusing on studies in Africa has shown that the WHO 2015 cutoffs of APRI>1.5 for staging significant fibrosis and APRI>2 for staging cirrhosis are inappropriately high in Africa.[34] The low sensitivity of APRI cutoffs in the WHO 2015 is also consistent with findings from studies conducted in other African countries, including Ethiopia and Nigeria,[35,36] which led to a substantial proportion of cirrhotic individuals missing treatment. The meta-analysis that informed the WHO 2024 guidelines[18] proposed lower APRI cutoffs, aiming to improve the identification of individuals with significant fibrosis and cirrhosis; however, one limitation of the meta-analysis is that the accuracy of these revised cutoffs still needs to be thoroughly evaluated in sub-Saharan African populations as only 7 out of 211 studies from the region were included. Our study indicates that the revised APRI cutoff (APRI>0.5) for staging significant fibrosis have a low sensitivity of 39.5% due to both APRI's low diagnostic accuracy and the delay between APRI and FibroScan testing. Although sensitivity improves to 61.1% when tests are performed within 3 days, it remains lower than the 72.3% reported in the meta-analysis that informed the cutoffs.[18] Therefore, FibroScan should be prescribed as the first-line diagnostic test for fibrosis, and in settings where it is not an option, transaminase and platelet count, used to calculate the APRI and FIB-4 scores, should be completed within 3 days.

The WHO 2024 guidelines broadened treatment eligibility not only by lowering APRI cutoffs but also by including all individuals with significant liver fibrosis, in contrast to the 2015 guidelines which focused on those with cirrhosis. [20] Additionally, the 2024 guidelines reduced the HBV DNA viral load threshold required to initiate treatment, and expanded eligibility to all adults and adolescents.[21] While these new guidelines may reduce missed diagnoses with higher sensitivity, our study shows that it results in individuals being initiated on non-essential treatment in a context where access to diagnostic testing is already limited due to high costs. There is so far no evidence of clinical benefits of expanding antiviral treatment among people

who are ineligible for treatment.[37] Expanding treatment to ineligible groups also presents a risk that limited treatment availability may prevent high-risk groups from receiving necessary therapy.[34] Additionally, existing HBV antiviral treatments have also been associated with potential side effects[7], and patient retention in care might be challenging.[31] In contrast to the WHO guidelines, the TREAT-B score demonstrated a good balance between sensitivity and specificity and has been validated in different studies.[38,39] Similarly, the HEPSANET score has also shown promising performance,[40] though additional studies are needed to further validate its applicability across diverse settings.

Our findings indicate that while access to HBV DNA quantification and FibroScan—both essential for determining treatment eligibility—is often delayed, the majority of individuals eventually receive these tests, with 87.8% undergoing HBV DNA testing and 85.9% receiving FibroScan. Despite costs being a challenge for individuals, our study shows participants' commitment to obtain all the required tests even after 1 year after their first visit. Healthcare providers should emphasize the importance of completing all necessary tests and encourage individuals to obtain and bring results whenever possible, and emphasize the importance of FibroScan as a priority to stage fibrosis. In cases where access to HBV DNA quantification is not possible, the TREAT-B and HEPSANET scores can serve as accurate alternatives for assessing treatment eligibility, and these guidelines can inform test prioritization in resource-limited settings. At the same time, structural changes in the healthcare system are essential to improve accessibility of HBV testing by subsidizing diagnostic tests through universal healthcare coverage.

This study provides a valuable real-world scenario on HBV care by highlighting the delays in diagnostic access in resource-limited settings. Unlike most clinical recommendations that assume simultaneous availability of all necessary tests, our analysis captures the delays individuals face in obtaining diagnostic tests and how these delays contribute to the diagnostic misclassification of fibrosis using non-invasive and accessible markers. Additionally, this is the first study to evaluate the performance of the newly recommended WHO 2024 guidelines in an African setting.

