

**IDENTIFYING THE GAPS TO ACHIEVING HEPATITIS B
VIRUS ELIMINATION IN AFRICA: THE GAMBIAN
PROLIFICA EXPERIENCE**

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DECLARATION OF ORIGINALITY

I declare that the work presented in this thesis is my own, and all else is appropriately referenced.

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31st October 2022

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DECLARATION OF CONTRIBUTORS

The work presented in this thesis is original and performed primarily by the author. All collaboration and assistance are described below:

Chapter 3

The PROLIFICA LIVER Clinic team helped me with completing patient assessment and collection of samples; Dr Harr F Njai (and the PROLIFICA lab team) ran the virology and biochemistry laboratory analyses; and Dr Erwan Vo Quang assisted in the data analysis.

Chapter 4

Dr Mindy Gore (Imperial College London) helped develop and validate the nested PCR assay OBI detection and Dr Damien Cohen (INSERM) and Dr Isabelle Chemin (INSERM) conducted some of the HBV genotyping analyses.

Chapter 5

Dr Mindy Gore (Imperial College London) helped to develop and validate the protocol for HBV DNA viral load quantification and genome sequencing. Dr Gora Lo (IRESSEF) and Prof Coumba Touré-Kane (IRESSEF) conducted the HIV viral load quantification.

Chapter 6

Sarwar Golam (Senior Data Manager, MRCG) and Dr Esu Ezeani (Head of MRCG HDSS) provided the dataset, and Professor Julien Randon-Furling and Cécile De Bezenac assisted with the data analysis.

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ORAL PRESENTATIONS

Conference on Liver Disease in Africa (COLDA), 29th September–1st October 2022, Cape Town, South Africa. Trend of hepatitis B birth dose vaccine coverage in The Gambia: 2015 – 2021

International Association for the Study of the Liver (IASL) annual conference, July 2021, Virtual. Scaling up hepatitis B care in sub-Saharan Africa: can we simply do it?

International workshop on viral hepatitis elimination, October 2018, Ho Chi Minh City, Vietnam. Epidemiology, surveillance, and elimination of viral hepatitis in The Gambia

EASL International Liver Conference, April 2016, Barcelona, Spain. Hepatitis B virus screening in HIV facilities: current coverage and consequences

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EASL International Liver Conference, 22-26 June 2022, London, United Kingdom. Hepatitis B birth dose coverage remains dramatically low in The Gambia and has been disrupted by the COVID-19 pandemic.

EASL International Liver Conference, 22-26 June 2022, London, United Kingdom.
Concordance between Xpert HBV and quantitative PCR to detect and quantify HBV viremia in West Africa.

EASL International Liver Conference, April 2018, Paris, France. Occult hepatitis B is frequent and associated with advanced liver disease in The Gambia

ABSTRACT

Background and aims

Chronic hepatitis B (CHB) remains a major global health problem. This PhD assessed The Gambia's progress towards WHO targets for reducing CHB mortality and new infections, identifying gaps and proposing solutions. As the first African country to adopt both infant and birth dose hepatitis B (HepB-BD) vaccinations, The Gambia provides a unique context for assessment.

Methods and results

A prospective cohort analysis (2012-2015) of adults with cirrhosis or hepatocellular carcinoma (HCC) found hepatitis B, C, and Delta seropositivity rates of 84.4%, 9.9%, and 9.9%. Most patients presented with advanced liver disease, with median survival of 17.1months for compensated cirrhosis, 11.3months for decompensated cirrhosis, and 1.5months for HCC. Ascites (HR:1.83) and portal thrombosis (HR:3.81) independently increased mortality among HCC patients, while Tenofovir treatment improved survival in CHB-related cirrhosis (HR:0.14; $p<0.001$).

In the population-based PROLIFICA cohort, occult hepatitis B infection (OBI) prevalence was 9.9% in the general population and 18.3% among advanced liver disease patients, and was significantly associated with liver disease (OR:2.8, $p=0.006$). Among HBsAg-negative people, OBI accounted for 12.9% of advanced liver disease cases.

Among HIV patients, HBV screening was low (21.5%) and only 26.1% of HIV-HBV co-infected patients received appropriate treatment. Co-infected patients had higher APRI scores with 98.1% (52/53) having detectable HBV DNA and 83.3% (10/12) had 3TC-resistance mutations linked to increased liver fibrosis ($p=0.05$).

Timely HepB-BD coverage in rural Gambia rose from 1.8% (2015) to 20.7% (2021). Births in health facilities (OR:0.76) and maternal education (OR:0.50) increased timely HepB-BD, while delays were associated with remote regions (OR:1.96), weekend births (OR:6.14), or increasing maternal age (OR:1.008 for every additional year). Timely vaccination declined from 10.1% pre-COVID to 5.4% during the first wave.

Summary

Despite progress, gaps in disease prevention, screening, and treatment remain. Integrated, resource-efficient strategies are needed to meet WHO elimination targets by 2030.

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ABBREVIATIONS

3TC	Lamivudine
95% CI	95% confidence interval
AASLD	American Association for the Study of The Liver
AIDS	Acquired Immune Deficiency Syndrome
ALT	Alanine aminotransferase
Anti-HBc	Antibody against hepatitis B core antigen
Anti-HBe	Antibody against hepatitis B e antigen
Anti-HBs	Antibody against hepatitis B surface antigen
APRI	Aspartate aminotransferase to platelet ratio index
AST	Aspartate aminotransferase
BMI	Body mass index
CD	Cluster of differentiation
CDC	Centres for Disease Control
CLIA	Chemiluminescence immunoassay
CHB	Chronic hepatitis B
CT	Computed Tomography
DBS	Dried blood spot
EASL	European Association for the Study of the Liver
EFSTH	Edward Francis Small Teaching Hospital
EIA	Enzyme-linked immunosorbent assay
ESLD	End stage liver disease
GBD	Global Burden of Disease

GGT	Gamma glutamyl transferase
GHIS	Gambia Hepatitis Intervention Study
GLCS	Gambia Liver Cancer Study
GPRI	GGT to platelet ratio index
HBcAg	Hepatitis B core antigen
HBcrAg	Hepatitis B core related antigen
HBeAg	Hepatitis B e antigen
HBeAb	Hepatitis B e antibody
HBIG	Hepatitis B immunoglobulin
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HBxAg	Hepatitis B x antigen
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HDV	Hepatitis D virus
HIV	Human Immunodeficiency Virus
HOC	Hands on Care
IARC	International Agency for Research on Cancer
ICL	Imperial College London
IFN	Pegylated-interferon alpha
IGHI	Institute of Global Health Innovation
INFANT-B	Intervention for the avoidance of neonatal transmission of hepatitis B in Africa

INSERM	French National Institute of Health and Medical Research
IU	International Unit
LBV	Laboratoire Bactériologie Virologie
LMIC	Low and Middle-Income Countries
LSM	Liver stiffness measurement
MATCH-B	Management of chronic hepatitis B in Africa study
MRC	Medical Research Council
MRCG	Medical Research Council Unit, The Gambia
MRI	Magnetic resonance imaging
NACP	National HIV/AIDS Control Program
NHP	National Hepatitis Program
NPHL	National Public Health Laboratories
OBI	Occult hepatitis B infection
OR	Odds ratio
ORF	Open reading frame
PCR	Polymerase chain reaction
PMTCT	Prevention of mother to child transmission
PROLIFICA	Prevention of Liver Fibrosis and Cancer in Africa
qPCR	Quantitative polymerase chain reaction
RCT	Randomised control trial
RR	Relative risk
RNA	Ribonucleic acid
RT-PCR	Real-time polymerase chain reaction

sSA	Sub-Saharan Africa
TDF	Tenofovir Disoproxil Fumarate
TE	Transient elastography
UCAD	Université Cheikh Anta DIOP de Dakar
USS	Ultrasound scan
VL	Viral load
WHO	World Health Organisation

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1. CHAPTER 1: GENERAL INTRODUCTION

1.1. The hepatitis B virus

The hepatitis B virus (HBV) is a member of the Hepadnaviridae family of hepatotropic viruses [1]. HBV is a small spherical DNA virus measuring 40 to 42 nm in diameter. The infectious virion (Dane particle) comprises a lipid envelope surrounding an inner viral core (nucleocapsid). The lipid envelop contains hepatitis B surface antigens (HBsAg) and the nucleocapsid contains the viral genome, which is composed of the HBV DNA, polymerase, and hepatitis B core antigen (HBcAg) [1, 2]. Figure 1.1 shows a schematic diagram of the HBV virion.

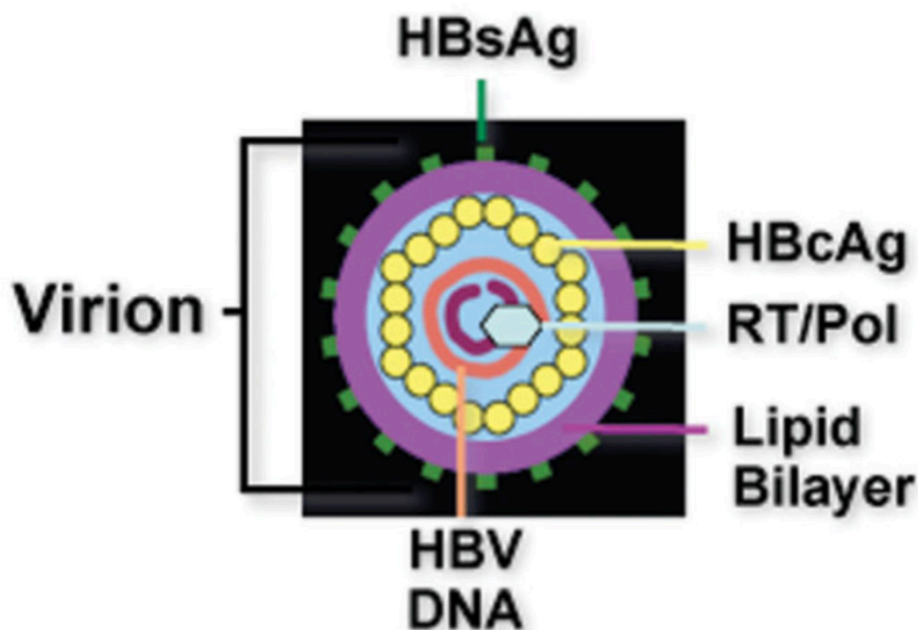


Figure 1.1. Schematic drawing of the HBV virion [1]

The HBV genome (figure 1.2) is a partially double stranded, relaxed-circular DNA (RC-DNA) of 3.2kb pairs. Inside the hepatocyte nucleus, the viral polymerase converts the RC-DNA into an extremely stable covalently closed circular DNA (cccDNA) by filling the partially single-stranded region of the genome [3]. The cccDNA integrates within the host genome and persists in the host nucleus for the lifespan of the hepatocyte, providing a transcriptional template for all RNA species that are translated into viral proteins.

The HBV genome encodes four overlapping open reading frames (ORFs): X, C, S and P. The X ORF encodes the hepatitis B x antigen (HBxAg) which is overexpressed in

the livers and tumours of chronic HBV carriers and interacts with several host proteins to increase HBV viral replication, gene expression and pathogenesis [4]. The C ORF has the core and precore regions that encode the HBcAg and the hepatitis B e antigen (HBeAg) respectively. The S ORF can be divided, both structurally and functionally, into the pre-S1, pre-S2 and S regions, and encodes the HBsAg. The P ORF encodes the polymerase (pol), a large protein of 800 amino acids that has three domains: a terminal protein domain for encapsidation and initiation of minus-strand synthesis, a reverse transcriptase domain for catalysing genome synthesis, and a ribonuclease H domain for degrading pregenomic RNA and facilitating replication [1]. The polymerase is a reverse transcriptase that is covalently attached to the 5' end of the minus strand of the HBV DNA. It is integral for HBV replication, involved in viral RNA binding and packaging, protein priming, template switching and DNA synthesis. It is therefore a key target for HBV treatment.

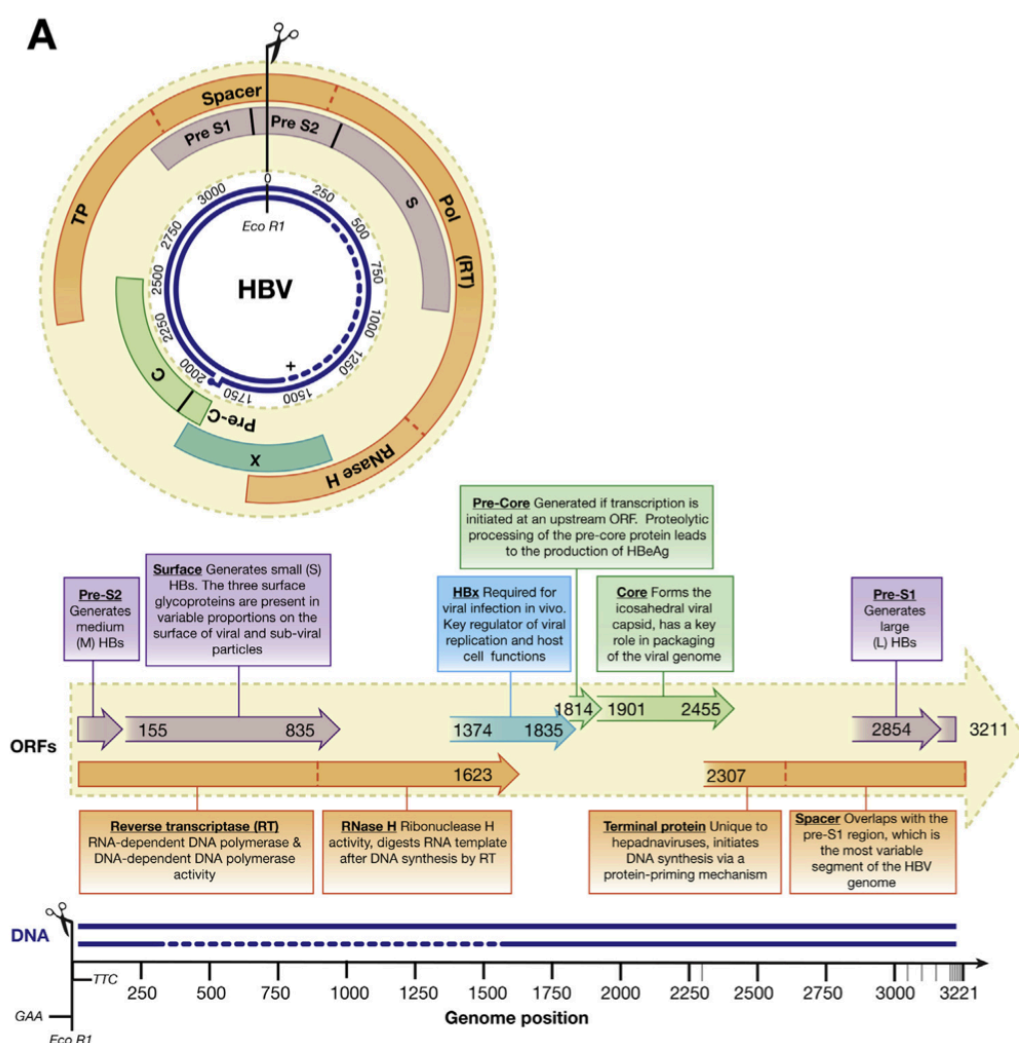


Figure 1.2. Structure of the HBV viral genome [3]

1.2. Hepatitis B virus replication cycle

HBV replication, demonstrated in figure 1.3, begins with encapsidation of the HBV virion into the host cell, likely involving the pre-S domain of the surface protein. After membrane fusion, the HBV nucleocapsid (core particle) is transported to the host nucleus where the single-stranded gap region in the viral DNA is repaired by the viral pol protein, followed by conversion of the viral DNA into cccDNA which serves as the template for viral messenger RNA transcription [3].

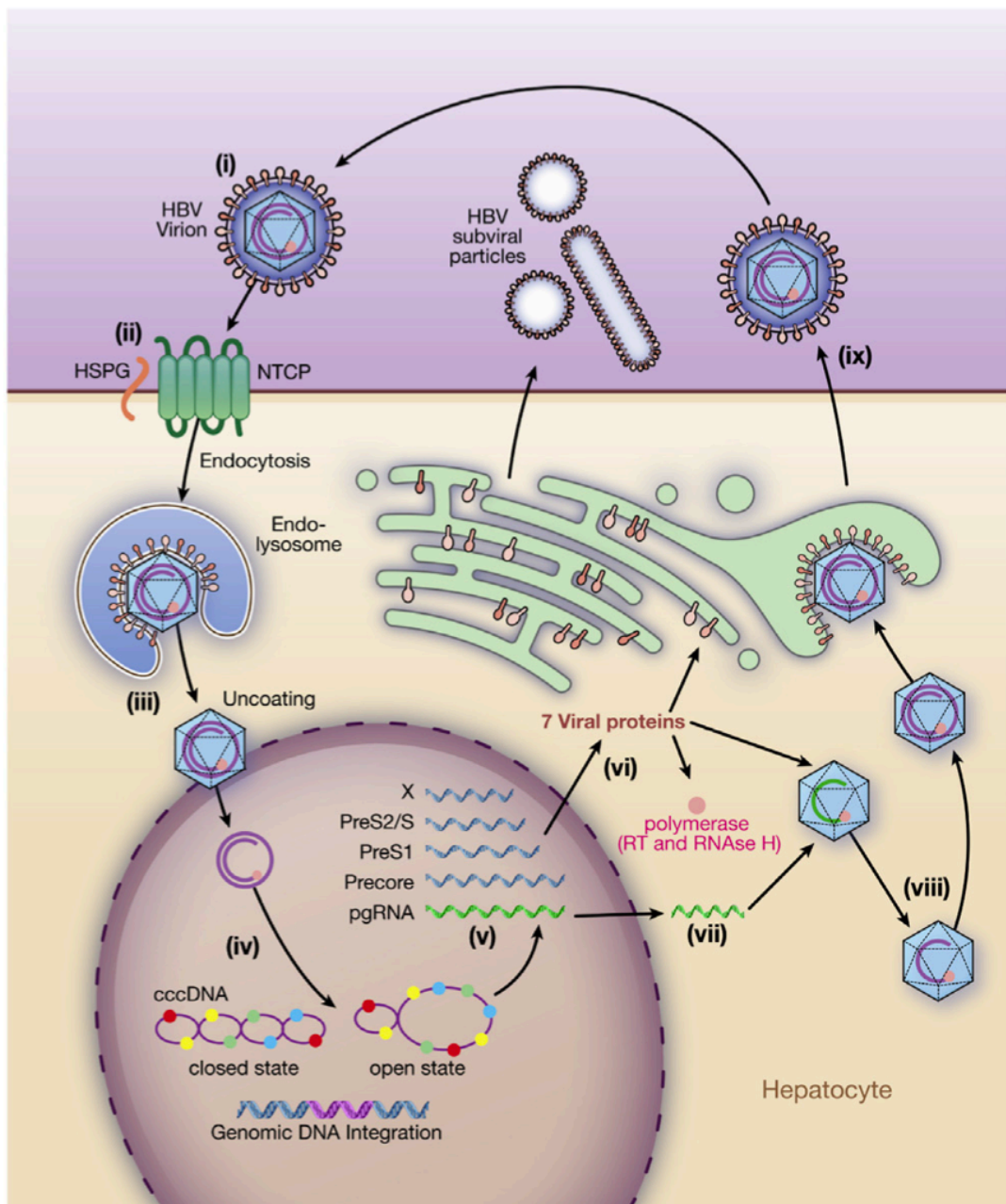


Figure 1.3. HBV life cycle [3]

All viral RNA are then transported to the cytoplasm for translation of viral proteins (envelope, core and polymerase) and polypeptides (X and pre-core). Viral proteins and a single molecule of genomic RNA are assembled in the cytosol to form new nucleocapsids. This genomic RNA serves as template for the synthesis of the first viral DNA strand by reverse transcription. The RNA strand is then degraded and the newly made first DNA strand serves as template for the second strand of viral DNA. Most of the resulting nucleocapsid buds into the endoplasmic reticulum to be enveloped and exported from the hepatocyte. Some nucleocapsids recycle their genomes into the nucleus for conversion [1-3].

1.3. Hepatitis B genotypes

Based on genomic sequence divergence of >8%, nine HBV viral genotypes (A-I) and several sub-genotypes have been described [5], each with characteristic geographic distribution (Figure 1.4). In addition, HBV genotypes differ in their predominant mode of transmission, disease severity, risk of progression to hepatocellular carcinoma (HCC) and the potential and timing of HBeAg seroconversion and HBsAg clearance [6, 7].

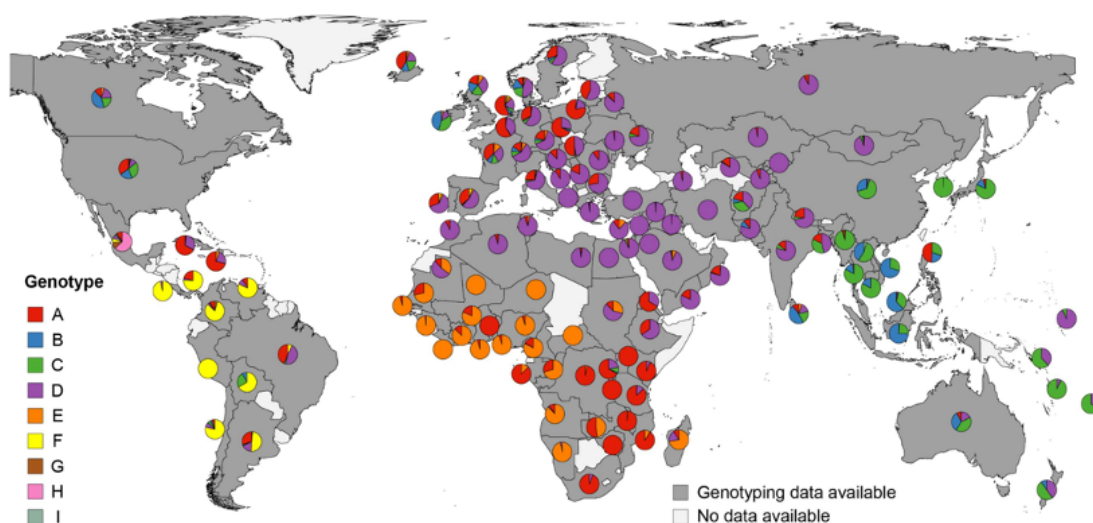


Figure 1.4. Global distribution of HBV genotypes [8]

Genotypes A and E, which are widespread in sub-Saharan Africa (sSA), are commonly transmitted horizontally whereas genotypes B and C, predominant in Asia, are typically transmitted perinatally [7, 9]. Genotypes A (in particular sub-genotype A1), C and F have been associated with earlier progression to HCC [9, 10], with genotype A associated with higher risk of HCC in sSA [10-13]. In a meta-analysis of 14,545 patients enrolled in 43 studies, genotype C had a higher risk of HCC compared to

genotypes A, B and D [14]. A tenth putative genotype (J) was characterised after isolation from one individual [15].

In addition to genotypes, four major HBV serological subtypes (*adw*, *ayw*, *adr* and *ayr*) and nine minor subtypes are identified by antigenic determinants of HBsAg [16, 17]. The geographic distribution of the HBV serotypes and their inter-relation with genotypes, is presented in table 1.1.

Table 1.1. Geographic distribution of HBV genotypes and serotypes (modified from Kao JH et al, 2002)

Genotype	Serotype	Areas of predominance
A	<i>adw2</i> , <i>ayw1</i>	North-western Europe, USA, central Africa
B	<i>adw2</i> , <i>ayw1</i>	Taiwan, Japan, Indonesia, China, Vietnam
C	<i>adw2</i> , <i>adrq+</i> , <i>adrq-</i> , <i>ayr</i>	East Asia, Taiwan, Korea, China, Japan, Polynesia, Vietnam
D	<i>ayw2</i> , <i>ayw3</i>	Mediterranean area, India
E	<i>ayw4</i>	West Africa
F	<i>adw4q-</i> , <i>adw2</i> , <i>ayw4</i>	Central and South America, Polynesia
G	<i>adw2</i>	France, USA
?	<i>ayr</i>	Borneo

1.4. Hepatitis B serology

Several HBV viral antigens and their respective antibodies can be detected in serum. The correct interpretation of these antigens and antibodies allows distinction between clinical forms and phases of HBV infection and immune status.

- a) **Hepatitis B surface antigen (HBsAg):** a protein found on the surface of the HBV, detectable both in acute and chronic infection. Its presence indicates an ongoing (current) infection.
- b) **Hepatitis B surface antibody (anti-HBs):** Antibody to the HBsAg, usually present during recovery from infection or following successful vaccination. It indicates immunity from HBV infection.
- c) **Hepatitis B core antigen (HBcAg):** Indicates ongoing infection and active viral replication. HBcAg cannot be detected in serum. However, immunohistochemical stains for HBcAg are used to confirm chronic HBV

infection as the cause of liver disease in biopsy tissue. Positive HBcAg staining also indicates active HBV replication.