One important limitation of this study is that given that this is a retrospective study, the analysis was based on routinely collected clinical data, and important contextual variables that may contribute to diagnostic delays—such as socioeconomic status, geographic location (e.g., rural vs. urban residence), or health system barriers—were not available. These unmeasured factors likely play a significant role in individuals' ability to access timely HBV related tests and should be considered in future research to better understand and address the structural barriers to timely diagnosis. Additionally, due to the nature of the study, reasons for loss to

follow up- such as patient death, seeking care elsewhere- were unknown. Secondly, we did not assess how delays in diagnostic testing may impact discordance in treatment eligibility decisions defined by the different criteria. The temporal gap between these tests could lead to inconsistencies in treatment decisions, however since the reference standard (EASL 2017) also relies on a combination of different tests (HBV DNA, ALT and FibroScan), and is similarly impacted by the delay, this analysis could not be captured in our study.

In summary, this study underscores the need to adapt HBV diagnostic tests and treatment eligibility criteria to the realities of resource-limited settings. By documenting the timing of diagnostic tests, it highlights how delays in accessing tests lead to diagnostic misclassification using non-invasive and accessible markers to stage fibrosis. Further studies on the impact of the delayed access to diagnostic tests can help to understand better its impact on patient care. Importantly, it also provides evidence supporting the use of context-appropriate alternatives, the TREAT-B and HEPSANET scores to determine treatment eligibility that use affordable tests. These findings emphasize the persistent need for revised diagnostic and treatment guidelines specific to the context of resource-limited countries.

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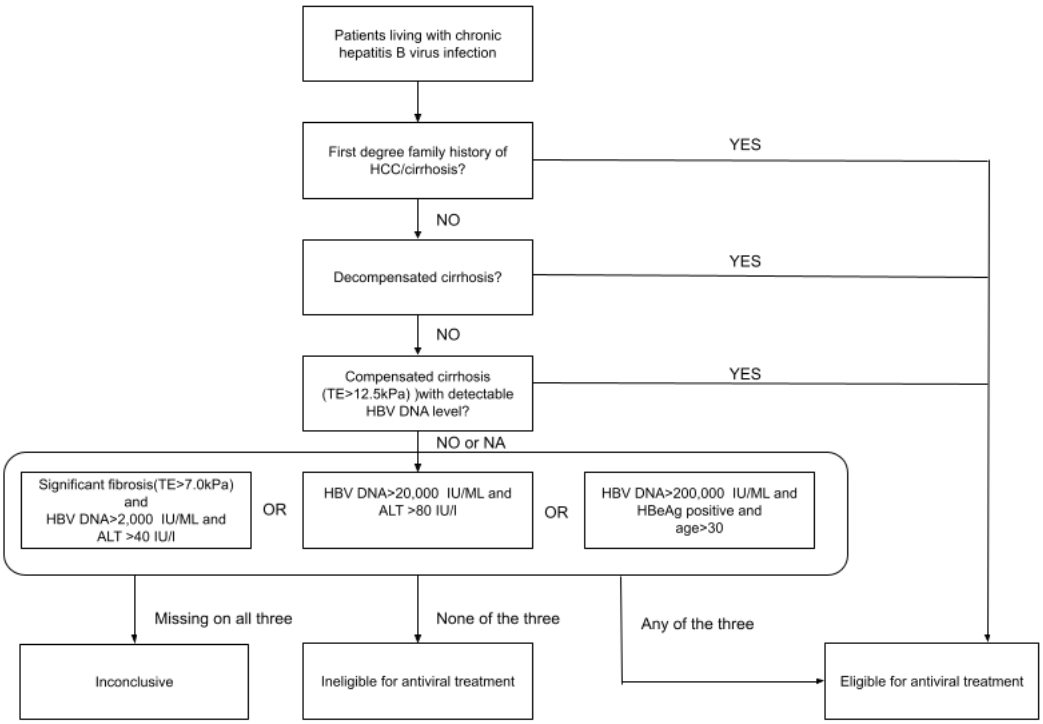
APPENDIX