- d) **Hepatitis B core antibody (anti-HBc):** Antibody to HBcAg, appears during acute HBV infection and persists for life.
 - a. **IgM anti-HBc:** indicates recent (acute) HBV infection, usually within 6 months. May be detected during flares or reactivation [18, 19].
 - b. **IgG anti-HBc:** in the absence of IgM, IgG positivity indicates chronic HBV infection.
- e) **Hepatitis B e antigen (HBeAg):** It is produced during viral replication and is detectable during acute and chronic infection. Its presence indicates ongoing infection, active replication, and infectivity. HBeAg is used to monitor treatment response. HBeAg negative HBV variants that do not produce HBeAg during replication have been described in sSA [20].
- f) **Hepatitis B e antibody (anti-HBe):** Production of anti-HBe usually follows HBeAg seroconversion. Detection of anti-HBe in previously HBeAg-positive individuals usually indicates low or absent viral replication, low viremia and is an indication for stopping antiviral therapy in eligible candidates [21].
- g) **HBV DNA:** The detection of HBV DNA in serum (or liver) indicates ongoing infection. Detection and quantification of HBV DNA is used for clinical staging of HBV infection and for monitoring disease progression and treatment response.

Table 1.2 summarises the interpretation of HBV serological markers for various disease stages and immune status.

Table 1.2. Interpretation of HBV serological markers. (Source: www.cdc.gov/hepatitis/hbv/pdfs/serologicchartv8.pdf; accessed 14th October 2021)

Serological markers	Result	Interpretation
HBsAg	Negative	Susceptible to infection
Anti-HBc	Negative	
Anti-HBs	Negative	

HBsAg	Negative	Immune due to natural infection
Anti-HBc	Positive	
Anti-HBs	Positive	
HBsAg	Negative	Immune due to hepatitis B vaccination
Anti-HBc	Negative	
Anti-HBs	Positive	
HBsAg	Positive	Acutely infected or HBV reactivation
Anti-HBc	Positive	
IgM anti-HBc	Positive	
Anti-HBs	Negative	
HBsAg	Positive	Chronically infected
Anti-HBc	Positive	
IgM anti-HBc	Negative	
Anti-HBs	Negative	
HBsAg	Negative	Three possible interpretations:
Anti-HBc	Positive	
Anti-HBs	Negative	

Novel HBV biomarkers have been described in an efforts to improve understanding and management of CHB [22]:

- a. **Ultrasensitive hepatitis B surface antigen (us-HBsAg):** Detectable using newer assays with limit of detection 0.2-5mIU/mL. us-HBsAg is used for early detection of acute HBsAg infection or to monitor reactivation or subclinical levels of HBsAg following seroclearance.

- b. **Quantitative hepatitis B surface antigen (qHBsAg):** An important marker of treatment response; correlated with levels of cccDNA in the liver. Used to predict development of liver fibrosis and HCC or HBsAg loss.
- c. **Serum HBV RNA:** Surrogate of cccDNA transcriptional activity. Serum HBV RNA levels can be used to differentiate HBeAg-negative phases of CHB infection, predict HBeAg seroconversion and predict sustained viral response in treated patients.
- d. **Hepatitis B core related antigen (HBcrAg):** Correlates with cccDNA. Serum levels can distinguish between active and inactive chronic HBV disease, predict risk of developing cirrhosis, monitor treatment response and predict HBeAg and HBsAg seroconversion.
- e. **Quantitative hepatitis B core antigen (qAnti-HBc or qAHBc):** Correlates with ALT during treatment. Can be used to monitor treatment response, identify occult hepatitis B infection, and predict development of liver fibrosis and inflammation.
- f. **HBV nucleic acid-related antigen (HBV-NRAg):** Qualitative test that correlates with HBV DNA positivity. Positive HBV-NRAg is associated with liver abnormalities, but its clinical utility remains unclear.

1.5. Routes of transmission of hepatitis B virus

HBV is transmitted through contact with blood or body fluids from an infected person to an uninfected susceptible person. Transmission can occur perinatally from mother-to-child, horizontally between children, through transfusion of blood or blood products, or from sexual contact and sharing sharp objects such as needles [23, 24]. The most common routes of transmission are perinatally from an infected mother, and horizontally from child to child, particularly in early infancy during the first 5 years of life.

Perinatal transmission is the predominant route of transmission in Asia [25-27]. In contrast, horizontal transmission between children is the main route of transmission in Africa, accounting for more than 80% of infections [28]. HBV MTCT is estimated to account for approximately 15% of infections in sub-Saharan Africa [29, 30].

The route and time of HBV infection are important factors for determining chronicity of infection [31, 32] and subsequent risk of liver disease and need/eligibility for antiviral therapy in adulthood [12]. More than 90% of infants infected perinatally or during early infancy go on to develop chronic infection, compared to 5% of immunocompetent

persons infected in adulthood [31]. In sSA, individuals infected perinatally have a greater risk of developing HCC and needing antiviral therapy in adulthood compared to those infected later in life [12].

1.6. Natural history of hepatitis B infection

HBV infection can be either acute (when infection lasts less than 6 months) or chronic (infection lasting longer than 6 months).

Acute infection is characterised by detection of HBsAg, HBV DNA and HBeAg in serum, usually 1-2 weeks after exposure to the virus. Approximately 3-4 weeks after infection, IgM anti-HBc levels peak in serum, but quickly decline and are replaced with IgG anti-HBc. HBeAg is usually cleared early in serum, replaced by anti-HBe which usually becomes detectable at the start of recovery. HBsAg and HBV DNA, on the other hand, remain detectable for the entire duration of acute HBV infection, usually until the recovery. Anti-HBs, the antibody associated with HBV immunity, rises at the end of the recovery period, and persists in serum after recovery [1]. Figure 1.5 demonstrates the typical course of acute HBV infection.

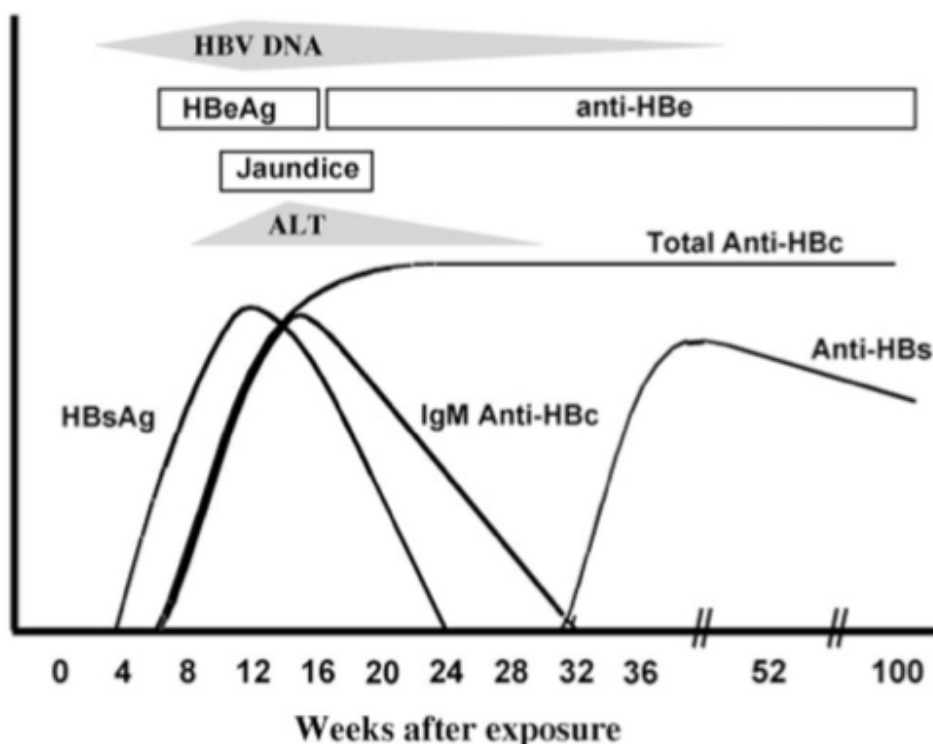


Figure1.5. Typical serological profile of acute hepatitis B infection [1]

In acute infections, the innate and adaptive immune response to HBV infection are efficient and timely [33]. As a result, most acute infections in immunocompetent adults are asymptomatic. When symptoms occur, they are usually mild to moderate and can include abdominal pain, fever, nausea, vomiting, diarrhoea, and jaundice. These are treated with supportive management, and infection is usually cleared within a few months. In rare instances, acute HBV infection may cause severe liver disease with acute liver failure requiring specialised care and/or liver transplantation. Case fatality in acute HBV infection is estimated at 0.5-1% [34].

The natural history of **chronic hepatitis B (CHB) infection** is complex and not fully understood. In CHB infection, HBsAg, HBV DNA and HBeAg persist more than 6 months and are often detectable in high titres in serum. Figure 1.6 illustrates the serological profile in CHB infection.

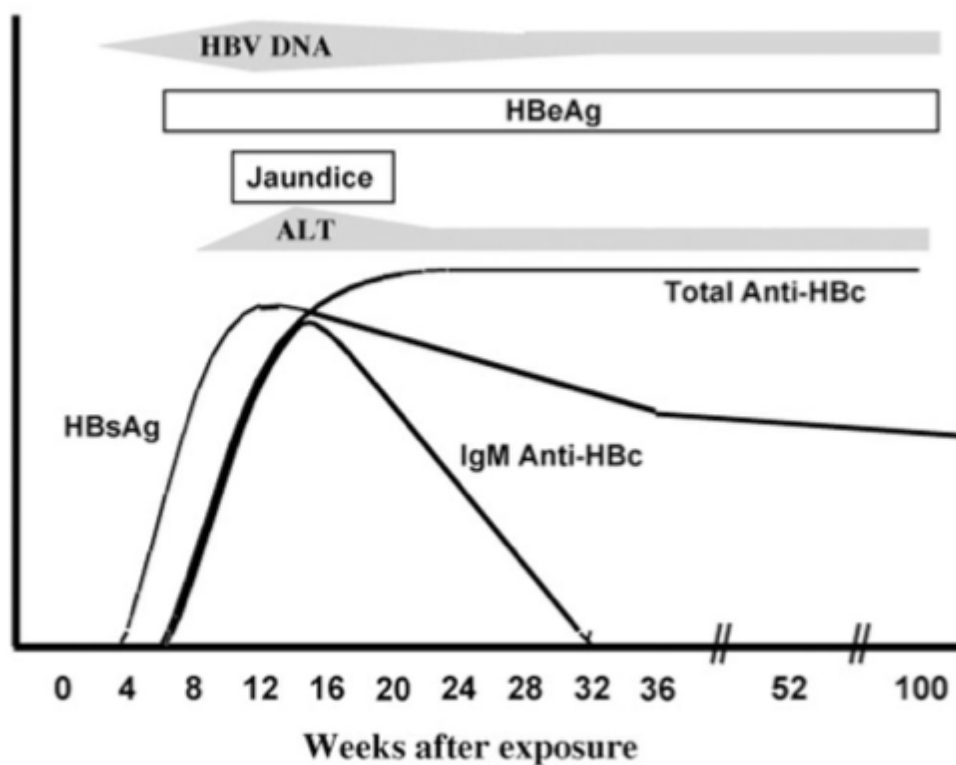


Figure 1.6. Serological profile of CHB infection

The clinical course of CHB varies significantly. The European Association for the Study of the Liver (EASL) recently proposed a new nomenclature for chronic HBV infection [33], dividing the natural history of chronic infection into non-sequential phases based on HBeAg status and presence or absence of liver inflammation (hepatitis). The phases, summarised in table 1.3, are:

- a. **Phase 1 – HBeAg-positive chronic infection:** Characterised by high HBV DNA levels with normal alanine aminotransaminase (ALT) and minimal or no liver fibrosis or inflammation (normal Fibroscan and ALT). This phase was previously termed the “immune tolerant” phase.
- b. **Phase 2 – HBeAg-positive chronic hepatitis:** Characterised by high HBV DNA levels, elevated ALT, moderate to severe liver inflammation and accelerated progression of fibrosis.
- c. **Phase 3 – HBeAg-negative chronic infection:** Characterised by low or undetectable HBV DNA (usually <2,000IU/mL), normal ALT, low fibrosis and no or minimal liver inflammation, with or without presence of anti-HBe. This phase was previously termed the “inactive carrier” phase.
- d. **Phase 4 – HBeAg-negative chronic hepatitis:** Characterised by high levels of HBV DNA, elevated (or fluctuating) ALT, liver fibrosis and inflammation, with or without detectable anti-HBe. HBeAg-negative chronic hepatitis is common in sSA (especially in The Gambia), and many patients have HBV variants with precore or core mutations that impair HBeAg expression [12, 35].
- e. **Phase 5 – Occult HBV infection phase:** Characterised by negative (undetectable) HBsAg, detectable HBV DNA, usually in very low levels (<200IU/mL), and presence of cccDNA in liver, with or without positive anti-HBc and/or anti-HBs. Patients often have normal ALT but may present with advanced liver disease if HBsAg loss occurred after onset of significant fibrosis or cirrhosis.

Table 1.3. New EASL nomenclature of the phases of chronic HBV infection [33]

	HBeAg positive		HBeAg negative	
	Chronic infection	Chronic hepatitis	Chronic infection	Chronic hepatitis
Quantitative HBsAg	High	High / intermediate	Low	Intermediate
HBeAg	Positive	Positive	Negative	Negative
HBV DNA	High (>20,000IU/mL)	2,000 to 20,000IU/mL	<2,000IU/mL	>2,000IU/mL
ALT	Normal	Elevated	Normal	Elevated (or fluctuating)
Liver disease	None / minimal	Moderate / severe	None	Moderate / severe

Old terminology	Immune tolerant	Immune reactive	Inactive carrier	
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The natural history of CHB has been mainly studied in Asian and Western populations. Consequently, the natural history of CHB in sSA is poorly understood. In Gambian patients predominantly infected with HBV genotype E and followed up for a median length of 28.4 years, HBsAg and HBeAg seroconversion were reported at 1% and 7.4% per annum respectively [12]. In this Gambian cohort, 5.5% had significant fibrosis and the HCC incidence rate was 55.5 per 100,000 carrier-years. Perinatal infection (from an infected mother), infection with HBV genotype A, and aflatoxin exposure were risk factors of chronic hepatitis and disease progression [12].

To achieve HBV elimination in Africa, it is important to better understand the natural history of CHB in Africans and to develop hepatitis B guidelines adapted to the African population and setting.

1.7. Immunopathogenesis – HBV-specific immune response

HBV infection in immunocompetent adults is generally self-limiting. In acute infection, the initial poor induction of intracellular innate responses in early stages of infection is followed by functionally efficient, multi-specific antiviral T-cell responses which increase as HBV viremia rises and eventually lead to resolution. Virus specific CD8⁺ T-cells secrete cytokines, primarily interferon-gamma (IFN γ) and tumour necrosis factor (TNF) that inhibit viral replication and support non-cytolytic clearance of HBV from hepatocytes. There is usually no detectable cellular infiltration of immune cells in the liver during recovery of acute HBV infection [32, 36]. After recovery of acute infection, the trace amounts of cccDNA that persist in the nucleus of hepatocytes help maintain continuous stimulation of the immune system, thereby ensuring persistent control of infection [36].

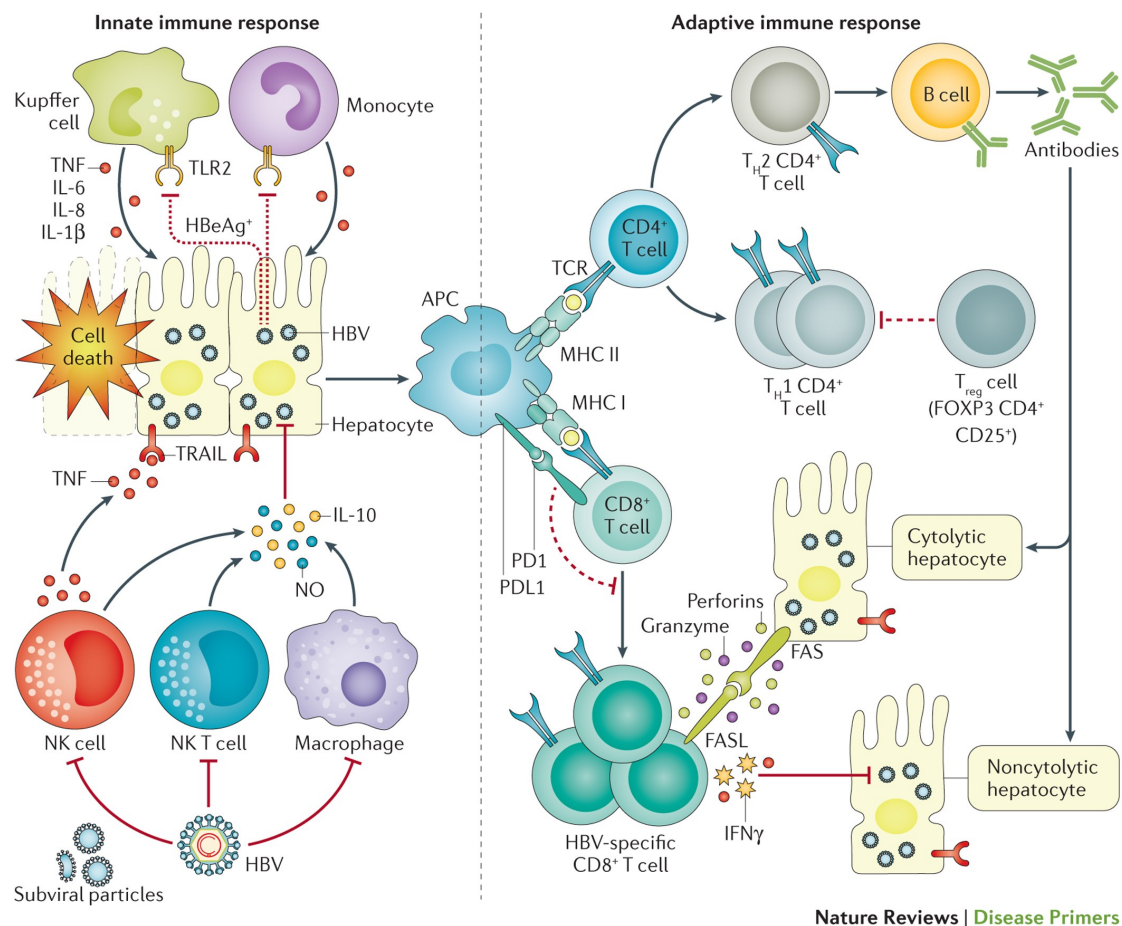


Figure 1.7. Hepatitis B specific immune responses [32].

Chronic HBV infection, on the other hand, mainly results from infections acquired early in life when the immune system has not matured. CHB infection is established through immune anergy and is characterised by failure to mount an effective and coordinated adaptive immune response. Following infection, HBV produces excessive quantities of subviral particles (HBsAg with or without HBeAg) to manipulate key innate immune responses including blocking recruitment of natural killer (NK) cells, NK T-cells and macrophages; decreasing cytokine secretion from Kupffer cells; and blocking retinoic-acid inducible gene I protein and Toll-like receptor signalling within hepatocytes [37-40]. In addition, HBx protein can block host viral restriction factors that silence cccDNA and inhibit degradation of viral proteins, thereby reducing HBV antigen presentation [32].

The immune responses to CHB infection is mainly described from studies examining the expression of immune markers in serum. To describe the intrahepatic immune events in CHB infection, a landmark study analysed biopsies from more than 100 treatment-naïve CHB patients and demonstrated a strong downregulation of the major

innate immune response pathways including interferon-stimulated gene, Toll-like and pathogen recognition receptor pathways and antiviral sensors [40]

1.8. Epidemiology of chronic hepatitis B infection

CHB is a major public health problem globally. In 2019, the WHO estimated that 3.8% (95% CI 3.0-5.5%) of the general population and 0.9% (95% CI 0.7-1.6%) of children under 5 years of age had CHB infection globally [41]. This translates to 296 million (228 million – 423 million) individuals living with CHB infection worldwide, of whom approximately 6 million (4 million – 11 million) are children less than 5 years old. Globally, it is estimated that 1.5 million (1.1 million – 2.6 million) individuals are newly infected with CHB annually [41, 42]. CHB accounts for 820,000 (450,000 – 950,000) deaths annually, mainly from cirrhosis and HCC [41, 42].

Figures 1.8 a and b illustrate the 2016 global burden of CHB infection in the general population and among children younger than 5 years [43].

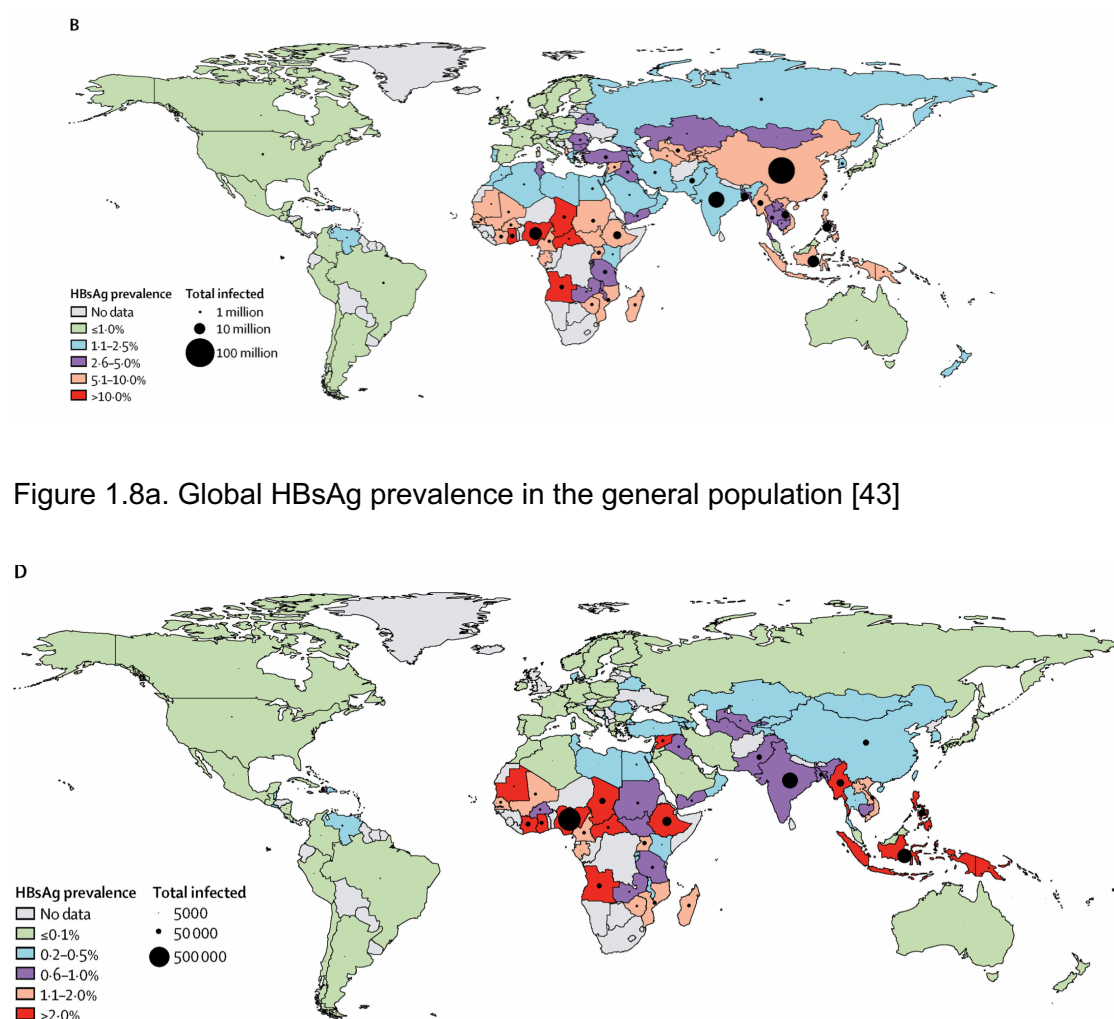


Figure 1.8a. Global HBsAg prevalence in the general population [43]

Figure 1.8b. Global HBsAg prevalence among children younger than 5 years [43]

In Africa, the CHB infection affects 82.3 million (62.1 million – 114.7 million) individuals with a prevalence of 7.5% (5.7-10.5) in the general population and 2.5% (1.7-4.0) among children under 5 years of age [41, 42]. The reported incidence of CHB infection in Africa was 990,000 (660,000–1,600,000) new cases in 2019, with approximately 350,000 new infections acquired annually from mother-to-child transmission [30]. The WHO estimates that 70% of new hepatitis B infections worldwide occur in Africa, with 4.5 million African children affected [44]. Currently, 33 countries in Africa have a hepatitis B prevalence of more than 1% in children younger than 5 years.

CHB infection accounts for 80,000 (47,000 – 110,000) deaths annually in Africa [41]. The Viral Hepatitis Scorecard 2021 found that Africa accounted for 26% of the global burden of hepatitis B (and C), with 125,000 reported deaths annually. In 19 African countries, the HBsAg prevalence in the general population is more than 8% [44].

Globally, only 10% (30.4 million) individuals with CHB infection have been tested and diagnosed, and <2% (6.6 million) of those in need of treatment were receiving antiviral therapy in 2019 [41]. Access to care is especially worse in sSA where it is estimated that only 2% of CHB infected individuals knew their status and 0.1% of individuals living with CHB infection were accessing treatment in 2019 [41]. Scaling up access to affordable low-cost diagnostics for screening HBsAg and quantifying HBV DNA viral load, improving provision of HBV antiviral drugs, and integrating timely hepatitis B birth-dose vaccination into routine new-born care practices and policies are urgently needed to eliminate CHB infection in Africa and meet the WHO targets in 2030 [45].

1.9. Liver disease in chronic hepatitis B infection

Cirrhosis and HCC are leading causes of morbidity and mortality globally, accounting for 1.16 million and 788,000 annual deaths respectively. Together, cirrhosis and HCC account for 3.5% of all deaths globally each year [46]. In Africa, HCC was the second and third leading cause of cancer in men and women respectively, with an estimated 36,844 incident cases and 36,067 deaths in 2018 [47]. Both the 2016 Global Burden of Disease (GBD) Cancer collaboration study [48] and a recent systematic review on the worldwide prevalence of HBV infection among patients with cirrhosis [49] showed that CHB infection is a major risk factor for cirrhosis and HCC (both absolute incidence and death) in Africa. Alberts et al estimated that 41% of patients with cirrhosis worldwide had CHB infection [49]. Individuals with CHB infection have a 20% risk of

developing cirrhosis within 1-13 years, with the risk of decompensated cirrhosis and HCC being 23% and 6% respectively [50, 51].

In sSA, 41% of cirrhosis and 50% of HCC cases are attributable to CHB infection [48, 49]. West Africa has the highest burden of HCC in Africa, with age-standardised incidence rate (ASIR) and age-standardised mortality rates (ASMR) of 16.9 (95% CI 13.0-22.4) and 18.1 (95% CI 14.1-23.4) per 100,000 person years respectively [52]. These results confirm previous reports that up to 60% of HCC cases in West Africa are attributable to CHB infection [53-55]. The Gambia Liver Cancer Study had estimated that the odds ratio of developing HCC was 16.7 (95% CI 9.7-28.7) in CHB mono-infected adults and OR 35.3 (95% CI 3.9-323) in CHB patients co-infected with hepatitis C [53].

In Asian and Western populations with CHB infection, the annual risk of progressing from compensated to decompensated cirrhosis was 2-5%/year [51, 56, 57], with risk increasing to 14% in patients with hepatic flares [58]. The 5-year cumulative incidence of HCC in CHB-infected patients with cirrhosis was 10% in Western populations and 17% in Asian populations [59], and the 5-year survival rate was 19% [60, 61].

The rate of liver disease progression among African adults with CHB infection is not well studied. In a Gambian cohort of adults with CHB infection selected from the general population, the prevalence of significant liver fibrosis was 5.5% with an HCC incidence of 55.5 per 100,000 (95% CI 24.9-123.5) carrier-years [12].

Across all populations including Africans, viral, host, and environmental factors have been identified as predictors of fibrosis, cirrhosis and HCC in patients with CHB infection. High HBV DNA viral load, HBeAg seropositivity, infection with genotype A (in particular sub-genotype A1) or C, age, gender, family history of HCC, earlier age at HBV infection, perinatal transmission and earlier birth order (reflecting perinatal transmission from younger, highly viraemic mothers) all increased risk of HCC [12, 53, 62-68]. Co-infection with hepatitis D, hepatitis C and HIV as well as exposure to Aflatoxin have also been identified as independent predictors of developing cirrhosis and/or HCC in Africans with CHB infection [64].

In addition, certain mutations in the HBV genome have been associated with greater risk of liver fibrosis, cirrhosis and HCC, notably the pre-core mutations G1862T/G1896A, basal core promotor mutation A1762T/G1764A and the genotype-independent pre-S2 deletion preS2 Δ 38-55 are associated with greater risk of fibrosis

and developing HCC [13, 64]. Among HBsAg-negative individuals, occult hepatitis B infection (OBI) has been found as a significant risk factor of cirrhosis and HCC [69, 70].

Access to facilities for screening, diagnosing, managing and monitoring CHB-related cirrhosis and HCC are limited in sSA. As a result, patients are often diagnosed very late with advanced disease, very poor prognosis and short survival from diagnosis [54, 64, 71, 72].

1.10. Management of chronic hepatitis B infection

1.10.1. Diagnosis of chronic hepatitis B infection

CHB diagnosis relies on the serological detection of HBsAg by enzyme-linked immunosorbent assay (EIA) in whole blood, serum or plasma. Rapid HBsAg point-of-care (POC) tests with good diagnostic accuracy have been developed to detect and diagnose CHB infection in settings where access to EIA testing may be limited or expensive [73]. Rapid HBsAg POC tests do not require sophisticated laboratories, constant electricity or highly trained personnel, thereby providing a cheap, easy to use and reliable alternative for CHB diagnosis in sSA. As of March 2020, three rapid POC HBsAg kits had acceptable analytical sensitivity to meet performance requirements for WHO prequalification [74]: Determine HBsAg 2 (manufactured by Alere Medical Co. Ltd), Vikia HBsAg (manufactured by bioMerieux SA) and SB BIOLINE HBsAg WB (manufactured by Standard Diagnostics Inc).

Rapid HBsAg POC tests have been deployed for HBV testing in the general population, health facilities and antenatal clinic in sSA. However, a recent systematic review and meta-analysis of 33 rapid POC HBsAg test kits reported lower sensitivity (72%) among patients with HIV infection [75]. Although not ideal, rapid HBsAg POC test, with or without anti-HBc test, is commonly used for HBV screening of blood products in blood transfusion services in sSA [76].

Individuals diagnosed with CHB infection will normally undergo additional laboratory investigations to determine the stage/phase of infection, presence/severity of liver disease and eligibility for antiviral therapy [33]. These investigations include HBeAg, HBV DNA viral load, complete blood count, liver function tests and assessment of liver fibrosis. Serum HBV DNA concentration, measured by real-time PCR and expressed in IU/mL, correlates with disease progression and is used to differentiate between chronic infection and chronic hepatitis [77-79].

1.10.2. Assessment of liver disease

All CHB patients should undergo liver assessment for presence of inflammation/fibrosis, features of cirrhosis and/or evidence of decompensation, ultrasound scan for HCC screening, and measurement of serum markers of liver inflammation [80].

Non-invasive assessments of liver fibrosis have been developed as alternatives to the more invasive and less accessible liver biopsy. The gold standard of these non-invasive tests is the point-of-care transient elastography (Fibroscan) test which is based on the propagation of a shear wave through the liver to measure liver stiffness as a surrogate for fibrosis [81, 82]. The diagnostic accuracy of transient elastography to predict significant fibrosis and cirrhosis in CHB infected patients has been well validated [83].

Given the costs associated with transient elastography, non-invasive biomarkers using few inexpensive and more accessible laboratory tests have been developed to predict significant fibrosis and cirrhosis in CHB infected patients in resource limited settings. These include APRI (aspartate aminotransferase-to-platelet ratio index), derived from AST and platelet count and calculated as $[\text{AST (IU/L)}/\text{AST (ULN)}] / [\text{platelet count (10}^9/\text{L)} \times 100]$; FIB-4, derived from AST, ALT, platelets and age, and calculated by $\text{FIB-4} = [\text{age (yr)} \times \text{AST (IU/L)}] / [\text{platelet count (10}^9/\text{L)} \times [\text{ALT (IU/L)}]^{1/2}]$; FibroTest, derived from alpha-2-macroglobulin, haptoglobin, apolipoprotein A1, gamma-glutamyl transpeptidase (GGT) and ALT; and GPR (gamma-glutamyl transpeptidase to platelet ratio), derived from GGT and platelet count [84-88].

High and low cut-offs for APRI have been proposed to predict presence of significant fibrosis and cirrhosis in CHB patients. Table 1.4 summarises the sensitivity, specificity, and positive and negative predictive values for APRI, FIB-4, FibroTest and GPR index to detect significant fibrosis and cirrhosis compared to transient elastography cut-offs [78, 88]. In West Africa, GPR index was shown to be more accurate than APRI and FIB-4 in staging liver fibrosis in CHB patients [88].

Table 1.4. Cut-off values of APRI, FIB-4, FibroTest and transient elastography for detecting significant fibrosis and cirrhosis [78, 88]

	APRI (low cut-off)	APRI (high cut-off)	FIB-4	FibroTest	GPR index	Transient elastography
Cirrhosis (METAVIR F4)	1.0	2.0	-	0.32-0.48	0.56	>11-14kPa
Significant fibrosis (METAVIR >F2)	0.5	1.5	1.45 (low) 3.25 (high)	0.58-0.75	0.32	>7-8.5kPa

Abbreviations: APRI, aspartate aminotransferase-to-platelet ratio index; GPR, gamma-glutamyl transpeptidase; kPa, kilopascal.

Current international guidelines recommend antiviral therapy for CHB patient with or at high risk of cirrhosis [33, 89, 90]. The first ever WHO guidelines on prevention, care and treatment of CHB infection [80] recommend using APRI in low- and middle-income settings (LMICs) to identify patients with significant fibrosis or cirrhosis and to determine treatment eligibility.

1.10.3. Treatment of chronic hepatitis B infection

i. Goals of treatment

Antiviral therapy for CHB infection aims to suppress HBV; reduce (or reverse) necroinflammatory change and hepatic fibrosis; and prevent cirrhosis, decompensation, liver failure, HCC and death. Currently available antiviral agents are highly effective in suppressing HBV replication and reducing risk of HCC [91] and have been shown to induce regression of fibrosis and cirrhosis [92].

Treatment is also indicated in pregnant women with high HBV DNA viral load >200,000IU/mL and/or positive HBeAg to prevent HBV mother-to-child transmission (MTCT) [93-95]. When indicated as prophylaxis, the goal of treatment is primarily for prevention of MTCT and treatment is usually discontinued shortly after delivery [80].

ii. Hepatitis B cure

In contrast with chronic hepatitis C infection, current HBV therapy with nucleos(t)ide analogues (NUC) or interferon are not curative [96]. Currently, 3 types of HBV cure are defined:

- a. **Partial cure:** defined by persistently low or undetectable HBV DNA in serum, normalization of serum transaminases, inactive liver disease with persisting presence of HBsAg [97]. Partial cure can be achieved on treatment with standard therapy with NUC. The presence of HBsAg and cccDNA pose risks of reactivation, which can be spontaneous or follow immunosuppression [98].
- b. **Functional cure:** defined as sustained undetectable circulating HBsAg (HBsAg loss) and HBV DNA after a finite course of treatment, with or without HBsAg seroconversion [97]. HBV functional cure is associated with reduced risk of HCC, cccDNA inactivity and improved clinical outcomes [96, 98]. However, HBsAg seroconversion is rare in CHB patients on NUC therapy, reported a rate of 1% per year [99].
- c. **Sterilizing cure:** defined by elimination of both cccDNA and integrated HBV DNA [97]. This is unlikely to be feasible in the foreseeable future.

A combination of factors make HBV cure difficult to achieve: persistence of cccDNA in the nucleus of the human hepatocyte, integration of HBV DNA genome into the host genome, and impaired immune response against HBV [96, 97]. cccDNA in the hepatocyte nucleus and integrated HBV DNA in host genome are protected from host immune response and NUC treatment. Thus, they serve as reservoirs for HBV replication and HBsAg production after HBV suppression following long term therapy. Therefore, therapies for HBV cure must target both cccDNA and integrated HBV DNA and will likely require multiple combination therapies with concerted action to suppress replication, inhibit antigen production and stimulate robust immune response [96, 97]. Numerous new HBV drugs are currently in development, including direct acting antivirals (DAAs) that target various stages of the HBV replication cycle, innate immunomodulators, therapeutic vaccines, checkpoint inhibitors and monoclonal antibodies [96, 97].

iii. Antiviral drugs

Eight antiviral agents comprising pegylated interferon (PEG-IFN) and six NUCs (Lamivudine, adefovir, entecavir, telbivudine, tenofovir disoproxil fumarate, tenofovir

alafenamide, and emtricitabine) are approved for CHB treatment. PEG-IFN therapy has a finite treatment duration and higher rate of HBsAg loss. A 1-year course of PEG-IFN α results in an overall HBsAg seroclearance rate of 2-3% at the end of treatment, increasing to 3-8% after 3 years of post-treatment follow-up. However, use of PEG-IFN in LMICs is limited by cost, adverse effects, and requirements for injection and careful monitoring. IFN is therefore not recommended by the WHO for CHB treatment [80].

The nucleot(s)ide analogue agents all target the HBV polymerase through different mechanisms of action and with different inhibitory capacity and resistance barrier. Entecavir, tenofovir disoproxil fumarate (TDF) and tenofovir alafenamide (TAF) have a high HBV resistance barrier and therefore make up the first line treatment for CHB infection.

CHB infected patients treated with entecavir had better viral suppression, improved liver histology and normalisation of ALT levels at 48 and 72 weeks compared to those treated with adefovir or lamivudine [100, 101]. Entecavir treated patients also had increased HBsAg loss and anti-HBe seroconversion (RR 2.83; 95% CI 1.27-6.33) compared to patients treated with lamivudine plus adefovir.

In HBeAg-positive patients, treatment with tenofovir was associated with viral suppression (RR 5.71; 95% CI 3.35-9.73) and ALT normalisation (RR 1.25; 95% CI 1.01-1.55) at 48 weeks compared to treatment with adefovir [102]. Tenofovir treatment was also associated with regression of fibrosis and resolution of cirrhosis. A network meta-analysis that included 5,073 HBeAg-positive and 2,604 HBeAg-negative nucleoside-naïve persons showed higher probability of viral suppression at 1 year in tenofovir treated patients (94.1% in HBeAg-positive and 97.6% in HBeAg-negative patients). Among patients on entecavir, complete viral suppression was achieved in 64.5% of HBeAg-positive and 91.9% of HBeAg-negative patients [80].

TAF is increasingly being recommended and/or used to in CHB treatment among key high risk populations, including older patients and/or those with renal, bone or metabolic co-morbidities [33]. The rates of virologic response is similar both HBeAg positive and HBeAg negative patients treated with TDF or TAF. However, TAF was shown to have higher proportion ALT normalisation and better safety profile with smaller decrease in bone mineral density and smaller median change in estimated glomerular filtration rate [103]. Table 1.5 summarises the HBsAg seroclearance rates with PEG-IFN or NUC therapy.

Table 1.5 HBsAg seroclearance rates with PEG-IFN or NUC therapy (modified from Wong GH et al).

	HBeAg-positive	HBeAg-negative
PEG-IFN α -2a (180ug) - EOT	3 (EOT; 5 in genotype A; 3 in non-genotype A)	7 (EOT; 11 in genotype A; 3 in non-genotype A)
PEG-IFN α -2a (180ug) – 3 years treatment	7	9
Entecavir (0.5mg) – 1 year	2	0
Entecavir (0.5mg) – 5 years	3	1
Tenofovir Disoproxil Fumarate (300mg) – 1 year	3	0
Tenofovir Disoproxil Fumarate (300mg) – 3 years	5	3
Tenofovir Alafenamide (25mg) – 1 year	1	0
Tenofovir Alafenamide (25mg) – 3 years	4	3

iv. Indications for antiviral therapy

Owing to the low rates of functional cure using current antiviral therapy, CHB treatment is often infinite. Therefore, indications for antiviral therapy have been targeted at CHB patients with or at high risk of severe liver disease, who are believed to benefit most from treatment. Current international guidelines [33, 89, 90] have used a combination of demographic, clinical and virological criteria to determine best indications for treatment. However, these guidelines are often complex and heterogenous, with different regions recommending varied criteria for treatment eligibility, monitoring, HCC surveillance and discontinuation of NUCs. Moreover, the European, American and Asia-Pacific guidelines often require sophisticated assessments that are not routinely available in CHB endemic LMICs, making these guidelines unfeasible for Africa.

In 2015, the WHO, with an aim to simplify CHB treatment and monitoring in LMICs, released its first ever guidelines for management of CHB [80]. The guidelines recommend antiviral therapy for CHB patients with evidence of cirrhosis, either by clinical criteria, Fibroscan score or APRI score >2.0. In patients without cirrhosis,

antiviral therapy is recommended in those with persistently abnormal ALT ($>19\text{U/L}$ for women and $>30\text{U/L}$ for men) and HBV DNA viral load $>20,000\text{IU/mL}$ [80]. Figure 1.9 shows the algorithm of the 2015 WHO recommendations for CHB management.

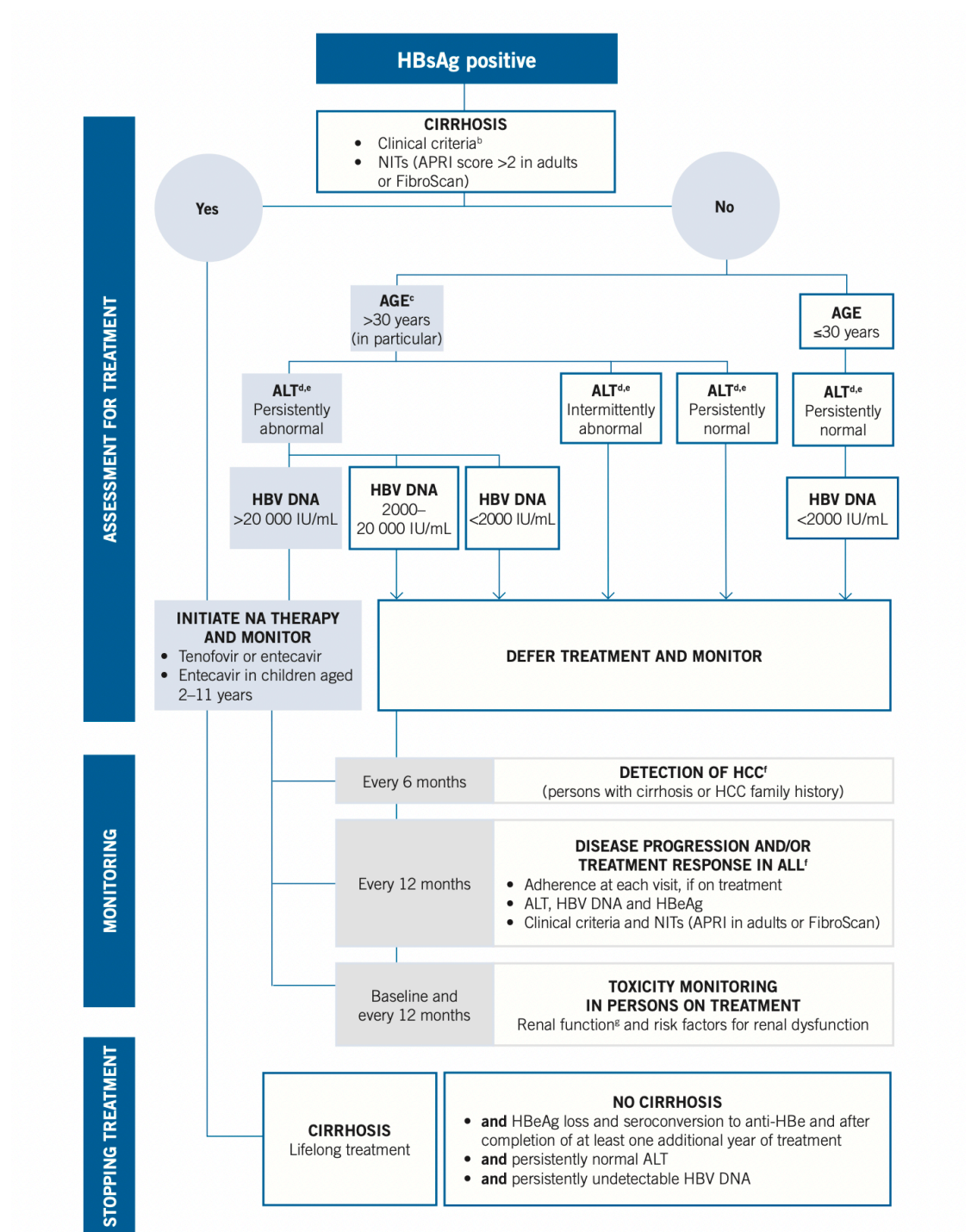


Figure 1.9. Algorithm of WHO recommendations on the management of persons with CHB infection

When benchmarked against the EASL clinical practice guidelines for CHB [33], the WHO guidelines, which were developed using mostly data from Asian and Western CHB cohorts, had poor sensitivity (49.0%) in detecting patients in need of treatment in a large CHB cohort in Ethiopia [104]. Only 35.7% of patients with compensated cirrhosis in this Ethiopian cohort fulfilled the WHO treatment eligibility criteria, with most patients (51.6%) who met treatment eligibility having decompensated cirrhosis. There is, therefore, an urgent call for revised international guidelines adapted to the CHB epidemic in Africa and aimed at prioritising earlier antiviral therapy for patients with advanced fibrosis and compensated cirrhosis [105]. Such guidelines will prevent development of cirrhosis, onset of decompensated cirrhosis and reduce risk of HCC [106].

v. Current treatment strategies

The first line of antiviral treatment for CHB infection recommended in all international guidelines comprises Tenofovir in the general adult population and entecavir in children aged 2-11year [33, 80, 89, 90]. Lifelong treatment is recommended for patients with cirrhosis at time of treatment initiation. In non-cirrhotic patients, antiviral therapy can be stopped after 2-3 years treatment with persistently undetectable serum HBV DNA. Studies have found that stopping NUCs in carefully selected patients, mainly HBeAg-negative patients with low HBsAg and persistently undetectable HBV DNA, can result in higher rates of HBsAg seroclearance over time [107, 108]. The annual incidence of HBsAg seroclearance was estimated at 1.8% among HBeAg-negative patients who stopped NUC treatment compared to 0.9% who continued treatment, with a cumulative HBsAg seroclearance incidence of 13% at 6 years of stopping treatment [109].

Stopping NUC treatment is however associated with high rates of clinical relapse. Jeng et al reported that 61% of patients who stopped NUC treatment had either rebound HBV DNA viral load $>2,000\text{IU/mL}$ or increased ALT >2 times upper limit of normal(ULN), and 2.3% had hepatic decompensation. With the limited capacity to diagnose and manage acute hepatic decompensation and flares in LMICs, particularly in Africa [64], there is no clear consensus on stopping treatment in CHB patients.

Whilst the Asian-Pacific clinical practice guidelines recommend treatment for HBeAg-negative patients with HBV DNA $>2,000\text{IU/mL}$ and ALT $>1-2\times$ ULN [90], the existing 2015 WHO guidelines recommend deferring antiviral therapy for these patients [80].

Earlier initiation of antiviral therapy in these patients, often considered to be in the “grey zone”, could reduce the risk of HCC [110]. It is expected that the revised WHO treatment guidelines for CHB infection, expected in 2023, will widen the scope of treatment and could lower the thresholds for HBV DNA (down to 2,000IU/mL) and non-invasive markers of fibrosis (APRI score down to 1 or 1.5). Local data from longitudinal African cohorts is urgently needed to determine optimal cut-offs and best treatment strategy for non-cirrhotic CHB patients in the region. The requirement to detect and continually assess HBV DNA to select and monitor CHB patients pose a barrier to decentralising CHB treatment in Africa, where capacity for HBVDNA quantification is often limited to major referral or tertiary centres in urban areas. As an alternative, DNA-free scoring systems have been developed to identify patients in need of treatment. The TREAT-B score [111], which uses ALT and HBeAg, presents an economically viable option for identifying CHB patients in need of treatment in Africa. For a TREAT-B score of 2 or more (HBeAg-positive PLUS ALT ≥ 20 U/L; or HBeAg-negative PLUS ALT ≥ 40 U/L), the specificity and sensitivity for treatment eligibility was 85% and 77% respectively.

There is increasing debate over a test-and-treat approach for CHB infection, where the existing treatment eligibility criteria are removed, and all HBsAg-positive patients receive antiviral therapy irrespective of evidence or risk of liver disease. Strong arguments are presented both for and against such **treat all strategy**. Whilst a treat all strategy will provide treatment earlier and could prevent development and progression of liver disease, a significant proportion of individual who do not need (or want) treatment will be exposed to infinite courses of antiviral therapy. The experts not in favour of a treat all strategy argue the approach will pose adherence challenges (clinically healthy patients are less likely to adhere to long term daily treatment regimen), increase risks of side effects and flares among those who discontinue therapy, and carry a significant economic burden (largely incurred from overtreating patients). Therefore, a treat-all strategy may not be feasible or cost-effective in Africa.