APPENDIX A: HBV diagnostic tests approximate costs in Burkina Faso

Test types	Cost in F CFA	Approx. EUR
HBsAg	3,000	~4.5
HBeAg	3,000	~4.5
Transaminase	3,000	~4.5
Platelet count	2,500	~4
HBV DNA (PCR)	20,000 - 25,000	~30-40
FibroScan	30,000	~45

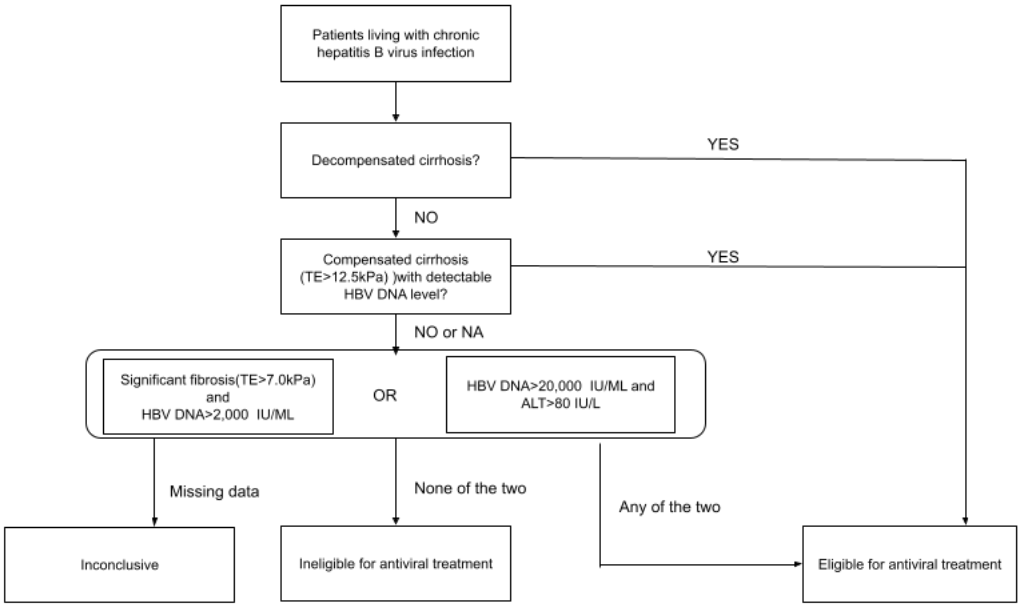
APPENDIX B: Treatment criteria algorithms

1. EASL 2017 (reference standard)



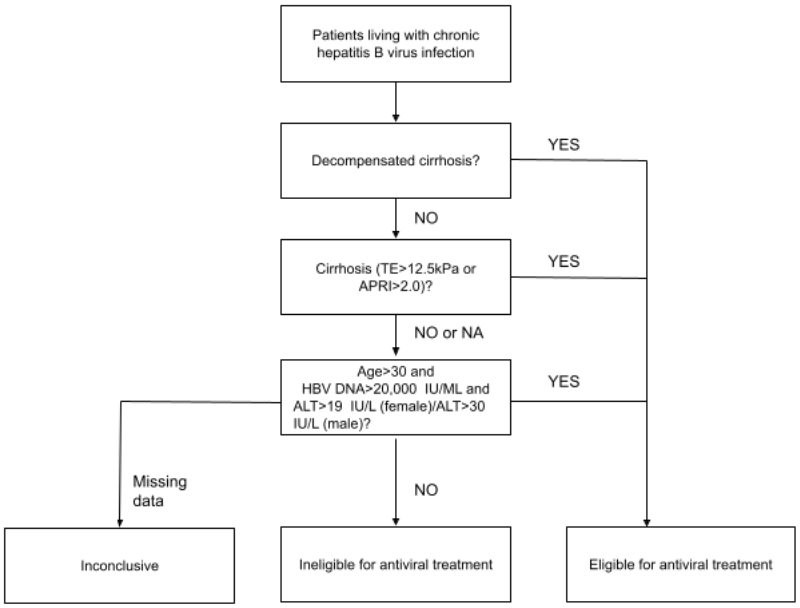
Note: TE thresholds used are from the recent metanalysis[18] since specific thresholds were not specified in the guidelines. Cut off for high HBV DNA was not set in the guidelines: and here it was arbitrarily set at HBV>200,000 IU/ml DNA following a previous study [28]