Finally, HBV antiviral therapy is now recommended as prophylaxis to prevent reactivation in immunosuppression and to prevent mother-to-child transmission (MTCT) of HBV. Among pregnant women with high HBV DNA $>200,000$ IU/mL and positive HBeAg positive, antenatal tenofovir prophylaxis reduced the risk of HBV MTCT [93]. In addition to the birth dose vaccination given with 24 hours of birth, the WHO in July 2020, recommended antiviral prophylaxis for HBsAg positive pregnant women with HBV DNA $>200,000$ IU/mL or positive HBeAg to prevent MTCT [112].

1.10.4. Prevention of chronic hepatitis B infection

Vaccination is the cornerstone of CHB prevention. With the availability of a safe, effective, and cheap vaccine against HBV, the WHO recommended universal childhood immunization through the Expanded Program of Immunization (EPI) since 1991. The infant hepatitis B vaccines, administered at 6, 10 and 14 weeks, have been adopted by the 190 WHO Member States as of the end of 2020 [113]. In 2020, the coverage of the three doses of the hepatitis B vaccine (HepB3) was 83% globally and 72% in Africa [114]. The WHO reported that the HepB3 coverage rate had declined globally and in Africa from 85% and 75% respectively in 2019, to 80% and 71% in 2021 (<https://immunizationdata.who.int/pages/coverage/HEPB.html>). Similar declines in coverage between 2019 and 2021 were also reported in The Gambia where the coverage of HepB3 declined from 93% in 2018 to 82% in 2021.

The hepatitis B vaccine has a protective efficacy of 83% against infection and 95% against chronic carriage [115-117] with longitudinal serosurveys demonstrating long-term immune response, vaccine efficacy and protection from CHB infection [118-120]. Vaccination significantly reduces both HBV carriage [115, 121] and incidence of HCC in children and young adults [122-127]. Since CHB infection is the leading cause of HCC in Africa, it is expected that routine infant HBV vaccination along with reduced HBV carriage will impact HCC incidence in the region. The Gambia Hepatitis Intervention Study [128] is expected to provide robust epidemiological data on the true impact of infant HBV vaccination in reducing HCC in Africa.

Overall, infant HBV vaccination had already prevented an estimated 210 million new CHB infections by 2010 and will have averted 1.1 million deaths by 2030 [27]. The impact of infant HepB3 vaccination in preventing death is expected to be more significant over time, given that most deaths from CHB occur later in life from cirrhosis and HCC. Therefore, it is estimated that infant HepB3 vaccination averted 640,000 deaths between 2000-2019 and could avert up to 1.6 million deaths (460,000-2.9 million) between 2020-2030 [129].

Although effective in reducing horizontal HBV transmission, the infant HBV vaccination alone does not prevent HBV mother-to-child transmission. Therefore in 2004, the WHO recommended the hepatitis B “birth dose” vaccine (HepB-BD) to be administered within the first 24 hours of birth [130]. Although 111 WHO Member States have adopted the HepB-BD, uptake of the timely administration of the vaccine has remained poor

globally at 42% in 2021. Coverage of timely HepB-BD is particularly poor in Africa where 17% of babies receive a timely HepB-BD and only 14 countries have incorporated universal HepB-BD vaccination in their national strategy (WHO Immunization Coverage, 14th July 2022. <https://www.who.int/news-room/fact-sheets/detail/immunization-coverage> Accessed 25th October 2022). Perinatal HBV infection is associated with higher risk of developing HCC and/or needing antiviral therapy in adulthood [12]. A recent modelling study estimated that increasing the coverage of timely HepB-BD vaccination in the WHO Africa region to 90% could avert 558,000 (412,000-830,000) HBV-related deaths among the 2020-2030 birth cohort [131]. Therefore, scaling up timely HepB-BD vaccination to prevent HBV MTCT is especially important in Africa.

In addition to HepB-BD and infant HepB3 vaccines, the WHO in 2020 recommended antiviral prophylaxis during third trimester in pregnant women with high HBV DNA viral load >200,000IU/mL or positive HBeAg, together with administration of hepatitis B immunoglobulin (HBIG) at birth where available [112]. Currently, HBsAg testing is not routinely offered in many antenatal services in Africa, and capacity to quantify HBV DNA or test HBeAg serology is scarce and limited to central reference centres. Therefore, implementing the current recommendations for antenatal antiviral prophylaxis remains challenging in many African settings.

1.11. Occult hepatitis B Infection

1.11.1. Definition and diagnosis

Occult hepatitis B virus infection (OBI) is defined as the presence of replication-competent HBV DNA in the liver tissue or blood of individuals tested negative for HBsAg using chemiluminescent or enzyme immuno-assays [132, 133]. The ideal diagnosis of OBI relies on HBV DNA detection by nucleic acid tests (NAT) including polymerase chain reaction (PCR) in liver tissue. However, given the complexity and lack of standardised assays for liver tissue assessment of HBV DNA, OBI is often diagnosed by serum HBV DNA detected in HBsAg-negative individuals. Recommended PCR methods include nested PCR targeting at least 3 different viral genomic regions, real-time PCR assays, or droplet digital PCR [134-136]. The serum concentration of HBV DNA in OBI is usually less than 200IU/mL (true OBI). Individuals with either “S-escape” or S promoter mutations (resulting in modified HBsAg that are not detectable by commercially available HBsAg assays) or with pre-S1/S2 variants

(that affect HBsAg expression) are often termed to have false OBI, characterised by serum HBV DNA >2,000IU/mL [137-143].

The pathophysiology of OBI is not well understood. OBI is often regarded as a phase in the natural history of CHB infection in which replicative activity and gene expression of HBV DNA and cccDNA are suppressed, resulting in extremely low rates of virion production [133, 144]. OBI is categorised as either seropositive OBI (anti-HBc and/or anti-HBs positive) or seronegative OBI (anti-HBc and anti-HBs negative) based on the HBV-specific antibody profiles [132]. Seropositive OBI, which comprises up to 80% of OBI cases, is thought to arise following HBsAg loss either following acute or chronic hepatitis B infection [132]. Seronegative OBI (also termed “primary OBI”) is estimated to comprise 1-20% of OBI cases and is thought to develop either from progressive loss of HBV antibodies or in individuals who remain antibody negative from the beginning of infection [132, 145, 146].

1.11.2. Epidemiology and impact

The prevalence of OBI varies depending on the population studied, sensitivity of assays (HBsAg and HBV DNA) used, volume of blood samples tested for HBV DNA and whether single or serial samples were tested [132]. Very few population-based surveys for OBI targeting the general population have been published, therefore the global burden of OBI in the general population has not been well established. A recent systematic review and meta-analysis estimated the global OBI prevalence in the general population was 0.82% (95% CI 0.69-0.96) [147]. Our group conducted a detailed systematic review and meta-analysis to estimate OBI prevalence in sub-populations (blood donors, patients with advanced liver disease, high risk groups and low risk groups) across different HBV endemicity regions. In areas of high HBV endemicity such as Africa, we found that OBI prevalence was 0.98% (0.44-1.72) among blood donors, 8.9% (5.5-13.1) in patients with advanced chronic liver disease, 12.0% (3.4-24.7) in high risk groups and 5.1% (2.7-8.0) in low risk groups [148]. The pooled sensitivity and specificity of anti-HBc to identify OBI was 77% (95% CI 62-88) and 76% (68-83) respectively.

OBI could have important clinical and public health impact. HBsAg-negative individuals with OBI, in particular those with seronegative OBI, could represent an important source of new HBV infections in blood transfusion services in Africa where HBsAg with or without anti-HBc testing is the primary screening tool for HBV in blood donors and

blood products [76]. Moreover, there is increasing evidence that OBI is associated with advanced liver disease, particularly HCC [149, 150]. Therefore, OBI must be taken into account when developing strategies to prevent new CHB infections and reduce HBV related morbidity and mortality; assessing cost-effectiveness of HBV interventions and estimating impact of prevention and control measures (such as DALYs and deaths averted by vaccinations).

1.12. Co-infection with hepatitis B and HIV

1.12.1. Epidemiology

Of the approximately 37 million individuals infected with HIV worldwide, 5-20% are coinfecting with CHB [151]. The rates of coinfection with HIV and HBV vary significantly between regions and sub-populations, often reflecting transmission patterns. In Africa, it is estimated that up to 28.4% of people living with HIV (PLHIV) have HBV coinfection, with a recent systematic review and meta-analysis estimating the prevalence of coinfection at 10.5% (95% CI 9.6-11.3). Coinfection was highest in Western Africa where reported median prevalence was 11.5%. Among pregnant women and children aged 18months-17years, the median prevalence of HIV-HBV coinfection was 3.8% (0-13%) and 7.4% (1.2-7.8%) respectively [152, 153]. The reported rate of OBI among PLHIV in South and West Africa was 10-33%.

1.12.2. Impact of HIV on chronic hepatitis B infection

HIV coinfection increases risk of chronicity in acute HBV infection, accelerates HBV replication and progression, weakens HBV immune response, lowers rates of clearance and seroconversion of HBeAg, HBcAg and HBsAg, and accelerates progression to cirrhosis and HCC [154-160]. Host immunosuppression manifested by CD4⁺ T cell depletion is the primary underlying route of HIV infection altering the natural course of HBV infection. Consequently, HIV-HBV coinfecting patients have higher HBV replication resulting in increased risk of cirrhosis and a six-fold higher risk of HCC incidence in cirrhotic patients [161]. Thio et al found that the risk of liver-related mortality increased 13-fold among men with HIV-HBV coinfection (14.2/1000) compared to men with either HIV-1 (1.7/1000) or HBsAg (0.8/1000) mono-infection [154]. Certain HBV mutations which are associated with increased risk of HCC, including T1762/A1764 and pre-S deletions, are found to be more common in HIV-HBV coinfecting patients [162].

1.12.3. Management recommendations

The WHO recommends routine HBV testing as part of the initial assessment for all HIV patients [163]. Treatment for HIV-HBV coinfection must start as early as possible irrespective of CD4 count, HBV DNA viral load or presence/risk of liver disease. Highly active antiretroviral therapy (HAART) for HIV-HBV coinfecting patients must comprise at least two HBV-active antiretroviral drugs – usually Tenofovir plus Lamivudine (3TC) or Emtricitabine (FTC).

Risk of hepatotoxicity can limit tolerability to antiretroviral and other treatment (such as anti-TB treatment) in patients with HIV-HBV coinfection in Africa [164]. Studies in Asia showed that HIV-HBV coinfecting individuals had 2-3 fold increase in hepatotoxicity (15.6-16.0 episodes per 100 person-years) compared to individuals with CHB (or hepatitis C) mono-infection (4.5-8.0 episodes per 100 person-years). There are conflicting reports that CHB infection could blunt the initial immune recovery (CD4 count) after initiation of HAART.

1.13. Elimination of hepatitis B virus as a public health threat

1.13.1. Global HBV elimination – rationale and targets

Given the available opportunities to prevent, diagnose, manage and monitor CHB infection and the expected economic impact of reducing CHB infection and HBV-related morbidity and mortality, there is strong rationale for HBV elimination.

The World Health Assembly (WHA), in response to the increasing burden of CHB morbidity and mortality and in view of the available preventive and management strategies for CHB infection, adopted a resolution in 2015 to improve viral hepatitis prevention, testing and treatment worldwide [165]. Subsequently, the United Nations General Assembly and the WHO respectively incorporated viral hepatitis elimination in the 2030 Agenda for Sustainable Development [166] and the WHO Global Health Sector Strategy on Viral Hepatitis 2016-2021 [167, 168].

The WHO aims to, by 2030, eliminate viral hepatitis as a major public health threat by reducing new HBV infections by 90% and HBV-related deaths by 65% targets (Figure 1.10).

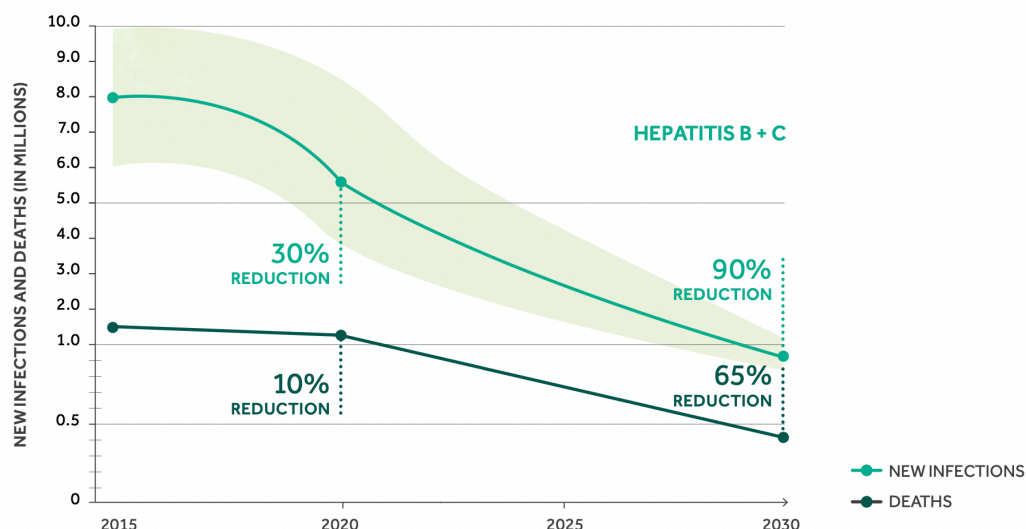


Figure 1.10. WHO impact targets for hepatitis B elimination [167]

In addition, interim targets to reduce new infections and deaths by 2020 were set, as well as service coverage targets for hepatitis B vaccination, prevention of mother-to-child transmission, other prevention strategies, diagnosis, and treatment. Table 1.6 summarises these key targets impact and service coverage targets and timelines.

Table 1.6. Summary of WHO impact and service coverage targets for HBV elimination

Target area	Baseline 2015	2020 targets	2030 targets
IMPACT TARGETS			
Incidence: new cases of HBsAg infection	8 million new infections	30% reduction; 1% prevalence of HBsAg among children	90% reduction; 0.1% prevalence among children
Mortality: HBV related deaths	1.1 million deaths	10% reduction	65% reduction
SERVICE COVERAGE TARGETS			
Coverage of HepB3 (third dose) infant vaccination	82%	90%	90%
Timely HepB-BD coverage and	38%	50%	90%

other PMTCT approaches			
Blood safety	39 countries do not routinely test all blood donations for transfusion-transmissible infection. Only 89% of donations screened in a quality-assured manner	95% of donations screened in a quality-assured manner	100% donations screened in a quality-assured manner
HBV diagnosis	<5% infections diagnosed	30%	90%
HBV treatment	<1% receiving treatment	5 million people receiving HBV treatment	80% of eligible CHB patients treated

1.13.2. Current progress and challenges in Africa

The WHO Viral Hepatitis Scorecard 2021 estimates that 70% of new hepatitis B infection occur in Africa with 33 countries having HBsAg prevalence >1% among children younger than 5 years and approximately 4.5 million children younger than 5 years infected with CHB in the region [44]. Routine infant HepB3 vaccination coverage in Africa was 72% in 2021, with only 27 countries achieving 90% coverage (an increase from 23 countries in 2019). Coverage of timely birth dose vaccination was 10% in 2021 with only 14 African countries adopting birth dose vaccination within national services. Only 2% of Africans with CHB infection knew their status, of whom only 0.1% had access to treatment, and only 80% of blood donations are screened with quality assurance [44].

In response to the WHO call for hepatitis elimination, 28 African countries have now set up national hepatitis programs, either as standalone programs or integrated within existing HIV services. Twenty-one countries have developed strategic plans for

hepatitis prevention and control and 17 countries have hepatitis testing and treatment guidelines.

Insufficient implementation, standalone programs, low hepatitis awareness and inadequate financing are key challenges to the viral hepatitis response in Africa. In 2021, the WHO established the 2021-2030 Framework for Integrated Multisectoral Response to Tuberculosis, HIV, Sexually Transmitted Infection and Hepatitis in the African region. One of the aims of this framework is to support the introduction of birth dose vaccination in 35 countries and increase diagnosis and treatment by 30%.

1.14. Hepatitis B infection in The Gambia

1.14.1. The Gambia – generalities

The Gambia, with a total area of 11,295 km² and estimated population of 2.4 million inhabitants, is the smallest country in mainland Africa. It is a predominantly rural country with increasing migration of the population to urban areas. Total fertility rate is 4.4 births per woman with 79% of pregnant women attending 4 or more antenatal clinic visits and 84% of births attended by a skilled provider (88% in urban areas, 75% in rural areas). Infant immunization coverage is high with 77% of children aged 12-23 months receiving all age-appropriate vaccinations [169].

Life expectancy in the Gambian population was estimated at 65 years in 2020, an improvement over the last decade. According to the latest 2020 WHO data, liver-related mortality represented 2.51% of all deaths in The Gambia. With the challenges of the Gambia Cancer Registry and the lack of national registries for cirrhosis, it is likely that liver-related deaths are underestimated. Advanced liver disease is not well diagnosed due to the lack of liver dedicated centres, liver specialists and high-quality diagnostic tools. There are only two liver clinics (one in the MRC Unit The Gambia and second at the Edward Francis Small Teaching Hospital) and one consultant gastroenterologist in The Gambia.

1.14.2. History of hepatitis B research and implementation in The Gambia

The Gambia has a long and very strong track record for hepatitis B research. One of the earliest hepatitis related studies, investigating hepatitis associated antigen, cirrhosis and primary liver cancer in The Gambia, was published in the Transactions of the Royal Society of Tropical Medicine and Hygiene in January 1975 [170]. Since then, several landmark studies from The Gambia have improved knowledge on HBV

epidemiology [171, 172], the patterns, modes and risk factors of transmission [28, 172-175], natural history of infection and factors associated with virus clearance [12, 20, 176], efficacy and cost-effectiveness of vaccination [116, 117, 120, 177], immune response and level of protection following hepatitis B vaccination [118, 178, 179], long-term protection after infant vaccination [119, 180, 181], and risk factors of advanced liver disease in CHB infection [53, 62, 68, 182-184]. Studies have also evaluated the use of dried blood spots to detect HBsAg and alpha-fetoprotein in field-based research and clinical care [185], the applications of real-time PCR to quantify HBV DNA viral load [186, 187], and validated rapid point-of-care tests for detection of HBsAg in field and laboratory settings [73].

In July 1986, The Gambian Ministry of Health in collaboration with the MRC Unit The Gambia and the WHO through the International Agency for Research on Cancer (IARC), set up the Gambia Hepatitis Intervention Study (GHIS). Over a 4-year period, the study progressively introduced the then new hepatitis B vaccine into the routine infant vaccination program [128]. The study followed up vaccinated infants and unvaccinated controls over 30 years to study the impact of infant hepatitis B vaccination on HBV infection, the protection against chronic carriage and duration of robust immune response [115, 188-191]. The final phase of the GHIS study, which was concluded in 2020, is expected to demonstrate the impact of infant hepatitis B vaccination in preventing cirrhosis and HCC in adulthood.

Building on the GHIS study, The Gambia became the first African country to implement routine hepatitis B infant vaccination in 1991, and the first to adopt hepatitis B birth dose vaccination in 1997. The Gambia has consistently maintained high coverage (>90%) of the three-dose infant hepatitis B (HepB3) vaccination, administered with other EPI vaccines. However, coverage of the timely hepatitis B birth dose (HepB-BD) vaccine, administered within 24 hours of birth, has remained poor, at 1.1% in 2015 [192]. As the WHO aims to eliminate viral hepatitis by 2030 and African countries begin to set up national hepatitis programs, it became increasingly important and urgent to demonstrate best practice for scaling up hepatitis B interventions (such as screening and treatment) and to simplify diagnosis, assessment for treatment eligibility, and monitoring. The acceptability, feasibility and cost-effectiveness of a screen-and-treat program for CHB infection in endemic African settings was demonstrated in The Gambia [193, 194] and DNA-free scores developed to simplify selecting CHB patients for treatment [111].

In 2016, the Gambian Ministry of Health set up a National Hepatitis Programme (NHP), integrated within the existing National HIV/AIDS Control Programme (NACP). With support from key stakeholders including the WHO Country Office and the MRC Unit The Gambia, the NHP developed a National Viral Hepatitis Prevention and Control Strategic Plan 2018-2022 and a National Viral Hepatitis Prevention and Control Policy 2018-2022 [195, 196]. The strategic plan and control policy were launched by the Ministry of Health in October 2017, setting the scene for coordinated national interventions towards hepatitis elimination. The Gambia is currently developing its first national guidelines for the prevention and management of chronic hepatitis, adapted from the 2015 WHO guidelines, and is exploring effective ways to implement viral hepatitis services within existing programs (such as the HIV services). Although systematic HBsAg screening has been implemented in all national blood banks and HIV treatment centres, it is not systematically offered in other groups, in particular in pregnant women at risk of mother-to-child transmission.

Despite the long track record of hepatitis B research and vaccination in The Gambia, knowledge and awareness about viral hepatitis is poor [193, 197]. Increasing hepatitis awareness is central in the national strategic plan and upcoming guidelines. As such, campaigns have been initiated with the support of the NHP, the Hepatitis Patients' Association and Civil Society Organisations to increase hepatitis awareness, prioritizing healthcare workers and women of childbearing age.

1.14.3. Prevalence of chronic hepatitis B infection and burden of liver disease

The Gambia is highly endemic with CHB infection, with 8.8% (95% CI 7.9-9.7) of the adult general population, 13.0% (12.1-13.9) of blood donors and 9.0% of pregnant women infected [193, 198]. Prevalence of CHB infection among children younger than 5 years is less studied in The Gambia. However, horizontal transmission in early infancy was the primary route of CHB transmission in The Gambia, with rates of infection increasing with age during the first decade of life [28].

Serial serosurveys and longitudinal studies of historic CHB cohorts have increased our understanding of the natural history of CHB infection in The Gambia. HBV genotype E is the predominant circulating genotype (up to 95%) in The Gambia with more than 90% of individuals having HBeAg-negative CHB infection [12, 111, 199]. Over a median follow up period of 28.4 years, HBsAg and HBeAg clearance was observed

among treatment naïve CHB patients at a rate of 1.0% (0.8-1.2) and 7.4% (6.3-8.8) respectively.

Significant liver fibrosis was reported in 5.5% (95% CI 3.5-9.0) of CHB patients followed up in The Gambia [12]. The risk factors of severe liver disease include infection with HBV genotype A, chronic aflatoxin B1 exposure and perinatal CHB infection. Among patients screened from the general population and blood bank, 4.4% (95% CI 2.5-7.7) and 9.7% (95% CI 6.8-13.6) were eligible for treatment, respectively, with males being more likely to meet treatment eligibility criteria (OR 4.35, 95% CI 1.50-12.58) [193]. High HBV DNA viral load is an important risk factor of cirrhosis and HCC [20], with more than two-thirds of HCC cases in The Gambia attributable to CHB infection [53, 200]. The incidence of HCC in treatment-naïve CHB patients was estimated at 55.5 (24.9-123.5) per 100,000 carrier-years [12].

1.15. The PROLIFICA programme in The Gambia

In 2011, the PROLIFICA (Prevention of Liver Fibrosis and Cancer in Africa, www.prolifica.africa) program was set up in The Gambia to advance research and improve capacity for prevention, diagnosis and treatment of viral hepatitis and liver disease. The PROLIFICA program, funded mainly by the European Council and MRC UK, launched several research projects to characterise the CHB epidemic, generate evidence for best practice to scale up interventions, develop alternative simplified strategies for diagnosis and management adapted to a low-income setting, study new biomarkers for early detection of HCC, and assess the cost-effectiveness of interventions. Findings from the PROLIFICA program have informed the WHO elimination targets, policy and guidelines, and have supported the Gambia national strategic plan.

The PROLIFICA program has now set up the largest longitudinal African cohort of CHB infected adults screened from the general population and blood banks. Patients in this cohort, enrolled between 2011-2014, were reassessed annually with detailed clinical, epidemiological, biochemical and virological assessment. The longitudinal data and serial samples collected during annual follow-up provide a unique resource to study CHB evolution and treatment outcomes in African patients. This cohort, and the larger PROLIFICA program in The Gambia, hosted the studies that make up this PhD thesis.

I joined the PROLIFICA group in The Gambia as research clinician in 2012 and have remained at the forefront of all clinical activities since then. During the period before

my PhD (2012-2016), I was responsible for enrolling participants into the clinical cohorts, conducting liver assessments, evaluating treatment eligibility and following up and monitoring treated and treatment-naïve patients. Between 2018-2022, as part of my PhD, I led the PROLIFICA research group in The Gambia, re-invited all patients in the original PROLIFICA cohort for reassessment, expanded the clinical cohort, and helped set up additional projects on HBsAg antenatal screening, HBV PMTCT and HBsAg testing in infants born to HBsAg positive mothers.

2. CHAPTER 2: AIMS AND OBJECTIVES OF PhD

2.1. Rationale

CHB infection is a major public health problem. It is estimated that 296 million individuals have CHB infection, of whom 82 million individuals live in sub-Saharan Africa [41, 42]. Globally, CHB infection accounted for more than 800,000 deaths in 2019, an increase by 5.9% from 1990 and 2.9% from 2015 [42]. Most CHB related deaths occur from cirrhosis and hepatocellular carcinoma (HCC).

HCC is a leading cause of malignancy in adult men and women in West Africa [54, 201]. Most HCC cases are diagnosed very late, with very short survival from diagnosis [54, 64], making HCC a major cause of premature death in West African adults. CHB infection is the most common cause of HCC in West Africa, with more than two-thirds of HCC cases among individuals aged less than 50 years being attributable to chronic HBV infection [53, 202].

In 2015, the World Health Organisation (WHO) set targets to eliminate viral hepatitis as a public health threat by 2030 [167]. The ambitious WHO targets of reducing new hepatitis infections by 90% and hepatitis related deaths by 65% can only be achieved by scaling up prevention, diagnosis, and treatment interventions. Currently, there is no evidence on best practice for scaling up these interventions in Africa. As a result, scale up of interventions is lowest in the African region, which continues to record the highest incidence and prevalence of CHB infection in the general population and under 5-year-olds [42]. Moreover, modelling data has predicted that, with the current status quo, Western Africa will be the last region in the world to achieve the WHO elimination targets for prevention and care [27].

It is therefore urgent to characterise the HBV epidemic in West Africa and describe the current challenges and opportunities for HBV prevention, diagnosis and management. The PROLIFICA research program in The Gambia provides a unique opportunity to study the CHB evolution in the general and key populations, investigate the rates and risk factors of disease progression in adults, the impact of antiviral therapy on preventing advanced liver disease and HBV-related death, and the drivers and rates of HBV mother-to-child transmission. The PROLIFICA program also offers a platform to assess the feasibility of adaptive HBV prevention strategies in The Gambia, including universal incorporation of timely hepatitis B birth dose vaccination and HBsAg antenatal screening.

2.2. Aim

The main aim of this PhD was to describe the long-term (7-10 year) evolution of chronic HBV infection and HBV-related morbidity and mortality among treated and treatment-naïve African adults recruited from the general population in The Gambia.

2.3. Objectives

Specifically, this PhD aimed to address the following objectives:

- i. Assess the mortality from CHB infection among a population-based cohort of adults followed up in The Gambia
- ii. Describe the epidemiology of advanced liver disease in The Gambia, stratified by age and underlying cause
- iii. Define the prevalence and clinical significance of occult hepatitis B infection (OBI) in The Gambia
- iv. Assess the uptake of HBV testing and treatment among HIV-infected patients in The Gambia
- v. Assess the trend of timely hepatitis B birth dose (HepB-BD) vaccine coverage in The Gambia since 2015, and the impact of the COVID-19 pandemic on HepB-BD coverage

3. CHAPTER 3: Advanced liver disease in The Gambia: clinical characteristics and outcomes

3.1. Abstract

Background & aims

Advanced liver disease (cirrhosis and HCC) is a major cause of premature death in Africa. However, due to the lack of curative treatment for cirrhosis and HCC, the clinical relevance of screening and surveillance in Africa remains unclear. In this study, I described the clinical characteristics and outcomes of patients with cirrhosis and HCC in The Gambia and assessed the impact of treatment with Tenofovir Disoproxil Fumarate (TDF) on survival among HBV infected patients.

Methods

I conducted a prospective cohort analysis of consecutive adults diagnosed with cirrhosis or HCC between 2012-2015 in The Gambia, enrolled in the PROLIFICA program. HBV-positive patients with cirrhosis, without HCC, were offered TDF. Primary outcome was overall survival estimated using the Kaplan-Meier method. Multivariable Cox proportional hazards models with the inverse probability of treatment weighted (IPTW) were performed to determine the effect of TDF.

Results

Of 529 patients enrolled in this study, 336 patients (252 with HCC and 84 with cirrhosis) were analysed. Patients were predominantly male (75.3%) with median age 42 years (IQR: 33-55). HBV, HCV and HDV serologies were positive in 84.4%, 9.9% and 9.9%, respectively. Sixty-four percent of HCC patients had multifocal tumour, with a median size of 7.5 cm (IQR: 5.4-10.8). Median survival was 17.1, 11.3 and 1.5 months among patients with compensated cirrhosis, decompensated cirrhosis, and HCC, respectively (log rank $p < 0.0001$).

In HBV-positive patients with cirrhosis, the median turnaround time between cirrhosis diagnosis and TDF initiation was 4.9 months. TDF treatment was associated with

improved survival in patients with HBV-related cirrhosis (IPTW-adjusted Cox analysis HR: 0.14; 95%CI: 0.06-0.34, $p<0.001$). Ascites (HR: 1.83, 95%CI: 1.09–3.09) and portal thrombosis (HR: 3.81, 95%CI: 1.99–7.30) were independent risk factors of mortality in HCC cases.

Conclusions

Early screening and treatment programs of cirrhosis and HCC alongside simplified treatment guidelines are urgently required in Africa.

3.2. Introduction

Cirrhosis and hepatocellular carcinoma (HCC) are leading causes of death globally, accounting for 1.16 million and 788,000 annual deaths, respectively. Together, cirrhosis and HCC account for 3.5% of all deaths each year, worldwide [46]. Chronic infections with hepatitis B and C virus are a major cause of cirrhosis and HCC, especially in sSA where the prevalence of these infections is high and access to curative treatments for advanced liver disease is scarce [49, 64]. In 2021, the World Health Organisation (WHO) estimated that incident cases of HBV and HCV infection in Africa were 990,000 and 210,000 respectively [41]. HCC was the second and third leading cancer in African men and women, respectively, with an estimated 36,844 incident cases and 36,067 deaths in 2018 in Africa [47].

West Africa has the highest burden of HCC in Africa, with an age-standardised incidence rate (ASIR) and age-standardised mortality rates (ASMR) of 16.9 (95%CI: 13.0-22.4) and 18.1 (14.1-23.4) per 100,000 person-years, respectively [52]. HCC is the most common cancer in men in nine West African countries (Burkina Faso, Cape Verde, The Gambia, Ghana, Guinea, Guinea Bissau, Liberia, Mauritania, Senegal). In 2018, 50% and 21% of HCC cases in Africa were estimated to be attributable to HBV and HCV, respectively [52]. A recent systematic review and meta-analysis estimates that 54% of cirrhosis cases are attributable to HBV or HCV in the WHO Africa region [49]. In The Gambia, the Global Cancer Observatory (<https://gco.iarc.fr>) estimates that the 5-year HCC incidence in the general population is 15.43 per 100,000 population. Among HBV-infected adults in The Gambia, the HCC incidence is 55.5 per 100,000 (95%CI 24.9-123.5) carrier-years [12].

Despite the high disease burden, screening, surveillance and treatment programmes for cirrhosis and HCC are almost non-existent in sSA. Consequently, mortality rate in Africans with cirrhosis or HCC is only poorly estimated [64]. In a region with limited access to curative therapy for cirrhosis and HCC, including liver transplantation or surgical resection[64, 71], the clinical relevance of implementing screening programmes for cirrhosis or HCC in people known to have viral hepatitis remains unclear. Widely available treatments in the region are limited to antiviral therapy and palliative management to alleviate symptoms.

To fill knowledge and capacity gaps in cirrhosis and HCC in sSA, the PROLIFICA programme in 2011 established a clinic specialized in liver care at the MRC Unit The Gambia to build capacity, provide access to diagnostics and management, and provide

antiviral therapy to HBV-positive patients with advanced liver disease, according to the EASL criteria [203, 204]. In this study, I analysed data from prospective patients with advanced liver disease, seen at the PROLIFICA specialised liver clinic in The Gambia, to study: (i) the characteristics, underlying aetiologies, risk factors and clinical outcomes of cirrhosis and HCC, and (ii) the effectiveness of antiviral therapy using Tenofovir Disoproxil Fumarate (TDF) on overall survival of patients with HBV-related cirrhosis.

3.3. Methods

Study population

Between 2012-2015, the PROLIFICA programme in The Gambia recruited consecutive patients with advanced liver disease to study biomarkers of cirrhosis and HCC and to assess the association between birth order and the risk of HCC[68, 205]. The study was hosted in the PROLIFICA liver clinic at the MRC Unit The Gambia, which was the only healthcare facility providing diagnosis of cirrhosis and HCC throughout The Gambia. Using the referral system established by the Gambia National Cancer Registry[206], individuals with suspected cirrhosis or HCC in The Gambia were systematically referred to the liver clinic, allowing the study to have representative samples of patients with advanced liver disease in the country. The study was approved by the Gambia Government/MRCG Joint Ethics Committee.

Clinical assessment

Following an informed consent, patients referred to the liver clinic were asked to complete a questionnaire about demographic (place of birth, age, sex), environmental (excessive alcohol intake, defined as more than 20g/day; smoking), and clinical information (WHO performance status, past medical history, medication history including over-the-counter and herbal medication use, and family history of liver disease). A physical examination included anthropometric measurements (BMI, arm circumference, hip-waist ratio), assessment of signs of liver disease, fasting liver stiffness measurement (LSM) [207]using transient elastography (Fibroscan 402, Echosens, Paris, France) and a non-contrast abdominal ultrasound scan. Blood and urine samples were collected for laboratory investigations. Unless contraindicated, a liver biopsy of both non-tumoral and tumoral tissue was performed in patients with suspected mass on ultrasound.

Laboratory analysis

In all patients, serum biochemistry (Architect C4000, Abbott, USA), full blood count (Medonic M51, Boule, Sweden), alfa-fetoprotein (AFP) (Architect i1000 SR, Abbott, USA), hepatitis B surface antigen (HBsAg) (Determine, Alere, USA), HCV RNA and HIV-1 and 2 antibodies (EIA, Genscreen ULTRA HIV Ag-Ab, Bio-Rad, USA) were assessed. Aflatoxin B1 exposure was assessed by examining for AGG->QGT mutation at codon 249 of the p53 tumour suppressor gene (p53249S) in cell-free DNA [208].

HBsAg-positive patients were also tested for hepatitis B e antigen (HBeAg) (ELISA-ETI-EBK Plus, Diasorin, Saluggia, Italy) and anti-HDV (ETI-AB-DELTAK-2, Diasorin, Italy). HBV DNA level was quantified using an in-house real-time PCR assay (detection limit 50 IU/ml) that was validated against a commercial qPCR (Abbott, Wiesbaden, Germany) [187]. HBV genotype was determined in HBsAg-positive samples with detectable viremia. Prothrombin time was not routinely available in The Gambia. As a result, the severity of cirrhosis based on Child-Pugh or MELD scores were not estimated.

Clinical outcomes

Cirrhosis was defined either histologically (Metavir score: F4) or by a Fibroscan score ≥ 9.5 kPa in patients without biopsy. A cut-off for LSM of ≥ 9.5 kPa had a 100% sensitivity and 89% specificity to indicate cirrhosis diagnosed by histopathology in this Gambian cohort [88]. Signs and symptoms of liver decompensation (jaundice, ascites, hepatic encephalopathy, or variceal bleeding) were clinically ascertained [209].

HCC was confirmed either histologically in patients with biopsy, or clinically using one of the following criteria: (i) confirmed focal liver lesion ≥ 2 cm consistent with HCC on ultrasound and AFP ≥ 200 ng/mL, irrespective of cirrhosis; (ii) liver cirrhosis with suspected focal liver lesion ≥ 2 cm suggestive of HCC irrespective of AFP level; or (iii) focal liver lesion suggestive of HCC without documented size and AFP ≥ 200 ng/mL [210]. For the quality of my analysis, I excluded patients with AFP > 200 ng/ml but no visible mass on liver ultrasound. Contrast CT scan of the liver was not accessible during the study period.

Management of cirrhosis and HCC

Radiological or surgical treatment for HCC was not available in The Gambia during the study period; only palliative care was offered to patients with confirmed HCC [211]. In the absence of contraindications, ascites was managed with oral diuretics (furosemide and/or spironolactone) and/or paracentesis without albumin infusion. Spontaneous bacterial peritonitis (SBP) was treated with broad spectrum antibiotics including penicillin or fluoroquinolones as per international guidelines [212]. At the time of the study, there was no endoscopy in The Gambia thus endoscopic intervention was not routinely available for patients at risk of variceal bleeding. Vasopressin or its analogues were also not available in The Gambia during the study period. A summary of the clinical practice for managing decompensated cirrhosis at the time of the study is presented in Table 3.1.

None of the patients ever had anti-viral therapy at the time of their inclusion. HBsAg-positive patients with cirrhosis were offered free treatment with oral TDF 300mg daily, adjusted to the estimated glomerular filtration rate (eGFR). TDF was initiated at a subsequent visit to the clinic, once the results of HBsAg, anti-HIV, HBV viral load, and serum creatinine were available using the blood samples collected at the first visit. Patients lost to follow-up in our study were unlikely to benefit from antiviral treatment since the PROLIFICA liver clinic was the only healthcare facility within The Gambia where nucleos(t)ide analogues were prescribed and dispensed for HBV mono-infected patients. HCV antiviral treatment was not available in The Gambia at the time of the study. Table 3.1 summarises the clinical practice available in The Gambia for managing decompensated cirrhosis and HCC during the study period.

Table 3.1: Clinical practice in The Gambia [211] at the time of the study, according to the EASL guidelines [212, 213] on the management of decompensated cirrhosis (1) and HCC (2)

EASL Guidelines	The Gambia
Cirrhosis	
Effects of targeting key pathogenic events in prevention of cirrhosis progression: In patients with decompensated cirrhosis, the aetiological factor, should be removed, particularly alcohol consumption and hepatitis B.	Yes
Ascites: 1- A diagnostic paracentesis is recommended in all patients with new onset grade 2 or 3 ascites, or in	Yes

those hospitalised for worsening of ascites or any complication of cirrhosis. 1- Neutrophil count and culture of ascitic fluid culture (bedside inoculation blood culture bottles with 10 ml fluid each) should be performed to exclude bacterial peritonitis.	Yes
Gastrointestinal bleeding: 1- Patients in whom decompensation develops should have EGD performed to screen for gastro-oesophageal varices, unless previously diagnosed and treated. 2- Patients with medium-large varices should be treated with either NSBBs or EBL.	No No
Spontaneous bacterial peritonitis 1- A diagnostic paracentesis should be carried out in all patients with cirrhosis and ascites without delay at hospital admission to rule out SBP. 2- The administration of albumin (1.5 g/kg at diagnosis and 1 g/kg on day 3) is recommended in patients with SBP.	Yes No
Renal impairment: 1- In case of no obvious cause of AKI, AKI stage >1A or infection-induced AKI, 20% albumin solution should be used at the dose of 1 g of albumin/kg of body weight (with a maximum of 100 g of albumin) for two consecutive day.	No
HCC	
Surveillance: Patients at high risk of developing HCC should be entered into surveillance programmes.	No
Diagnosis: Diagnosis of HCC in cirrhotic patients should be based on non-invasive criteria and/or pathology	Yes
Diagnosis: Non-invasive criteria can only be applied to cirrhotic patients for nodule(s) ≥ 1 cm, in light of the high pre-test probability and are based on imaging techniques obtained by multiphasic CT, dynamic contrast-enhanced MRI	Yes on liver US only Contrast hardly accessible No access to MRI

(evidence high; recommendation strong) or CEUS (evidence moderate; recommendation weak).	
Staging systems and treatment allocation: Staging systems for clinical decision making in HCC should include tumour burden, liver function and performance status	Incomplete (INR and PT not done routinely)
Liver resection: Surgical resection is recommended as treatment of choice in patients with HCC arising on a non-cirrhotic liver	No
Liver transplantation: LT is recommended as the first-line option for HCC within Milan criteria but unsuitable for resection	No
Local ablation and external radiation/ Transarterial therapies/ Systemic therapies	No
Palliative and best supportive care Management of pain. Psycho-oncological support. Adequate nutrition	Only management of pain (3)

Abbreviations: EGD: esophagogastroduodenoscopy; NSBBs: non-selective beta-blockers; SBP: spontaneous bacterial peritonitis; CT: computerized tomography; CEUS: contrast-enhanced ultrasound scan; HCC: hepatocellular carcinoma LT: liver transplantation.

Follow-up and survival analysis

In July 2019, as part of this PhD, I retrieved patients' telephone numbers and home addresses from the PROLIFICA database and conducted a follow up for all the patients enrolled in this study. Overall survival was ascertained by clinic attendance among patients retained in care or by telephone call or home visit for patients who were lost to follow-up (not retained in care in the PROLIFICA clinic). For patients who died, the date, place and potential causes of death were confirmed either by reviewing case notes if patient died at the MRC clinic, or by verbal autopsy with close relatives if patient died elsewhere.

Statistical analysis

Data were presented as counts, percentages or median with interquartile range (IQR). Clinical characteristics were compared between three groups of patients: compensated cirrhosis, decompensated cirrhosis, and HCC. Continuous variables were examined for significance using Student's t-test or Mann–Whitney test, and categorical variables using Chi-squared test or Fisher's exact test. Patient survival was assessed according to the Kaplan–Meier method and compared by the log-rank test between the three groups.

Among HCC patients, factors associated with mortality were identified using the Cox proportional hazard regression. Variables found to be marginally associated with mortality in the univariable analysis ($p < 0.20$) were further included in the multivariable model. Among HBsAg-positive patients with compensated or decompensated cirrhosis without HCC, the survival rate was compared between those that received TDF and those untreated. Imbalance between cirrhotic cases who benefited from TDF and those who did not (due to loss to follow-up) was accounted for by using an inverse probability treatment weighting (IPTW); propensity scores (PS) were calculated by logistic regression including the following potential confounding factors for the association between TDF initiation and death: age, gender, decompensated cirrhosis, HBeAg status, total bilirubin, albumin, and platelet counts. All analyses were performed using RStudio statistical software (Version 1.4.869 X Rstudio, Inc) and statistically significant tests were based on two-tailed P values, with $p < 0.05$ considered to indicate statistical significance.

3.4. Results

Baseline characteristics

Between June 2012 and July 2015, 529 consecutive patients aged ≥ 15 years with suspected cirrhosis or HCC were enrolled in the study. Of these, 193 were excluded from this analysis because of incomplete baseline data ($n=119$) or diagnosis other than cirrhosis or HCC ($n=74$). Among the 336 patients included in this analysis, 252 had HCC and 84 had cirrhosis without HCC (Figure 3.1).

The study population was predominantly male ($n=253/336$, 75.3%) with median age of 42 years (IQR: 33-55) and median BMI of 19.6 kg/m² (IQR: 17.6-22.0). About half of the patients ($185/336$, 55.1%) had a WHO performance status ≥ 2 at diagnosis. Only 34/330 (10.3%) reported occasional alcohol intake but none acknowledged excessive alcohol intake.

Markers for viral hepatitis were positive in 317/327 (96.9%) patients: HBsAg (223/327, 62.2%), anti-HCV antibodies (31/311, 9.9%), and anti-HDV antibodies (22/223, 9.9%). Occult HBV infection (OBI) was found in 53/104 (51.0%) of HBsAg-negative patients. Overall, 276/327 (85.9%) patients had evidence of HBV infection, either HBsAg or OBI positive. Fifty-seven percent (58/102) of patients had aflatoxin exposure, with no statistically significant differences between patients with cirrhotic and HCC. Baseline characteristics of the study population are summarized in Table 3.2.

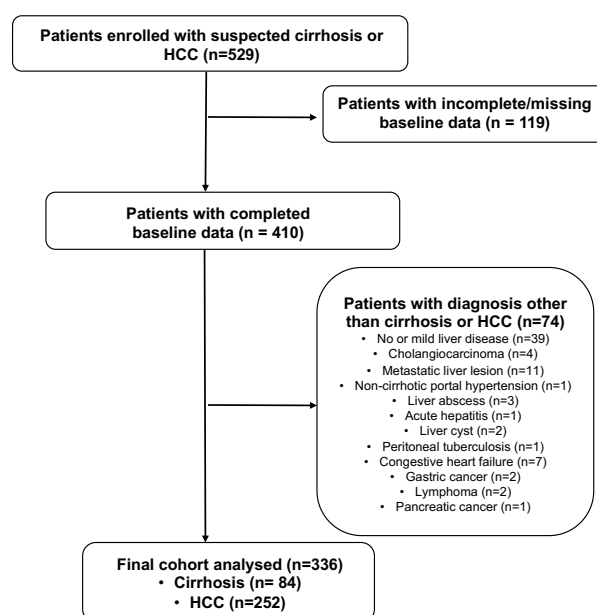


Figure 3.1. Study flow diagram

Patients with HCC presented at late stage with 63.9% presenting with multifocal tumour. The median tumoral size was 7.5 cm (IQR: 5.4-10.8) and median serum AFP was 1,986 ng/mL (IQR: 315-8,000 ng/mL). Two-thirds (66%) of HCC patients were positive for HBsAg.

Among the 84 patients with cirrhosis without HCC, 67.9% (57/84) had decompensated cirrhosis at presentation, mainly with ascites (48%). Similar to HCC cases, 75.0% (63/84) of patients with cirrhosis were positive for HBsAg, with 14.3% (9/63) being positive for anti-HDV antibodies.

Table 3.2: Characteristics of the study population (n=336)

Variables	Overall	HCC	Cirrhosis	p-value*
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	(n = 336)	(n = 252)	without HCC (n = 84)	
Age, years	42 (33-55)	43 (35-56)	36 (28-47)	<0.001
Male gender	253 / 336 (75.3%)	192 / 252 (76.2%)	61 / 84 (72.6%)	0.475
Performance status 0/1/2/3/4	16%/29%/25%/18%/12%	9%/31%/28%/19%/14%	36%/25%/18%/16%/5%	<0.001
Current or past smoking history	162 / 330 (49.1%)	126 / 248 (50.8%)	36 / 82 (43.9%)	0.278
Alcohol intake (any)	34 / 330 (10.3%)	23 / 248 (9.3%)	11 / 82 (13.4%)	0.285
BMI (kg/m ²)	19.6 (17.6-22.0)	19.2 (17.4-21.3)	21.2 (18.2-24.3)	0.002
Decompensated liver cirrhosis		NA	57 / 84 (67.9%)	
Ascites	130 / 318 (40.9%)	91 / 237 (38.4%)	39 / 81 (48.1%)	0.123
Variceal bleeding	22 / 329 (6.7%)	17 / 246 (6.9%)	5 / 83 (6.0%)	0.780
Hepatic encephalopathy	20 / 325 (6.1%)	18 / 243 (7.4%)	2 / 82 (2.4%)	0.105
Jaundice	161 / 324 (49.7%)	130 / 242 (53.7%)	31 / 82 (37.8%)	0.013
Haemoglobin (g/dL)	12.0 (10.0-13.9)	11.8 (9.8-13.7)	12.9 (10.4-14.4)	0.028
Platelets (x10 ⁹ /L)	211 (143-316)	238 (168-342)	140 (100-218)	<0.001
AST (U/L)	166 (68-312)	209 (104-370)	58 (34-92)	<0.001
ALT (U/L)	49 (31-89)	58 (36-104)	32 (25-61)	<0.001
GGT (U/L)	296 (113-495)	339 (182-563)	76 (46-198)	<0.001
Total Bilirubin (μmol/L)	24 (14-54)	25 (15-76)	20 (11-41)	0.051
Creatinine (μmol/L)	68 (55-88)	68 (53-92)	69 (57-84)	0.869
Sodium (mmol/L)	139 (135-142)	138 (135-141)	141 (137-143)	<0.001
Albumin (g/L)	34 (29-39)	33 (29-38)	35 (28-41)	0.427
Any positive viral hepatitis biomarkers**	317 / 327 (96.9%)	235 / 243 (99.2%)	82 / 84 (97.6%)	>0.999
Any positive HBV biomarkers ***	276 / 327 (84.4%)	204 / 243 (84.0%)	72 / 84 (85.7%)	0.693

Positive HBsAg	223 / 327 (62.2%)	160 / 243 (65.8%)	63 / 84 (75.0%)	0.120
Positive HBsAg & detectable HBV DNA****	186 / 212 (88.0%)	140 / 149 (94.0%)	46 / 63 (73.0%)	<0.001
Positive HBsAg & undetectable HBV DNA****	26 / 212 (12.0%)	9 / 149 (6.0%)	17 / 63 (27.0%)	<0.001
HBsAg negative and detectable HBV DNA (OBI) *****	53/104 (51.0%)	44/83 (53.0%)	9/21 (42.9%)	0.111
Positive HCV Ab	31 / 311 (9.9%)	23 / 227 (10.1%)	8 / 84 (9.5%)	0.874
Positive HDV Ab	22 / 223 (9.9%)	13 / 160 (8.1%)	9 / 63 (14.3%)	0.081
Positive HIV Ab	19 / 319 (6.0%)	15 / 235 (6.4%)	4 / 84 (4.8%)	0.790
Exposure to aflatoxin B1	58 / 102 (56.9%)	50 / 86 (58.1%)	8 / 16 (50.0%)	0.546
HBV Parameters				
Positive HBeAg	57 / 212 (26.9%)	44 / 149 (29.5%)	13 / 63 (20.6%)	0.482
HBV DNA	1,231 (79- 158,045)	1,650 (112- 157,660)	421 (22-122,149)	0.031
HBsAg levels	4,642 (1,267- 9,263)	3,356 (633- 7,753)	5,551 (2,301- 9,811)	0.154
HBV genotype				0.007
A	32 / 128 (25.0%)	27 / 81 (33.3%)	5 / 47 (10.6%)	
A+E	4 / 128 (3.1%)	3 / 81 (3.7%)	1 / 47 (2.1%)	
E	92 / 128 (71.9%)	51 / 81 (63.0%)	41 / 47 (87.2%)	
HCC characteristics				
Single mass		73 / 202 (36.1%)	NA	
Multiple masses		129 / 202 (63.9%)	NA	
Median tumour size (cm)		7.5 (5.4-10.8)	NA	
AFP (ng/mL)	369 (15-5,169)	1,986 (315- 8,000)	10 (4-28)	<0.001

Partial/complete portal thrombosis					0.001
No thrombosis	306 / 336 (91.1%)	222 / 252 (88.1%)	84 / 84 (100%)		
Complete thrombosis	7 / 336 (2.1%)	7 / 252 (2.8%)	0 / 84 (0%)		
Partial thrombosis	23 / 336 (6.8%)	23 / 252 (9.1%)	0 / 84 (0%)		
Histologically confirmed	69 / 336 (20.5%)	41 / 252 (16.3%)	28 / 84 (33.3%)		<0.001

Values are expressed as median (interquartile range, IQR) or numbers (%).