2. EASL 2012

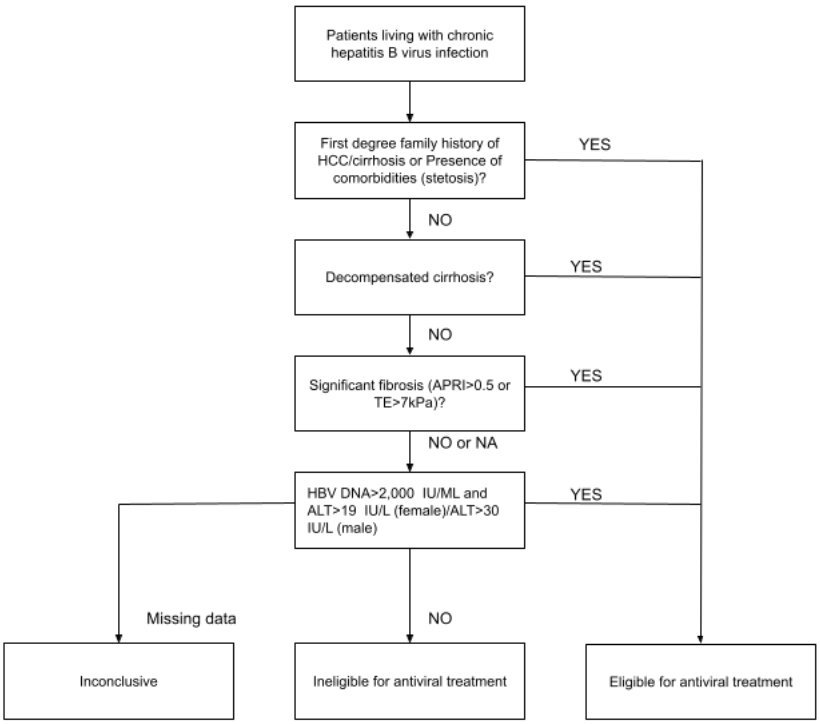


Note: TE thresholds used are from the recent metanalysis[18] since specific thresholds were not specified in the guidelines.