*Calculated by Student's t-test (or the Mann–Whitney test, if appropriate) and chi-squared test (or Fisher's exact test, if appropriate)

** Any positive viral hepatitis biomarkers: HBsAg, HCV Ab, HDV Ab, detectable HBV DNA at any level

*** Any positive HBV biomarkers: HBsAg, detectable HBV DNA at any level

**** Detectable HBV DNA among positive HBsAg patients: ≥ 50 IU/mL

***** OBI was defined as negative HBsAg and detectable HBV DNA: ≥ 50 IU/mL

Abbreviations: BMI, body mass index; HBsAg, Hepatitis B surface antigen; HCV, hepatitis C virus; HDV, hepatitis D virus; HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; OBI, occult HBV infection

Survival outcomes among patients with HCC and cirrhosis

Survival data was available for 243 of 336 patients (72.3%), comprising 70 patients with cirrhosis and 173 with HCC, with a median follow-up time of 2.0 months. Among patients with cirrhosis (n=70), median survival was 17.1 months (IQR: 11.2->24.0), with actuarial survival rates at 3, 6, 12 and 24 months being 79%, 68%, 55% and 47%, respectively. Among patients with HCC (n=173), median survival was 1.5 months (IQR: 1.1-11.2) with actuarial survival rates at 3, 6, 12 and 24 months of 30%, 14%, 7% and 3%, respectively. Survival rate was significantly higher among patients with cirrhosis compared to HCC (log-rank, $p < 0.0001$) (Figure 3.2a).

I further stratified patients with cirrhosis according to the presence of liver decompensation. In 21 patients with compensated cirrhosis, the actuarial survival rates at 3, 6, 12 and 24 months were 95%, 95%, 72% and 72%, respectively (Figure 3.2b). In 49 patients with decompensated cirrhosis, the median survival was 11.3 months and actuarial survival rates at 3, 6, 12 and 24 months were 72%, 57%, 48% and 38%, respectively. Survival rates were significantly different between compensated cirrhosis, decompensated cirrhosis and HCC patients (log-rank, $p < 0.0001$). (Figure 3.2b)

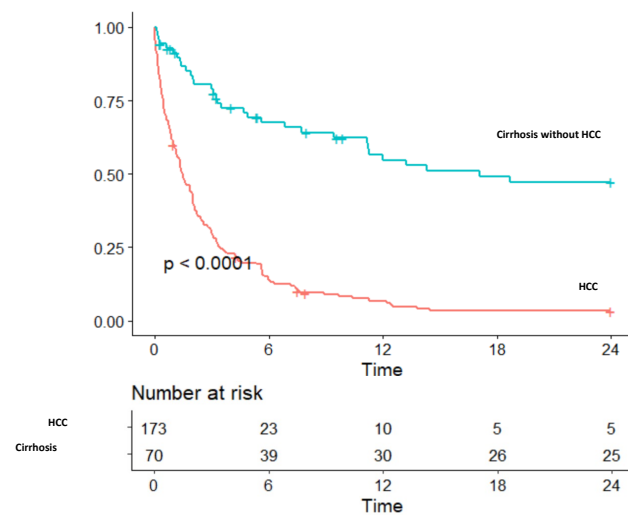


Figure 3.2a. Cumulative overall survival in patients with cirrhosis (n=70) and HCC (n=173)

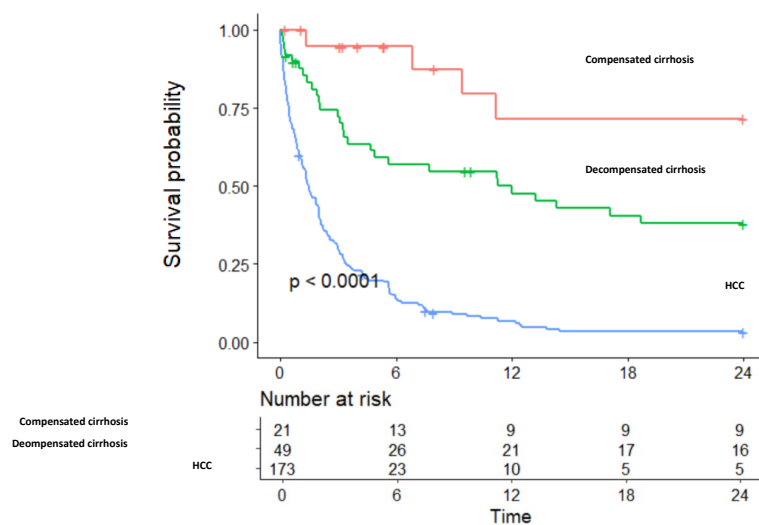


Figure 3.2b. Cumulative overall survival in patients with compensated cirrhosis (n=21), decompensated cirrhosis (n=49) and HCC (n=173)

Risk factors of mortality among patients with HCC

Among 174 HCC patients with known cause of death, 165 (94.8%) died of liver-related cause. Ascites (HR: 1.83, 95% CI: 1.09–3.09) and partial or complete portal thrombosis (HR: 3.81, 95% CI: 1.99–7.30) at baseline were independent predictive factors of mortality in patients with HCC. The results of the Cox proportional hazards regression analysis for the risk of mortality are shown in Table 4.3.

Table 3.3. Univariable and multivariable analyses to identify factors associated with mortality among patients with HCC (n=173)

Parameters	Numb er of parti cips	Perso n- month s at risk	Num ber of even ts	Incide nce rate (100 0 pers on mont hs)	Crude analysis			Adjusted analysis		
					HR	95% CI	P value	HR	95% CI	P valu e
Age (1 year increment)					1.00	0.98-1.00	0.35	1.01	0.99-1.02	0.54
Female	39	102	38	372	Ref	Ref	Ref	Ref	Ref	Ref
Male	133	432	125	289	0.82	0.57-1.20	0.29	0.71	0.37-1.38	0.32
No smoking status	85	264	79	299	Ref	Ref	Ref	Ref	Ref	Ref
Smoking	86	270	83	307	1.01	0.73-1.30	0.95			
BMI >18.5 kg/m ²	96	302	91	301	Ref	Ref	Ref	Ref	Ref	Ref
BMI ≤18.5 kg/m ²	57	263	79	300	1.00	0.74-1.50	0.82			
No ascites	95	382	89	233	Ref	Ref	Ref	Ref	Ref	Ref
Ascites	68	136	65	478	1.70	1.20-2.40	<0.01	1.83	1.09-3.09	0.02
Negative HBsAg	54	163	50	307	Ref	Ref	Ref	Ref	Ref	Ref
Positive HBsAg	114	356	109	306	1.00	0.99-1.00	0.94			
No exposure to aflatoxin B1	31	60	31	517	Ref	Ref	Ref	Ref	Ref	Ref
Exposure to aflatoxin B1	39	85	37	435	0.94	0.57-1.60	0.82			
Albumin >35 g/L	68	320	62	194	Ref	Ref	Ref	Ref	Ref	Ref
Albumin ≤35 g/L	95	202	92	455	2.00	1.40-2.70	<0.01	1.38	0.86-2.22	0.18
AST ≤40 U/L	10	68	7	103	Ref	Ref	Ref	Ref	Ref	Ref
AST >40 U/L	145	420	140	333	2.2	1.00-4.60	0.05	2.37	0.70-8.02	0.16
ALT ≤40 U/L	47	188	45	239	Ref	Ref	Ref	Ref	Ref	Ref
ALT >40 U/L	116	311	110	354	1.3	0.91-1.80	0.15	0.84	0.48-1.49	0.56
Total Bilirubin <40 μmol/L	59	296	53	179	Ref	Ref	Ref	Ref	Ref	Ref
Total Bilirubin ≥40 μmol/L	102	201	100	498	2.1	1.5-2.90	<0.01	1.51	0.92-2.48	0.10

Creatinine ≤110 μmol/L	118	382	110	288	Ref	Ref	Ref	Ref	Ref	Ref
Creatinine >110 μmol/L	22	66	21	318	1.40	0.85- 2.20	0.2	1.07	0.54- 2.10	0.85
Platelets ≤150 x10 ⁹ /L	33	70	32	457	Ref	Ref	Ref	Ref	Ref	Ref
Platelets >150	125	420	118	281	0.64	0.43- 0.94	0.02	0.83	0.49- 1.38	0.47
AFP ≤200 ng/mL	46	157	43	274	Ref	Ref	Ref	Ref	Ref	Ref
AFP >200 ng/mL	121	380	115	303	1.10	0.75- 1.50	0.73			
Performanc e status <2	63	287	59	206	Ref	Ref	Ref	Ref	Ref	Ref
Performanc e status ≥2	92	228	87	382	1.60	1.10- 2.20	<0.01	1.38	0.86- 2.21	0.19
No portal thrombosis	147	500	138	276	Ref	Ref	Ref	Ref	Ref	Ref
Partial or complete portal thrombosis	26	37	26	703	2.00	1.30- 3.00	<0.01	3.81	1.99- 7.30	<0.0 1
Single mass HCC	57	157	55	350	Ref	Ref	Ref	Ref	Ref	Ref
Multifocal tumour HCC	85	230	81	352	1.00	0.74- 1.50	0.83			
Size of HCC nodule <2cm	22	104	21	202	Ref	Ref	Ref	Ref	Ref	Ref
Size of HCC nodule ≥2cm	151	433	143	330	1.4	0.86- 2.20	0.19	1.08	0.56- 2.06	0.82

Abbreviations: BMI: body mass index; HBsAg: hepatitis B surface antigen; AST: aspartate aminotransferase; ALT: alanine aminotransferase; AFP: alpha-fetoprotein; HCC: hepatocellular carcinoma

Impact of TDF treatment on survival in HBsAg-positive patients with cirrhosis

Among 84 non-HCC patients with cirrhosis, 63 (75.0%) were positive for HBsAg, of whom 7/63 (11.1%) were lost to follow-up (did not attend follow up visit and could not be contacted by telephone). Of the remaining 56 patients, 31 (55.4%) attended their second visit and started antiviral therapy (treated group) while 25 (44.6%) did not continue care and therefore did not receive TDF treatment (untreated group).

The main baseline characteristics of the treated and untreated patients are presented in Table 3.4. The median turnaround time between the first diagnosis of cirrhosis and initiation of antiviral therapy in the treated group was 4.9 months (IQR: 3.2-7.3). At 12 and 24 months after treatment initiation, 15/16 (94%) and 15/16 (94%) of living patients with cirrhosis had viral suppression (Table 3.4).

Table 3.4. Comparison of baseline characteristics between treated and untreated HBsAg-positive patients with cirrhosis (n=56)

Variables	Untreated group (n=25)	Treated group (n=31)	P-value*
Age, years	32 (28-38)	36 (26-41)	0.781
Male gender	18 / 25 (72.0%)	23 / 31 (74.2%)	0.854
Alcohol intake (any)	5 / 24 (20.8%)	3 / 31 (9.7%)	0.276
Current or past smoking history	10 / 24 (41.7%)	12 / 31 (38.7%)	0.824
BMI (kg/m ²)	21.5 (18.6-24.0)	21.5 (18.9-24.9)	0.688
Ascites	9 / 25 (36.0%)	15 / 30 (50.0%)	0.297
Decompensated cirrhosis	14 / 25 (56.0%)	22 / 31 (71.0%)	0.245
Haemoglobin (g/dL)	13 (12-15)	12 (10-14)	0.198
Creatinine (µmol/L)	74 (62-87)	63 (57-74)	0.081
Albumin (g/L)	38 (26-43)	30 (28-40)	0.531
AST (U/L)	51 (32-102)	60 (44-76)	0.481
ALT (U/L)	35 (22-74)	34 (26-54)	0.599
Total Bilirubin (µmol/L)	20 (11-34)	19 (14-43)	0.507
Platelet count (x10 ⁹ /L)	143 (98-212)	120 (62-160)	0.142
Positive HCV Ab	3 / 25 (12.0%)	2 / 31 (6.5%)	0.647
Positive HDV Ab	4 / 25 (16.0%)	4 / 31 (12.9%)	0.999
Positive HIV Ab	2 / 25 (8.0%)	0 / 31 (0%)	0.195
Positive HBeAg	3/24 (12.5%)	9/29 (31.0%)	0.109
HBV viral load (median, IQR)			
Baseline HBV DNA	285 (17-18,448)	2,329 (109-235,576)	0.368
Month 6 HBV DNA	NA	0 (0, 76)	
Month 12 HBV DNA	NA	0 (0, 0)	
Month 18 HBV DNA	NA	0 (0, 0)	

Values are expressed as median (IQR) or numbers (%).

*Calculated by Student's t-test (or the Mann–Whitney test, if appropriate) and chi-squared test (or Fisher's exact test, if appropriate)

Abbreviations: HBeAg, hepatitis B e antigen; HBV, hepatitis B virus, NA : not applicable, SMD, standardized mean difference

Over a median follow-up period of 11.6 months (IQR: 3.3-24), the 3, 6, 12 and 24 month overall cumulative survival probabilities of patients with cirrhosis were 89%, 77%, 63% and 56%, respectively (Figure 3.3). When stratified by TDF treatment, untreated patients had 3, 6, 12 and 24 month overall cumulative survival probabilities of 77%, 55%, 36% and 24%, compared to 97%, 90%, 77% and 71% among the treated group, with an unadjusted hazard ratio of 0.21 [95% CI, 0.08–0.52], $p < 0.001$. In multivariate analysis, the risk of death was statistically lower in the treated group (adjusted HR: 0.13 [95% CI, 0.04–0.40], $p < 0.001$).

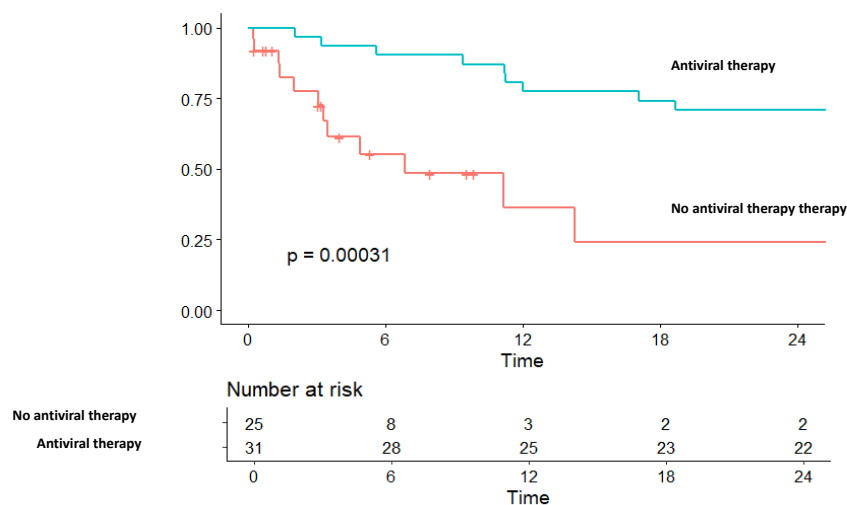


Figure 3.3. Cumulative overall survival in untreated and treated HBsAg-positive patients with cirrhosis without HCC (n=56)

IPTW analysis of clinical outcome among treated and untreated HBsAg-positive patients with cirrhosis

All 56 HBV-positive patients with cirrhosis were included in the IPTW survival analysis. The baseline characteristics with standardized mean difference (SMD) before and after IPTW analysis are listed in Table 3.5. The 3, 6, 12 and 24 months overall cumulative survival probabilities were 79%, 53%, 37% and 15% in the untreated group and 98%, 93%, 81% and 77% in the treated group (log-rank, $p < 0.001$), with a hazard ratio of 0.14 (95% CI, 0.06–0.34, $p < 0.001$).

Table 3.5. Comparison of baseline characteristics between treated and untreated HBV-positive patients with cirrhosis before and after IPTW analysis (n=56)

Variables	Before IPTW			After IPTW		
	Untreated group (n= 25)	Treated group (n= 31)	SMD	Untreated group (n= 25)	Treated group (n= 31)	SMD
Age, years	32 (28-38)	36 (26-41)	0.144	36 (27-45)	36 (27-45)	0.009
Male gender	18 / 25 (72.0%)	23 / 31 (74.2%)	0.049	18/25 (72.0%)	24/31 (77.4%)	0.066
Decompensated cirrhosis	14 / 25 (56.0%)	22 / 31 (71.0%)	0.315	17/25 (60.0%)	20/31 (64.5%)	0.042
Creatinine (µmol/L)	74 (62-87)	63 (57-74)	0.529	66 (55-80)	67 (54-80)	0.018
Albumin (g/L)	38 (26-43)	30 (28-40)	0.200	34 (26-43)	34 (26-43)	0.004
Total Bilirubin (µmol/L)	20 (11-34)	19 (14-43)	0.185	19 (10-33)	19 (10-33)	0.072
Platelets (x10 ⁹ /L)	143 (98-212)	120 (62-160)	0.387	132 (80-208)	132 (80-208)	0.103
Positive HBeAg	3 / 24 (12.5%)	9 / 29 (31.0%)	0.452	10/25 (40.0%)	13/31 (41.9%)	0.059
Baseline HBV DNA	285 (17-18,448)	2,329 (109-235,576)	0.227	269 (16-26,736)	270 (17-26,735)	0.136

Values are expressed as median (IQR) or numbers (%).

Abbreviations: SMD, standardized mean difference; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; DNA: deoxyribonucleic acid

3.5. Discussion

Clinical characteristics and prognosis of patients diagnosed with cirrhosis and HCC have been poorly documented in sSA. The Gambia has long recognized HBV infection as an important public health problem, being the first African country to integrate HBV vaccination in its national infant immunization program and maintained the vaccination schedule starting immediately after birth [116]. This study suggests that HBV occupies an important proportion of cases of advanced liver disease in The Gambia, and additional efforts are urgently needed to improve the prevention, diagnosis, and care of end-stage liver disease in The Gambia.

I found that HBV infection remains the main cause of cirrhosis and HCC in The Gambia with two-thirds of the study patients being positive for HBV – either HBsAg or OBI. Young men are predominantly affected and present at advanced disease stage with very poor median survival (1.5 months).

This study reports high effectiveness of antiviral therapy using TDF to improve survival in HBV-positive patients with cirrhosis. To the best of my knowledge, the impact of TDF on survival in patients with HBV-related cirrhosis has not been reported, particularly in resource-limited settings with limited access to curative therapies for cirrhosis or HCC. The study, however, highlights major difficulties to rapidly introduce antiviral therapy for all cirrhotic patients. According to the current international guidelines [33], HBV-positive patients with cirrhosis are eligible for antiviral therapy if they have detectable viral load. With a significant delay in rendering the result of HBV viral load, the median interval between the first assessment and treatment initiation was 4.9 months in patients with cirrhosis. During this time and despite active follow-up, 32 of 63 (50.8%) HBV-positive patients with cirrhosis were either lost to follow-up (7/63) or did not return to clinic for reassessment and treatment initiation (25/63). International guidelines should be modified to allow TDF introduction in HBV-positive patients with cirrhosis, without need for HBV DNA measurement.

A substantial proportion of patients had past or ongoing HDV co-infection, with HDV antibodies detected in 8.1% and 14.3% of patients with HCC and cirrhosis, respectively. These HDV antibody rates are much higher than the 2% estimated HDV prevalence among community-screened HBsAg-positive adults in The Gambia [193]. Despite high anti-HDV prevalence reported in this study and others in Africa [214], routine HDV screening is not currently performed in most African countries, and HDV RNA quantification is not available. As a result, previous studies that identified HBV as a main cause of HCC in sSA did not report the rate of HDV coinfection [53, 71].

This study suggests that HCV infection contributes approximately 10% of HCC and cirrhosis cases in The Gambia. Recently, two studies have estimated similar rates of HCV infection among patients with cirrhosis and HCC, with 13% of cirrhosis and 21% of HCC cases in Africa being attributable to HCV infection respectively [49, 215]. I also found in this study and in the study reported in Chapter 5 of this thesis [70] that OBI was observed in a non-negligible proportion of HBsAg-negative patients with cirrhosis (42.9%) or HCC (53.0%). Yet, OBI remains a neglected global health issue.

The late presentation and short survival reported in this study is in line with the results from two HCC survival studies in Africa [71, 216]. A multicentre African retrospective study [71] analysed 1,315 patients with HCC from eight African countries and reported late stage presentation with a median tumour size of 8cm and a dramatically low median survival of 2.5 months (IQR 2.0-3.1), higher than the 1.5 months median survival among HCC patients observed in this study. Poverty, limited confidence in Western medicine, trust in and accessibility of traditional medicine, or distance from suitable hospitals could explain the delay in seeking care for advanced chronic liver disease. Anthropological studies suggested that people in sSA are reluctant to seek modern medicine when they develop end-stage liver disease [217].

This multicentre African study [71], however, did not analyse patients with end-stage liver disease without HCC. Therefore, my study fills an important knowledge gap. In the absence of cirrhosis registries and surveillance programs, survival data in patients with cirrhosis in sSA is scarce [218]. I found that late presentation is common among patients with cirrhosis in this Gambian cohort, with more than two-thirds of cirrhotic patients presenting with a clinical sign of decompensation. Patients with decompensated cirrhosis (n=49) had a median survival of 11.3 months and a survival rate at 12 months of only 48%. These results are similar to previously published data on survival among decompensated cirrhotic patients before the era of highly effective antiviral treatments [219]. Together with the reported effectiveness of antiviral therapy in HBV-related cirrhosis, my results strengthen the urgent need for screening programs, simplified treatment guidelines adapted to resource-limited settings, and increased access to HBV treatment in highly endemic areas.

Although TDF treatment suppressed viral replication and improved survival in patients with cirrhosis, the 2-year survival rate at 71% is well below the 1- and 3- year cumulative probability of survival from liver related deaths (99.4% and 97.8%, respectively) reported in Western and Asian studies [220-222]. This suboptimal survival among patients with cirrhosis in The Gambia could be explained by late patient presentation (two-thirds had decompensated cirrhosis at diagnosis), prolonged time interval (4.9 months) between clinical diagnosis of cirrhosis and initiation of antiviral therapy, and limited access to facilities for adequate management of cirrhosis. Although access to low cost TDF across Africa is increasing, it must be emphasised that decompensated cirrhosis is a complex systemic disease with multi-organ dysfunction that requires a sophisticated management beyond the etiologic treatment. Such comprehensive management is yet to be widely implemented in sSA, therefore

prevention of decompensation through screening, surveillance and early treatment is paramount.

Limitations

I acknowledge that our study has some limitations. First, due to the unavailability of routine INR or prothrombin time, I was unable to evaluate the severity of cirrhosis using the Child-Pugh or MELD scoring systems. CT scan with contrast was not routinely available in The Gambia at the time of the study, making it difficult to properly characterize and stage HCC. To account for these limitations and avoid overestimation of HCC, I excluded patients with AFP >200ng/ml who did not have a visible mass on liver ultrasound.