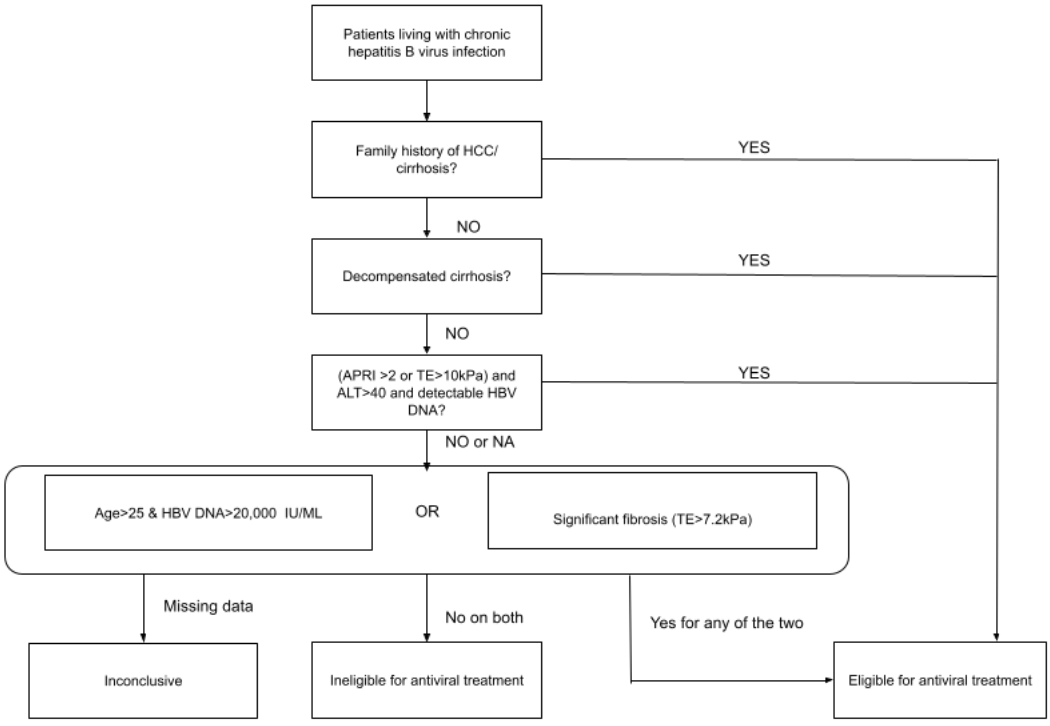
3. WHO 2015



4. WHO 2024

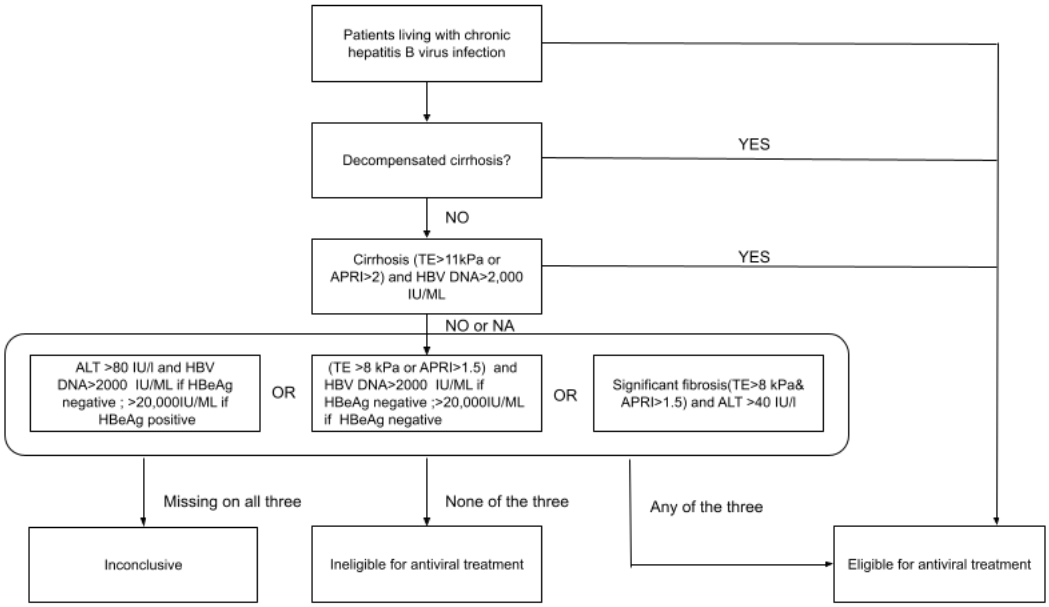


5. 2019 National recommendations