Secondly, HCV RNA and HDV RNA were not performed to evaluate the proportion of patients with active HCV and HDV infections. Thirdly, the efficacy of TDF in patients with cirrhosis was estimated using real-world data rather than data generated from a randomized control trial. Because of an important turnaround time between the diagnosis of cirrhosis and availability of HBV DNA viral load, which was a pre-requisite for TDF initiation, those who started TDF must have survived at least for this interval; this selection bias might have contributed, in a certain extent, to the observed association between TDF and improved survival in this study. I attempted to minimise this bias by performing IPTW analyses. Finally, this study was not a multi-country study. However, the findings in The Gambia are likely to be similar in most sSA countries where similar standard of sub-optimal care for cirrhosis and HCC are observed [64].

3.6. Conclusion

This study fills important knowledge gaps on the clinical characteristics and outcomes of end-stage liver disease in Africa, the estimated survival rates in patients with HCC and cirrhosis, the risk factors of mortality in HCC and the effectiveness of TDF to improve survival in HBV-positive patients with cirrhosis. These results highlight urgent needs for improved and decentralised treatment and surveillance programs for viral hepatitis and end-stage liver disease and could influence locally adapted guidelines and policy in the region.

A manuscript of this chapter is under review in Lancet Global Health

4. CHAPTER 4: Occult hepatitis B infection in The Gambia: prevalence and clinical significance

4.1. Abstract

Background

Prevalence and clinical outcomes of occult hepatitis B infection (OBI) have been poorly studied in Africa.

Methods

Using the PROLIFICA cohort, I compared the prevalence of OBI between HBsAg-negative healthy adults screened from the general population (controls) and HBsAg-negative patients with advanced liver disease (cases). Then I estimated the population attributable fraction (PAF) for the effect of OBI on advanced liver disease.

Results

OBI prevalence was significantly higher among cases (15/82, 18.3%) than controls (31/330, 9.4%, $p=0.03$). After adjusting for age, sex, and anti-hepatitis C virus (HCV) serology, OBI was significantly associated with advanced liver disease (OR, 2.8; 95% CI, 1.3–6.0; $p=0.006$). In HBsAg-negative people, the proportions of advanced liver disease cases attributable to OBI and HCV were estimated at 12.9% (95% CI, 7.5%–18.1%) and 16.9% (95% CI, 15.2%–18.6%), respectively.

Conclusions

OBI is endemic and an independent risk factor for advanced liver disease in The Gambia, West Africa. This implies that HBsAg-negative people with liver disease should be systematically screened for OBI. Moreover, the impact of infant hepatitis B immunization to prevent end-stage liver disease might be higher than previous estimates based solely on HBsAg positivity.

4.2. Introduction

More than 296 million individuals have chronic infection with hepatitis B virus (HBV) globally [41]. Chronic hepatitis B (CHB) infection is a major cause of cirrhosis [49], hepatocellular carcinoma (HCC) [52], and death [41]. By 2030, the World Health Organization (WHO) aims to reduce new HBV infections and HBV-related deaths by 90% and 65%, respectively [41, 167]. These ambitious goals can only be achieved with sustained interruption of transmission and significant scale-up of HBV screen-and-treat interventions [27].

In resource-limited settings where HBV is highly endemic and nucleic acid testing is scarce, HBV screening relies on hepatitis B surface antigen (HBsAg) testing, either using serology or more often rapid point-of-care tests. HBsAg screening, however, fails to detect occult hepatitis B infection (OBI), which is characterized by either loss or repression of HBsAg, or HBV pre-S/S mutations [132, 223].

OBI is defined as the presence of replication-competent HBV DNA in the liver tissue or blood of individuals tested negative for HBsAg using chemiluminescent or enzyme immuno-assays [132, 133]. HBsAg-negative patients with true OBI, characterised by low serum HBV viremia $<200\text{IU/mL}$ [132], could represent an important source of new HBV infections especially in blood transfusion services in Africa where HBsAg testing is the primary screening tool for HBV and nucleic acid testing or hepatitis B core antibody (anti-HBc) screening are not routine [76]. In addition, subjects with OBI are at risk of developing HCC [149] and should benefit from regular screening for liver complications. HBsAg-negative cirrhotic patients with undiagnosed OBI could miss potentially life-saving antiviral treatment opportunities [33].

The prevalence of OBI in sSA, its clinical significance in liver disease, and its public health impact on the HBV elimination goals remain unclear. Studies on OBI in Africa are limited and have mainly been conducted in selected populations: HIV-infected adults [224-227], health care workers [228], blood donors [229, 230], and patients with HCC [69]. Across the various populations, the reported prevalence of OBI in Africa range from less than 1% to more than 60%. However, the prevalence of OBI in the general population in sSA remains poorly documented [231] and no studies so far have investigated the association between OBI and advanced liver disease in sSA.

Given the high rate of OBI identified among HBsAg negative patients with advanced liver disease in Chapter 3, I used samples and data from two large-scale PROLIFICA studies in The Gambia to (i) estimated the prevalence of OBI in the general adult population, (ii) determined the clinical significance of OBI as a cause of advanced liver disease, and (iii) estimated the fraction of advanced liver disease attributable to OBI.

4.3. Methods

Study population

The PROLIFICA program in The Gambia concurrently conducted 2 large studies: the population-based screen-and-treat study [193] and the facility-based hepatocellular carcinoma case control (HC4) study [68].

Between December 2011 and January 2014, the test-and-treat study screened the general adult population living in 54 randomly selected enumeration areas (27 rural and 27 urban) in western Gambia. All inhabitants aged at least 30 years and living in each selected enumeration area were eligible and invited for HBsAg screening. From this screening intervention, all HBsAg-positive participants, as well as about 1 in 10 randomly selected HBsAg-negative participants from each enumeration area, were invited to the PROLIFICA liver clinic for a full assessment. HBsAg-negative controls were randomly selected using statistical software (STATA 11, Stata Corporation). In addition, 39 HBsAg-negative people identified through historical population-based serosurveys conducted in Keneba and Manduar villages in West Kiang District [68] were also recruited.

During the same period, the HC4 study enrolled consecutive patients with suspected advanced liver disease referred to the specialised outpatient liver clinic at the MRC Unit The Gambia, which was the only liver clinic in the country at the time and received all suspected cases of liver disease. Therefore, participants enrolled in the test-and-treat and the HC4 studies are, respectively, representative of the general adult population and population with advanced liver disease in The Gambia.

At enrolment, all HBsAg-positive and HBsAg-negative participants enrolled in these two studies had standardized clinical and biochemical assessments that included comprehensive medical history (including past medical, family and drug history), enquiry of known risk factors of liver disease (diet, alcohol intake), fasting liver stiffness measurement (LSM) [207] using transient elastography (Fibroscan 402, Echosens,

Paris, France) and non-contrast abdominal ultrasound scan. Serum and plasma was collected from all participants for liver biochemistry, virology and other investigations. Aliquots of samples were stored in -80°C freezers in the MRC Gambia biobank and patients gave consent for further analysis of data and samples. The studies were approved by the MRC-Gambia Government Joint Ethics Committee and all participants signed an informed consent.

In this OBI study, I included all HBsAg-negative participants from the two PROLIFICA studies [68, 193].

OBI case-control study

In this OBI study, cases were HBsAg-negative adults with advanced liver disease enrolled in the HC4 study and controls were HBsAg-negative adults without evidence of liver disease enrolled from the test-and-treat study.

DNA extraction

I extracted DNA from 200 µL plasma aliquots collected from HBsAg-negative participants. DNA extraction was with either the QIAamp DNA Blood MiniKit (QIAGEN) or the automated Arrow Nucleic Acid Extraction and Cell Separation Instrument (DiaSorin).

HBV molecular analysis

I set up a nested polymerase chain reaction (PCR) assay with limit of detection [LOD] <5 IU/mL to detect HBV DNA in HBsAg-negative plasma. The nested PCR used previously published primers [232] to amplify two regions of the HBV genome: 310 bps of the surface region in the primary PCR reaction, and 228 bp of the polymerase region in the nested (second) reaction. In all samples with positive HBV DNA on nested PCR, I used a highly sensitive in-house quantitative PCR technique validated against commercial assays [187] to quantify HBV viremia and determine viral load.

HBV serology

Participants enrolled in the test-and-treat study were screened for HBsAg in the community using the Determine rapid point-of-care kit, which has very good diagnostic performance in The Gambia [73]. To confirm negative HBsAg serology in all samples with detectable HBV DNA, I repeated the HBsAg serology using either

chemiluminescent microparticle immunoassay (Architect, Abbott; LOD, 0.1 IU/L) or the ultrasensitive Elecsys HBsAg II assay (Roche; LOD, 0.05 IU/L). All OBI-positive samples were then tested for anti-HBc using an enzyme-linked immunosorbent assay (ELISA; Abnova).

Occult hepatitis B infection

OBI was defined as detectable HBV DNA in plasma with negative HBsAg serology. In a sub-analysis, I further restricted the OBI definition to HBsAg-negative participants with HBV DNA levels <200 IU/mL (also called true OBI) to exclude individuals with “false” OBI due to S mutations (“S-escape” or S promoter mutations) or pre-S1/S2 variants and characterised by serum HBV DNA >2,000 IU/mL [137-143].

PreS/S mutation screening

In samples with detectable HBV DNA, I ran a second nested PCR targeting the preS/S region amplifying 315 bps and 113 bps in the primary and nested reactions, respectively.

Assessment of liver disease

To estimate liver fibrosis, I analysed results of LSM (Fibroscan) and liver/abdominal ultrasound scan. I used previously validated cut-off values of 7.9 kPa and 9.5 kPa to diagnose significant fibrosis ($\geq$ F2) and cirrhosis (F4) respectively [88]. Abdominal ultrasound assessed features of cirrhosis, portal hypertension, and/or hepatic mass. HCC was diagnosed either histopathologically or clinically in patients who met at least 2 of the following: 1 or more hepatic mass(es) $\geq$ 2 cm consistent with HCC on abdominal ultrasound, α -fetoprotein $\geq$ 200 ng/mL, and cirrhosis.

Statistical analysis

Prevalence of OBI in the general population and among patients with advanced liver disease was estimated in HBsAg-negative participants enrolled in the community-based test-and-treat and HC4 studies, respectively. Categorical variables were compared using Chi-squared test or Fisher’s Exact test, and continuous variables were compared using Kruskal-Wallis test. To assess the clinical significance of OBI, I used logistic regression to determine the association between OBI and advanced liver disease in HBsAg-negative people. Potential confounders (age, sex, alcohol consumption [never vs ever], family history of liver cancer, positive anti-HCV, and HIV

serologies) associated with advanced liver disease in univariable analysis ($p < 0.05$) were further included in multivariable logistic regression.

The population attributable fraction (PAF) and its 95% confidence interval (95% CI) were estimated for the effect of OBI and HCV on advanced liver disease [233]. All analyses were performed using STATA 13.1.

4.4. Results

Prevalence of OBI in the general population

Between December 2011 and January 2014, the test-and-treat study screened 5,980 adults living in urban and rural communities representative of western Gambia [193]. Of these, 5,485 were HBsAg negative individuals; 479 of them were randomly selected and 305 accepted further clinical and virological assessment. Compared to HBsAg-negative adults who did not accept the invitation ($n=174$), those who accepted were more likely to be female (60.0% [183/305] versus 54.0% [94/174]; $p=0.2$) and older (median age, 45 years; interquartile range [IQR], 36-57 versus 40 years; IQR, 34-53; $p=0.005$). Of the 305 HBsAg-negative participants who completed the clinical assessment, 14 did not have plasma samples and were excluded from this OBI analysis, leaving 291 HBsAg participants from the community.

An additional 39 HBsAg-negative people from the historical community-based screening in Keneba and Manduar [68] were included in this study, giving a total of 330 HBsAg-negative controls without advanced liver disease recruited from general population (Figure 4.1).

Thirty-one (31/330) HBsAg-negative individuals had detectable HBV DNA, giving an OBI prevalence of 9.4% (95% CI, 6.2-12.6). To assess whether having a HBsAg-positive household member was associated with OBI, I restricted the analysis to only individuals who had other household member(s) tested for HBsAg ($n=259$). There was no statistically significant association between OBI and HBsAg-positive household member; the prevalence of OBI was 12.6% (11/87) and 8.7% (15/172) in those with and without HBsAg-positive household member(s), respectively ($p=0.3$). When true OBI was defined as HBsAg-negative test and HBV DNA viral load <200 IU/mL, the prevalence of OBI in the general population was 2.7% (9/330).

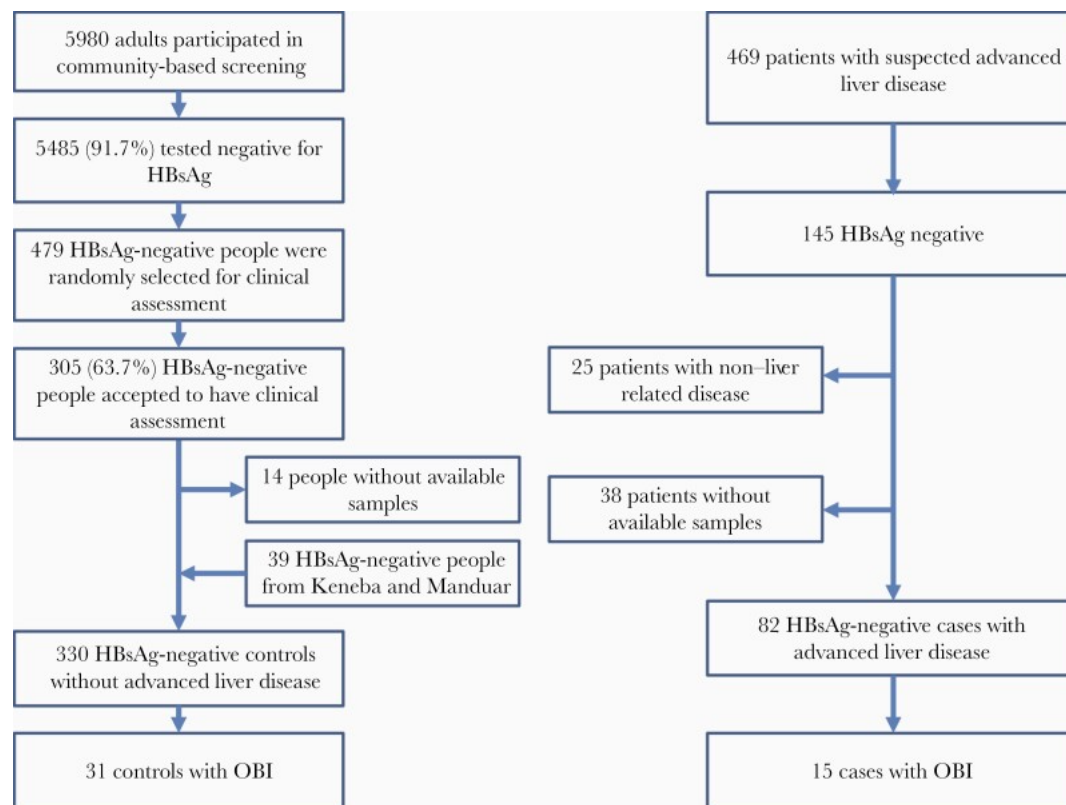


Figure 4.1. OBI study flow diagram

Prevalence of OBI among patients with advanced liver disease

During the same period (2012-2015), the HC4 study enrolled 469 patients with suspected advanced liver disease. Of these, 145 were HBsAg negative; 63 HBsAg-negative patients were excluded from this analysis (25 had non-liver disease and 38 had no samples available; figure 5.1). Therefore, 82 HBsAg-negative patients with confirmed advanced liver disease (29 with fibrosis/cirrhosis and 53 with HCC) were included in this analysis.

The prevalence of OBI among all HBsAg-negative patients with advanced liver disease was 18.3% (15/82; 95% CI, 9.7%-26.8%). When analysis was restricted to true OBI (as defined by HBV viral load detectable but <200 IU/mL), the prevalence of OBI among patients with advanced liver disease was 13.4% (11/82). HBsAg-negative patients with significant fibrosis or cirrhosis but without HCC (n=29) had the highest OBI prevalence (37.9%; 95% CI, 19.1%-56.7%). The general characteristics of the HBsAg-negative participants are described in Table 4.1.

Table 4.1. General characteristics of the study population

Characteristic	Healthy controls from the general population (n=330)	Cases with advanced liver disease		P value ^a
		Significant liver fibrosis and cirrhosis (n=29)	HCC (n=53)	
Age, y, median (IQR)	46 (36-59)	53 (33-67)	56 (48-65)	0.06
Male sex, No. (%)	129 (39)	20 (69)	38 (72)	<0.001
BMI, median (IQR)	22.4 (19.8-26.0)	18.9 (15.6-22.4)	19.6 (17.3-22.1)	<0.001
Alcohol, No. (%)	22 (7)	1 (4)	8 (15)	0.05
Family history of liver cancer, No. (%)	6 (2)	0	2 (4)	0.6
Positive HCV serology, No. (%)	9 (3)	4 (14)	11 (22)	<0.001
Positive HIV serology, No. (%)	12 (4)	0	3 (6)	0.6
ALT, IU/L, median (IQR)	22 (17-27)	46 (23-76)	52 (35-79)	<0.001
AST, IU/L, median (IQR)	27 (22-32)	78 (42-139)	114 (73-264)	<0.001
GGT, IU/L, median (IQR)	24 (18-34)	182 (54-434)	315 (139-516)	<0.001
Platelet/mm ³ , median (IQR)	216 (172-268)	264 (161-385)	269 (186-369)	0.01
LSM, kPa, median (IQR)	4.5 (3.7-5.5)	26.0 (8.8-66.4)	58.9 (27.0-75.0)	<0.001
OBI, No. (%)	31 (9)	11 (38)	4 (8)	<0.001

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index; GGT, γ -glutamyl transferase; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; IQR, interquartile range; LSM, liver stiffness measurement; OBI, occult hepatitis B infection

^a P values were obtained using Kruskal-Wallis test for continuous variables and Chi-squared test for categorical values.

Characteristics of participants with OBI

Table 4.2 summarizes the characteristics of participants with OBI. All 31 community-screened controls with OBI had no evidence of liver disease. Compared to controls with OBI (n=31), cases with OBI (n=15) were predominantly males (73%, p=0.03) and had higher median LSM (13.0 kPa [IQR, 7.9-48.0] vs 4.7 kPa [4.3-6.0]; p<0.001), higher median ALT (45 IU/L [IQR, 24-89] vs 20 IU/L [IQR, 17-30], p<0.001), higher median AST (82 IU/L [IQR, 29-142] vs 27 IU/L [IQR, 25-31], p<0.001), and had lower HBV DNA levels (2.7 log IU/mL [IQR, 2.1-3.4] vs 3.7 log IU/mL [IQR, 3.4-4.0], p=0.08).

Table 4.2. Clinical characteristics of people with OBI by disease status

Characteristic	Controls with OBI (n=31)	Cases with OBI (n=15)	P value
Age, y, median (IQR)	44 (38-66)	53 (35-56)	0.8
Sex			
Male, no. (%)	12 (39)	11 (73)	0.03
Female, no. (%)	19 (61)	4 (27)	
BMI, kg/m ² , median (IQR)	21.9 (20.3-23.4)	21.8 (19.6-22.8)	0.5
Alcohol, no. (%)			
Never	28 (93)	14 (100)	1.0
Ever	2 (7)	0	
Family history of liver disease, no. (%)			
No	31 (100)	15 (100)	NA
Yes	0	0	
ALT, IU/L, median (IQR)	20 (17-30)	45 (24-89)	<0.001
AST, IU/L, median (IQR)	27 (25-31)	82 (29-142)	<0.001
GGT, IU/L, median (IQR)	21 (15-28)	199 (24-354)	<0.001
Platelet/mm ³ , median (IQR)	203 (174-245)	256 (186-305)	0.2
LSM, kPa, median (IQR)	4.7 (4.3-6.0)	13.0 (7.9-48.0)	<0.001
HBV DNA, log IU/mL, median (IQR) ^a	3.7 (3.4-4.0)	2.7 (2.1-3.4)	0.08
HBV DNA level, IU/mL, no. (%)			
<50	7 (23)	9 (73)	0.003
50-2000	5 (16)	4 (7)	
2000-20,000	18 (58)	1 (7)	
>20,000	1 (3)	1 (7)	

Characteristic	Controls with OBI (n=31)	Cases with OBI (n=15)	P value
Quantitative HBsAg level, IU/mL, no.			
>0.05	NA	0	NA
<0.05	NA	15	
Anti-HBc serology, no. (%)			
Negative	2 (6)	0	
Positive	29 (94)	15 (100)	
Anti-HCV serology, no. (%)			
Negative	31 (100)	13 (87)	0.1
Positive	0	2 (13)	
Anti-HIV serology, no. (%)			
Negative	30 (97)	15 (100)	1.0
Positive	1 (3)	0	
HBV genotype, no. (%)			
A	0 (0)	5 (36)	NA
E	31 (100)	9 (64)	
Pre-S2 mutation, no. (%)			
Wild type	26 (84)	11 (79)	0.7
Mutation	5 (16)	3 (21)	

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; Abbreviations: BMI, body mass index; GGT, γ -glutamyl transferase; HBc, hepatitis B core; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HIV, human immunodeficiency virus; IQR, interquartile range; LSM, liver stiffness measurement; OBI, occult hepatitis B infection

^a After excluding those with detectable, but not quantifiable, HBV DNA

Pre-S2 deletion mutations were detected in 18% (8/45) of adults with OBI, with no significant difference between controls with OBI (5/31) and cases with OBI (3/14; $p=0.7$). All community-screened controls with OBI were infected with HBV genotype E. In contrast, all 4 HCC cases with OBI were infected with genotype A. There was no statistically significant difference in HCV and HIV prevalence between cases and controls with OBI. In 330 community-screened participants, there were no clinical or demographic differences between those with and without OBI (Table 4.3).

Table 4.3. General characteristics of community-screened adults with and without OBI

Characteristic	HBsAg-negative adults from general population (n=330)		P value
	OBI (n=31)	No OBI (n=299)	
Age, median (IQR)	44 (38-66)	46 (36-58)	0.6
Sex, no. (%)			
Male	12 (39)	117 (39)	0.9
Female	19 (61)	182 (61)	
BMI, median (IQR)	21.9 (20.3-23.4)	22.5 (19.8-26.3)	0.2
Alcohol, no. (%)			
Never	28 (93)	277 (93)	0.9
Ever	2 (7)	20 (7)	
Family history, no. (%)			
No	31 (100)	293 (98)	0.4
Yes	0	6 (2)	
Anti-HCV, no. (%)			
Negative	31 (100)	289 (97)	0.3
Positive	0	9 (3)	
Anti-HIV, no. (%)			
Negative	30 (97)	287 (96)	0.9
Positive	1 (3)	11 (4)	

Abbreviations: BMI, body mass index; HCV, hepatitis C virus; HIV, human immunodeficiency virus; IQR, interquartile range; OBI, occult hepatitis B infection

OBI is an independent factor associated with advanced liver disease

OBI prevalence was significantly higher among cases with advanced liver disease (18.3%, 95% CI: 9.7-26.8) compared to the control group from the general population (9.4%, 95% CI: 6.2-12.6, $p=0.03$) (Table 4.4). In addition to OBI, older age group (≥ 45 years old), male sex, and positive anti-HCV antibody were also associated with advanced liver disease in HBsAg-negative people in univariable analysis (Table 4.4). In multivariable analysis adjusted mutually for these variables, all except age remained significantly associated with advanced liver disease: male sex [odds ratio [OR]: 1.6; 95% CI 2.5-8.4, $p<0.001$], positive anti-HCV antibody [OR: 10.8, 95% CI 4.0-29.2, $p<0.001$] and OBI [OR: 2.8, 95% CI 1.3-6.0, $p=0.006$].

Using adjusted OR for the association between OBI and advanced liver diseases, the population attributable fraction (PAF) was obtained. In HBsAg-negative individuals, the

proportion of advanced liver disease cases attributable to OBI was estimated at 12.9% (95% CI: 7.5-18.1%). The PAF for HCV was 16.9% (95% CI 15.2-18.6).