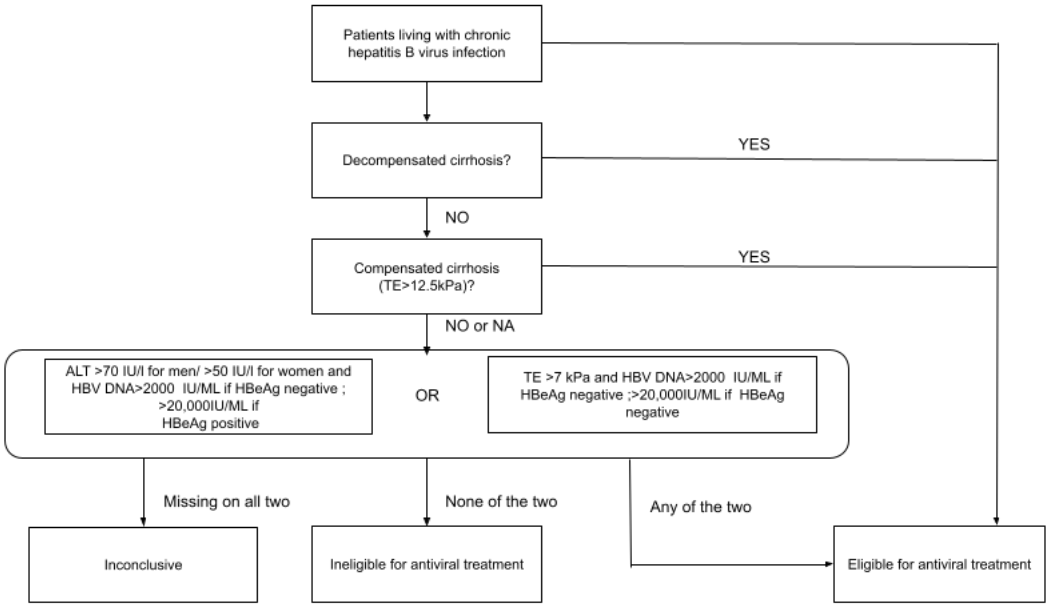


Note: TE thresholds used are from the recent metanalysis[18] since specific thresholds were not specified in the guidelines.

6. APASL 2015



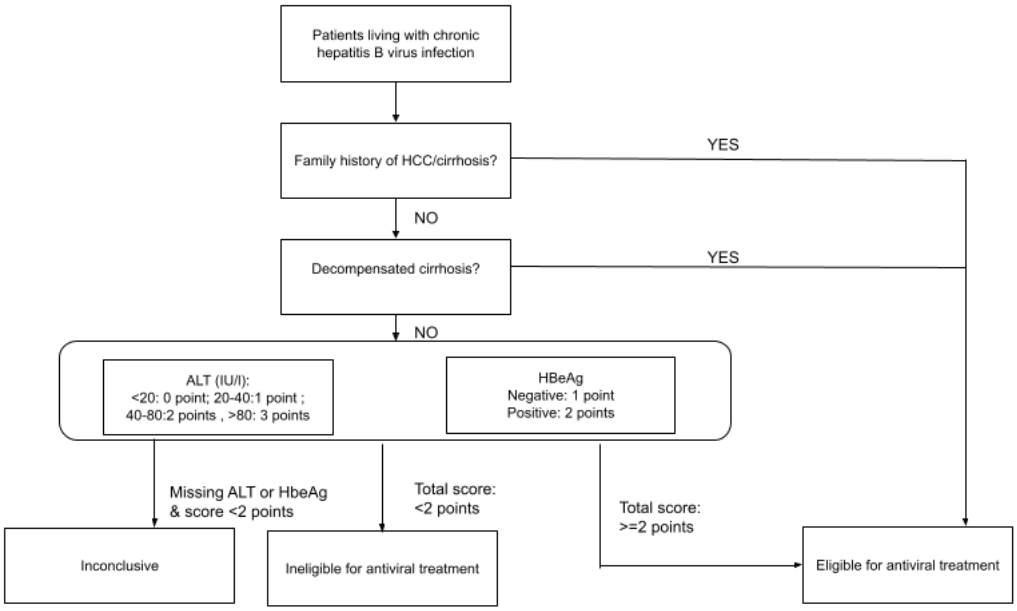
7. AASLD 2018



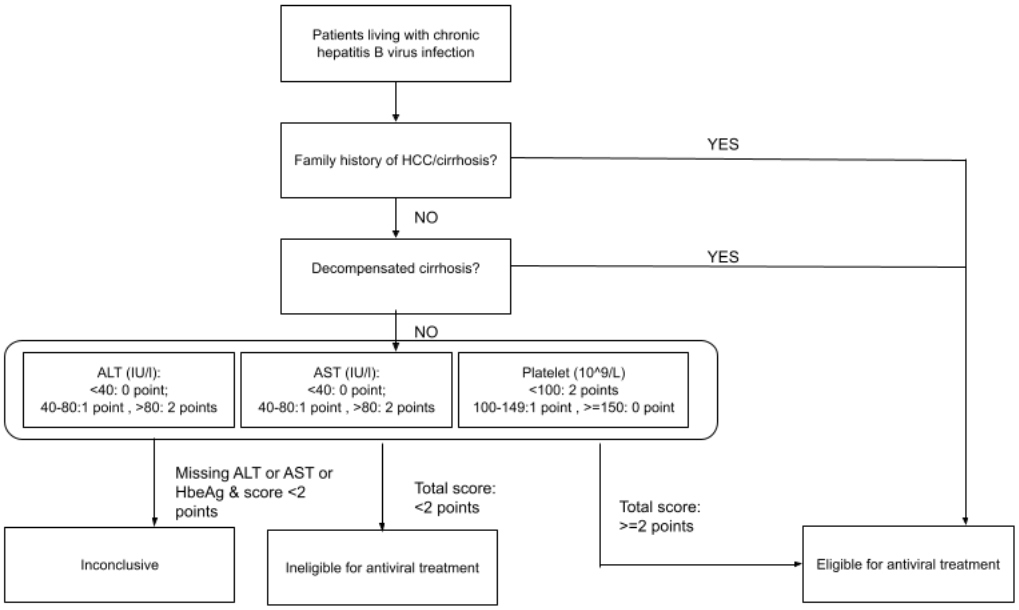
Notes:

- TE thresholds used are from the recent metanalysis[18] since specific thresholds were not specified in the guidelines.

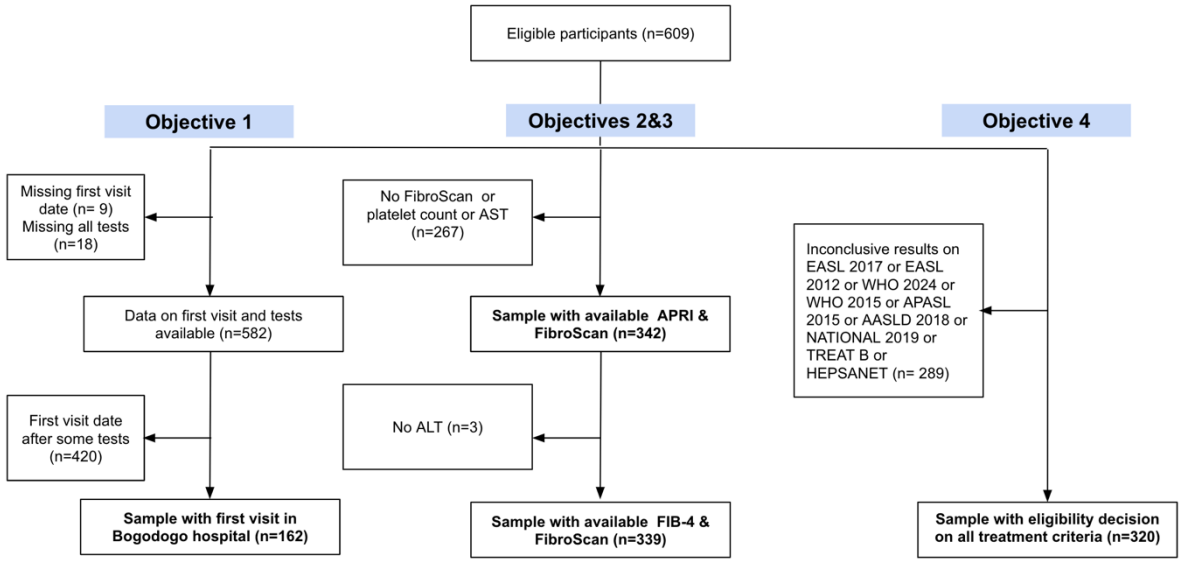
8. TREAT-B score



9. HEPSANET score (for district hospitals)



APPENDIX C: Flowchart illustrating complete case analyses conducted



APPENDIX D: Factors associated with test completion within 3 months

			Crude model	p-value*
	Completion <=3mths/Total	%	OR (95% CI)	
Age				
Less than 30	46/217	21.2%	1.00	0.08
30-50	46/313	14.7%	0.64[0.40,1.00]	
More than 50	18/79	22.8%	1.10[0.58,2.01]	
Sex				
Female	43/264	16.3%	1.00	0.32
Male	67/345	19.4%	1.24[0.82,1.90]	
Family history of HCC or cirrhosis				
No	105/585	17.9%	1.00	0.72
Yes	5/24	20.8%	1.20[0.39-3.07]	
Decompensated cirrhosis				
No	109/602	18.1%	1.00	0.78
Yes	1/6	16.7%	0.75[0.04-4.47]	
First visit location**				
Elsewhere (with a GP)	82/420	19.5%	1.00	0.07
Bogodogo	27/162	16.7%	0.70[0.47-1.02]	

**Likelihood ratio test: p-value<0.05* **n=585, excluding those with missing data on first visit location

RESUME EN FRANÇAIS

Titre: Retard dans l'accès aux différents tests diagnostiques et son impact sur le diagnostic précis des maladies hépatiques liées au VHB au Burkina Faso

Contexte: Les tests diagnostiques du virus de l'hépatite B (VHB) sont essentiels pour évaluer le risque de progression vers la fibrose et décider de l'éligibilité au traitement. L'accès, en particulier à la charge virale et au FibroScan, est souvent limité et retardé dans les contextes à ressources limitées. Bien que des efforts soient faits pour mettre en œuvre des marqueurs plus accessibles, leur précision varie selon les contextes, et peu de données existent sur l'impact des retards d'accès sur leur performance diagnostique.

Objectifs: Cette étude vise à :(1) décrire les retards dans la réalisation des tests diagnostiques prescrits pour le VHB, (2) évaluer l'impact de ces retards sur la précision diagnostique des marqueurs alternatifs de fibrose, à savoir APRI et FIB-4, (3) analyser le niveau d'accord et la précision des différents critères d'éligibilité au traitement antiviral.

Méthodes: Dans cette étude de cohorte rétrospective, les dossiers médicaux de toutes les personnes vivant avec le VHB (n = 630), suivies au Centre Hospitalier Universitaire de Bogodogo entre 2014 et 2022, ont été extraits. Cinq tests ont été prescrits : charge virale, FibroScan, HBeAg, transaminases et numération plaquettaire. Les dates des tests ont été enregistrées à la réception des résultats. Les personnes âgées de moins de 18 ans et celles ayant des co-infections ont été exclues de l'analyse. Pour l'évaluation de la précision diagnostique, le FibroScan a été utilisé comme test de référence pour le diagnostic de la fibrose, et les critères de l'Association Européenne pour l'Étude du Foie (EASL) 2017 comme référence pour l'éligibilité au traitement.

Résultats: Une majorité importante a complété les examens essentiels pour l'éligibilité au traitement: 95,1 % pour l'ALT, 87,5 % pour l'ADN du VHB et 85,9 % pour le FibroScan. Dans les 3 mois suivant la première consultation à Bogodogo, 57,6 % avaient effectué la charge virale et 63,0 % un FibroScan. Un retard de test de 1 mois à 1 an était associé à une augmentation du risque de mauvaise classification de la fibrose par rapport à un intervalle de 3 jours: OR = 2,28 (95% IC: 1,06–5,10) pour APRI et OR = 3,09 (95% IC: 1,20–8,69) pour FIB-4. L'accord avec les critères EASL 2017 était plus faible pour les recommandations de l'OMS de 2024 (kappa de Cohen = 0,36), comparé aux critères alternatifs basés sur des tests accessibles, notamment TREAT-B (0,66) et HEPSANET (0,67).

Conclusion: Nos résultats montrent que les retards diagnostiques réduisent la précision des marqueurs de fibrose accessibles, et que l'encouragement à l'utilisation du FibroScan pour le diagnostic de la fibrose reste essentiel. Dans les contextes où la charge virale n'est pas disponible, les décisions d'eligibilité au traitement peuvent s'appuyer sur des critères développées pour les contextes à ressources limitées, TREAT-B and HEPSANET.

Mots-clés: fibrose, retard de diagnostique, APRI, FIB-4, éligibilité au traitement antiviral