Table 4.4. Risk factors of advanced liver disease in HBsAg-negative adults

Variables	Cases with advanced liver disease, no. (%) (n=82)	Healthy controls from general population, no. (%) (n=330)	Crude OR		Adjusted OR ^a	
			OR (95% CI)	P value	OR (95% CI)	P value
Age, y						
<45	23 (29)	150 (45)	1.0	0.01	1.0	0.1
≥45	55 (71)	180 (55)	2.0 (1.2-3.4)		1.6 (0.9-2.8)	
Sex						
Female	24 (29)	201 (61)	1.0	<0.001	1.0	<0.001
Male	58 (71)	129 (39)	3.8 (2.2-6.4)		1.6 (2.5-8.4)	
Alcohol						
Never	71 (89)	305 (93)	1.0	0.2		
Ever	9 (11)	22 (7)	1.8 (0.8-4.0)			
Family history						
No	80 (98)	324 (98)	1.0	0.7		
Yes	2 (2)	6 (2)	1.4 (0.3-6.8)			
Anti-HCV						
Negative	64 (81)	320 (97)	1.0	<0.001	1.0	<0.001
Positive	15 (19)	9 (3)	8.3 (3.5-19.9)		10.8 (4.0-29.2)	
Anti-HIV						
Negative	78 (96)	317 (96)	1.0	0.9		
Positive	3 (4)	12 (4)	1.0 (0.3-3.7)			
OBI						

Variables	Cases with advanced liver disease, no. (%) (n=82)	Healthy controls from general population, no. (%) (n=330)	Crude OR		Adjusted OR ^a	
			OR (95% CI)	P value	OR (95% CI)	P value
Negative	67 (82)	299 (91)	1.0	0.03	1.0	0.006
Positive	15 (18)	31 (9)	2.2 (1.1-4.2)		2.8 (1.3-6.0)	

Abbreviations: BMI, body mass index; HCV, hepatitis C virus; HIV, human immunodeficiency virus; IQR, interquartile range; OBI, occult hepatitis B infection

^a Model included age, sex, anti-HCV and OBI

4.5. Discussion

This study reports a high prevalence of OBI in both the general population (9.4%) and among patients with advanced liver disease (18.3%), suggesting that OBI is an independent risk factor for advanced liver disease in The Gambia, West Africa. OBI prevalence has been poorly reported in the general population in sSA with most studies on OBI in Africa conducted in selected populations [69, 224-230]. From a community-based screening, the prevalence of OBI among HBsAg-negative adults in the general population was high (9.4%), similar to the prevalence of overt HBsAg (8.5%) previously reported in The Gambia [193]. This finding raises an important public health concern because current hepatitis B screening strategies in resource-limited countries are based on HBsAg serology alone and therefore cannot identify individuals with OBI.

In Uganda, the prevalence of OBI among an urban population screened in hospital in Kampala was 30% [231]. Studies conducted in HIV-infected individuals in Sudan and Mozambique also reported high OBI prevalence of 8.3% and 17.5%, respectively [224, 226]. In Nigeria, OBI was found in 17% of blood donors [230].

In this study, I did not identify any predictors of OBI among community-screened healthy adults. From a public health perspective, the absence of associated factors with OBI among the general population makes it difficult to develop targeted interventions to identify at risk individuals. Importantly, age did not differ between community-screened healthy controls with or without OBI, suggesting that OBI might not only be accounted for by HBsAg loss in the ultimate phase of the natural history of chronic hepatitis B infection [132, 133].

To date, the clinical outcomes of OBI in Africa have been poorly assessed. Using biochemical markers of liver fibrosis, Carimo et al found that HIV-infected subjects with OBI in Mozambique had higher risk of liver fibrosis than HIV-infected subjects without HBV infection [226]. However, the clinical impact of OBI in non-HIV populations exposed to the African environment is unknown.

This study found that OBI is more frequently observed in cases with advanced liver disease (18.3%) compared to community-screened healthy controls (9.4%). Interestingly, patients with HCC had a lower prevalence of OBI than patients with significant fibrosis or cirrhosis (8% vs 38%). This might reflect the natural history of hepatitis B in Africa where HCC is often observed without cirrhosis, therefore in an earlier phase in the natural history of chronic HBV infection before the spontaneous loss of HBsAg. A direct role of HBV genotype A, which has been associated with higher risk of HCC in Africa [12, 64], or aflatoxin exposure remains to be elucidated. A prospective analysis with serial sample analysis of a larger longitudinal cohort of participants with OBI may find answers.

Importantly, this study found that OBI is an independent risk factor for advanced liver disease (OR, 2.8; 95% CI, 1.3-6.0; $p=0.006$) in The Gambia. Moreover, OBI remained an independent factor of advanced liver diseases even when the analysis was restricted to patients with true OBI with HBV DNA viral load <200 IU/mL (OR, 7.0; 95% CI, 2.5-20.0; $p<0.001$). This is in line with a previous meta-analysis reporting a 2.9-fold increased risk of HCC in individuals with OBI [149], with increased relative risk of HCC in both HCV and non-HCV-infected populations. This meta-analysis included 16 studies with total of 3,256 subjects, mainly from Asia with no subjects from Africa. My study, therefore, provides additional evidence on the association between OBI and advanced liver disease in Africa.

Further, this study found that 12.9% (95% CI, 7.5-18.1) of advanced liver disease cases in HBsAg-negative people in The Gambia were attributable to OBI. Therefore, OBI is responsible for a significant proportion of advanced liver disease cases, further increasing the population attributable risk for overall HBV-related advanced liver disease in The Gambia. This finding implies that the impact of infant hepatitis B immunization in preventing HBV infection, and by extension reducing the incidence of HBV-related liver disease, may be much higher than previous estimates based on PAF using HBsAg positivity alone [52].

The findings in this study could have important clinical implications in the management of significant liver disease and HCC screening in Africa. Patients with cirrhosis and OBI (with detectable viral load) would be eligible for antiviral therapy according to current guidelines [33]. Antiviral therapy for HBV using tenofovir is becoming more accessible in many resource-limited countries, with generic drugs available at very low cost (US\$ 1-2/month). It is therefore urgent to improve the identification of OBI in patients with cirrhosis, who would otherwise miss opportunities to receive potentially life-saving treatment. Given these results of OBI as risk factor for advanced liver disease, along with results from Chapter 4 demonstrating the impact of tenofovir treatment in preventing death in patients with cirrhosis, HBsAg-negative individuals in Africa with advanced liver disease should be systematically tested for HBV DNA. Current screening strategies for HBV do not systematically include anti-HBc serology or HBV DNA measurement due to cost and logistical constraints. In addition, the results of this study suggest that individuals with OBI should be regularly screened for advanced liver disease and HCC. Both approaches imply additional costs for HBV-endemic countries where OBI may be highly prevalent and resources for these additional screenings severely limited.

The mechanisms underlying OBI infection remain debated. OBI is often considered as the ultimate phase of the natural history of chronic hepatitis B following HBsAg loss in which replicative activity and gene expression of HBV DNA and cccDNA are suppressed, resulting in extremely low rates of virion production [133, 144]. However, the similar age between healthy adults with and without OBI in our study does not support this hypothesis.

HBsAg variants resulting from mutations in the S gene may result in poor detection of HBsAg using commercially available serology assays [132]. In this study, only a minority of participants with OBI (16% of controls and 21% of cases) had detectable pre-S mutations, with no difference in HBsAg mutations according to liver disease severity. As a result, viral mutation cannot fully explain the high OBI prevalence among cases nor the relationship between OBI and advanced liver disease. Studies from the PROLIFICA group found that pre-S2 deletion mutants are only observed in coinfection of mutant and wild-type HBV, with the latter able to produce HBsAg [13]. The impact of OBI on HCC development is certainly related to direct liver carcinogenesis through the integration of HBV DNA into the host genome leading to the synthesis of pro-oncogenic proteins [132].

Limitations

This study has some limitations. First, I analysed samples collected at a single time point and did not perform liver biopsies to measure hepatic cccDNA levels. HBV DNA in OBI is only detected intermittently in blood [132] therefore analysing serial samples increases diagnostic accuracy and might even increase the estimated OBI prevalence. Liver biopsies are difficult to perform in sSA and their indication difficult to justify in healthy controls. Moreover, there are no widely available standards for detecting OBI in liver tissue [132, 133]. Second, I did not assess the long-term clinical impact of OBI in our study population. In particular, the rate of liver disease progression and loss of HBV DNA in patients with OBI in Africa is currently unknown. I will address this question in a longitudinal study. Third, I was unable to measure HCV RNA in the 2 anti-HCV positive individuals with OBI in this study. Fourth, I did not assess the role of aflatoxin exposure on liver disease severity in individuals with OBI, nor the prevalence of HBsAg-immune complexes. As a result, the mechanisms underlying the development of OBI and its impact on liver outcomes need to be further elucidated. Finally, I did not characterize alcohol use in detail. Alcohol use is rare in this Gambian cohort and alcohol has not been associated with risk of advanced liver disease in this cohort [12, 68, 193], possibly due to the difficulty in ascertaining its consumption in the cultural/religious context.

4.6. Conclusion

OBI is frequently observed in The Gambia and is an independent risk factor for liver fibrosis, cirrhosis, and HCC accounting for 12.9% of advanced liver disease cases in HBsAg-negative adults. Strategies to identify adults with OBI need to be developed in Africa. Access to low-cost HBV DNA or reliable alternatives to HBV nucleic acid testing is urgent. These findings could have important clinical, public health, and economic implications on HBV and liver disease management in Africa, as well as on the 2030 WHO elimination targets.

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5. CHAPTER 5: Hepatitis B testing and treatment in HIV patients in The Gambia: compliance with international guidelines and clinical outcomes

5.1. Abstract

Background

Compliance with WHO guidelines on HBV screening and treatment in HIV co-infected patients is often challenging in resource limited countries and has been poorly assessed in sub-Saharan Africa.

Methods

Between 2015 and 2016, I assessed physicians' compliance with WHO guidelines on HBV testing in HIV infected patients attending the largest HIV clinic in The Gambia. Then I offered HBsAg test to consenting HIV patients and compared presence and severity of liver disease between HIV-HBV co-infected patients and randomly selected HIV mono-infected controls.

Results

Among 870 HIV-infected patients regularly seen in this clinic, only 187 (21.5%, 95% CI 18.8–24.3) had previously been screened for HBsAg. Of them, 23 (12.3%, 95% CI 8.0–17.9) were HBsAg positive, none had any liver assessment, and only 6 (26.1%) had received a Tenofovir based highly active antiretroviral (HAART) regimen.

When offered, all 870 agreed to HBsAg testing during this study with 94/870 (10.8%, 95% CI 8.8–13.1) testing HBsAg-positive. Seventy-eight HIV-HBV co-infected patients and 40 HBsAg-negative controls (HIV mono-infected) completed full liver assessment. At the time of liver assessment, 61/78 (78.2%) HIV-HBV co-infected patients were on HAART of whom 7 (11.5%) received Tenofovir based HAART and 54 (88.5%) received Lamivudine based HAART with Tenofovir.

HIV-HBV co-infected patients had higher APRI score compared to controls (0.58 vs 0.42, $p=0.002$). HBV DNA was detectable in 52/53 (98.1%) coinfecting patients with 14/53 (26.4%) having HBV DNA $>20,000$ IU/L. 10/12 (83.3%) had at least one

detectable 3TC-associated HBV resistance, which tended to be associated with increase in liver fibrosis after adjusting for age and sex ($p=0.05$).

Conclusions

Compliance with HBV testing and treatment guidelines is poor in this Gambian HIV facility, putting co-infected patients at risk of liver complications. However, the excellent uptake of HBsAg screening and linkage to care suggests that improvements are feasible.

5.2. Introduction

Chronic hepatitis B (CHB) infection is a major public health problem and a leading cause of morbidity and mortality globally [41]. It affects approximately 296 million individuals worldwide and accounts for 820,000 deaths annually, mainly from cirrhosis and HCC [41, 42]. Without effective interventions and treatment, CHB infection will lead to an estimated 11.8 million deaths by 2030, primarily as a result of cirrhosis and hepatocellular carcinoma (HCC) [168]. Most of these deaths will occur in low-income countries (LICs) in sub-Saharan Africa (sSA) and Asia where CHB infection is endemic.

HIV infection is also endemic in sSA [41], and affects a significant proportion of patients with CHB infection. Western Africa in particular has one of the highest prevalence of HIV-HBV co-infection [152]. Uncontrolled HIV-HBV coinfection, due either to lack of or ineffective antiretroviral therapy (ART), increases the risk of liver-related mortality by 13-fold among men with HIV-HBV coinfection (14.2/1000) compared to men with either HIV-1 (1.7/1000) or HBsAg (0.8/1000) mono-infection [154]. Early and effective Tenofovir Disoproxil Fumarate (TDF) based ART, on the other hand, reduces risk of death among coinfecting patients underlying the need for implementing universal HBV testing and prompt ART treatment.

As a result, the WHO recommends HBsAg testing for all HIV-infected patients, and highly active antiretroviral therapy (HAART) containing at least two drugs effective against HBV for all HIV-HBV co-infected patients, irrespective of disease stage or CD4 count [163].

In this study, I assessed the compliance to the WHO guidelines of HBV testing and treatment and its impact on clinical outcomes in the largest HIV treatment centre in The Gambia.

5.3. Methods

Patients and study design

In a cross-sectional study, I enrolled consecutive HIV infected adults followed up at the Hands-on Care HIV clinic in Brikama (The Gambia) who have had at least two successful clinic visits and baseline investigations. After written consent, I reviewed patient case notes to determine the coverage of HBsAg testing for all HIV patients and

use of TDF-based ART for known HIV-HBV coinfecting patients. Then I screened all patients for HBsAg using the Determine[®] rapid point-of-care kit (Alere[®], USA). All HBsAg positive patients (HIV-HBV co-infected) and randomly selected HBsAg negative controls (HIV mono-infected) had confirmatory HBsAg serology test (Architect HBsAg, Abbott, USA) and were invited for further liver assessment.

Demographic and clinical information such as age, gender, past medical history, duration since HIV diagnosis and antiretroviral drug exposure were collected, and a clinical examination and bedside ultrasound were performed. Liver transaminases (Vitros 350 Analyzer, Ortho Clinical Diagnostics, USA), haemoglobin and platelet count (CELL-DYN 3700 sample loader, Abbott, USA), HIV RNA (Abbott Real Time HIV-1 assay, 40copies/mL limit of detection), CD4⁺ T-cell count (flow cytometry) and in-house HBV DNA viral load quantification [187] were measured for all patients.

Assessment of liver fibrosis

In accordance with WHO guidelines for settings without access to transient elastography (Fibroscan), I assessed liver fibrosis using non-invasive tests: aspartate aminotransferase (AST)-to-platelet ratio index (APRI) and FIB-4. APRI was calculated as $[\text{AST level (IU/L)} / \text{upper limit of normal for AST (IU/L)} \times 100 / \text{platelet count (10}^9\text{/L)}]$, and FIB-4 as $[\text{age (years)} \times \text{AST (IU/L)} / (\text{platelet count (10}^9\text{/L)} \times \text{ALT (IU/L)}^{1/2})]$. Significant fibrosis was defined as APRI >1.5 or FIB-4 >3.25 [80, 234].

HBV molecular analysis and polymerase gene sequencing

For all HBsAg positive patients, I extracted DNA from 500µL plasma using the QIAamp DNA Blood MiniKit (QIAgen, Germany), and quantified HBV DNA by TaqMan based in-house qPCR assay with lower limit of detection of 50IU/mL [187] using probe (200nM) sequence HBVTAQPR:5'FAM-CCTCTKCATCCTGCTGCTATGCCTCATC-3'MGBNFQ with forward and reverse primer (400nM) sequences HBVTAQ1:5'-GTGTCTGCGGCGTTTTATCA-3' and HBVTAQ2:5'-GACAAACGGGCAACATACCTT-3' respectively [235].

In patients with detectable HBV DNA viral load $\geq 20,000\text{IU/L}$, I sequenced the hepatitis B polymerase gene sequence to assess resistance mutations. For the sequencing, I amplified the region from nucleotide 101 to 1149 of the HBV polymerase (pol) gene containing the highly conserved tyrosine-methionine-aspartate-aspartate (YMDD) motif by standard PCR using high fidelity polymerases (Supermix High Fidelity

polymerase from Invitrogen or Q5[®] High-Fidelity DNA Polymerase from New England BioLabs) and primers sense 5'-AYTGTCTCTTCCAYMTCRTC-3' and antisense 5'-GGGGTAAAGGTTTCAGRTAYTG-3'. Genotype of the amplified sequence was assessed using the NCBI genotyping tool [236].

Statistical analyses

Demographic, clinical and virological characteristics of the study participants were presented according to their HBsAg status, and comparison made between HIV mono-infected and HIV-HBV co-infected patients using the chi-squared test, Fisher's exact test, or Wilcoxon rank-sum test. Factors associated with significant fibrosis (APRI >1.5) were identified using univariable logistic regression. As the number of participants with APRI >1.5 was small, multivariable analysis was not performed.

Ethical approval

The MRC Scientific Coordinating committee and the joint Gambia Government/MRC Ethics committee approved this study (SCC1388). Consenting of participants followed group and individual sensitization, explanation of a study protocol and sufficient time for questions and discussion with spouse/family.

5.4. Results

Study population and compliance with guidelines

Between September 2015 and February 2016, 870 HIV-infected patients registered at the Hands-on Care HIV clinic were invited to participate in this study. All invited patients agreed to take part in the study. Review of patient case notes showed that only 187 (21.5%, 95% CI 18.8–24.3) patients had previously been tested for HBsAg, with 23 (12.3%, 95% CI 8.0–17.9) found to be HIV-HBV coinfectd. None of the HIV-HBV co-infected patients had a prior liver assessment based on liver transaminases, ultrasound, measurement of liver fibrosis or liver biopsy. All 23 HIV-HBV co-infected were receiving ART, with only 6 (26.1%, 95 CI 10.2–48.4) being on a TDF-based regimen.

After HBsAg screening, 94/870 tested positive giving a seroprevalence of 10.8% (95% CI 8.8–13.1). Of these, 15/94 (16%) were lost to follow up, and 1 died before the liver assessment. The remaining 78 HIV-HBV coinfectd patients underwent full liver assessment, along with 40 randomly selected HBsAg negative controls (Figure 5.1).

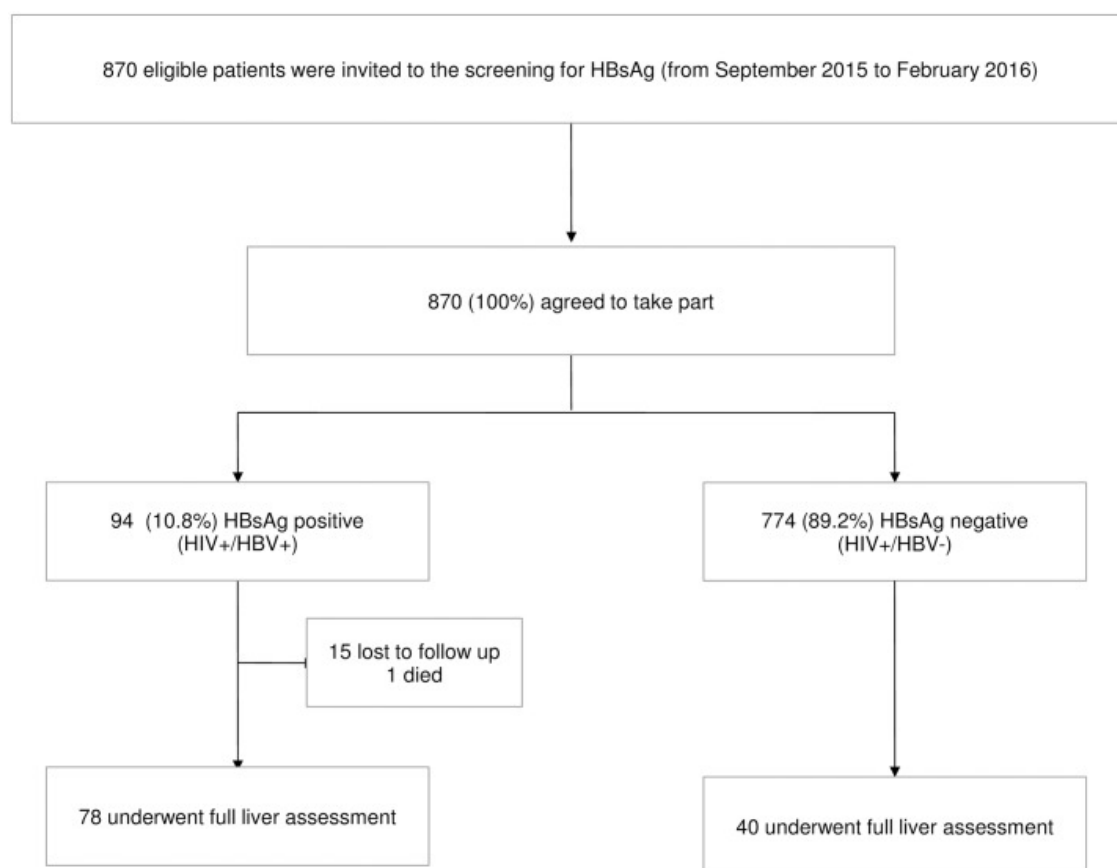


Figure 5.1. Flow diagram of the study population

Of the 78 co-infected patients who completed liver assessment as part of this study, 61 (78.2%, 95% CI 67.4–86.1) received ART, with 54/61 (88.5%, 95% CI 77.4–94.5) receiving Lamivudine (3TC) without TDF and only 7/61 (11.5%) receiving a TDF-based regimen. Median transaminase was within normal limits with only 5 patients (6.4%) having ALT above the upper limit of normal (ULN) and 1 (1.3%) with ALT >2xULN. Table 5.1 provides the characteristics of the study population by HBsAg status.

Table 5.1. Characteristics of the study population by HBV coinfection

Parameters	HIV-HBV (n=78)	HIV (n=40)	P value
Median age (years, IQR)	38 (35-48)	42 (35-46)	0.5
Male sex (n, %)	8 (20)	16 (21)	0.9
Median BMI (Kg/m ² , IQR)	21 (19-24)	22 (20-25)	0.4
HIV infection type (n, %)			
HIV1	58 (78)	34 (90)	0.2
HIV2	3 (4)	2 (5)	
HIV1+2	13 (18)	2 (5)	

Parameters	HIV-HBV (n=78)	HIV (n=40)	P value
Median duration since HIV diagnosis (months, IQR)	34 (14-68)	66 (20-82)	0.04
CD4+ T-cell count (n, %) <200 cells/mm ³ ≥200 cells/mm ³	24 (34) 46 (66)	13 (34) 25 (66)	1.0
Median CD4+ T-cell count (cells/mm ³ , IQR)	293.5 (147- 444)	302 (170- 411)	0.7
HIV RNA (n, %) Undetectable Detectable	30 (53) 27 (47)	26 (70) 11 (30)	0.09
Median HIV RNA (log copies/mL, IQR)	4.1 (3.2-5.0)	4.2 (3.2-4.6)	0.7
HBV DNA (n, %) Undetectable 50-2,000 IU/mL 2,000-20,000 IU/mL ≥2,000 IU/mL	1 (1) 46 (60) 12 (16) 18 (23)	NA	NA
Median ALT (IU/L, IQR)	15 (12-25)	17 (11-26)	0.9
Median AST (IU/L, IQR)	28 (24-40)	24 (19-30)	0.004
Median platelet count (x10 ⁹ /L, IQR)	186 (152-220)	198 (163- 238)	0.1
APRI (n, %) <0.5 0.5-1.5 ≥1.5	29 (39) 38 (51) 7 (10)	27 (69) 12 (31) 0	0.005
Median APRI (IQR)	0.58 (0.43- 0.88)	0.42 (0.35- 0.53)	0.002
FIB-4 (n, %) <1.45 1.45-3.25 ≥3.25	30 (40) 39 (53) 5 (7)	25 (64) 14 (36) 0	0.03
Median FIB-4 (IQR)	1.72 (1.22- 2.34)	1.35 (1.06- 1.63)	0.004
ART regimen (n, %) Never treated 3TC without TDF TDF	17 (22) 54 (69) 7 (9)	5 (13) 29 (74) 5 (13)	0.5
Duration of 3TC exposure (n, %)*, ** <12 months 12-60 months ≥60 months	14 (23) 32 (52) 15 (25)	7 (21) 13 (38) 14 (41)	0.2

*This analysis only includes those who have ever received ART

** 3TC without a second HBV active anti-viral drug

Abbreviations: ALT, alanine aminotransferase; APRI, AST to platelet ratio index; ART, antiretroviral therapy; AST, aspartate aminotransferase; BMI, body mass index; HIV, human immunodeficiency virus; RNA, ribonucleic acid; TDF, Tenofovir Disoproxil Fumarate; 3TC, Lamivudine

Assessment of liver fibrosis

Compared to HIV mono-infected patients, HIV-HBV coinfecting patients had significantly higher median APRI (0.42 vs 0.58, $p = 0.002$) and FIB-4 (1.72 vs 1.35, $p = 0.004$), suggesting higher rate of liver fibrosis in coinfecting patients. Table 5.2 shows the factors associated with significant liver fibrosis.

Table 6.2. Factors associated with significant liver fibrosis (APRI 1.5) in HIV-infected participants (n=113)

	Prevalence of significant fibrosis (%)	Crude odds ratios	
		OR, 95% CI	P value
Age group <40 ≥40	5/58 (9) 2/55 (4)	1.0 0.4 (0.1-2.2)	0.3
Sex Women Men	6/90 (7) 1/23 (4)	1.0 0.6 (0.1-.6)	0.7
BMI <25 ≥25	7/86 (8) 0/27 (0)	1.0 NA	0.1
Steatosis No Yes	6/105 (6) 1/8 (13)	1.0 2.4 (0.2-22.4)	0.5
HIV infection type HIV1 HIV2 HIV1+2	3/89 (3) 0/4 (0) 3/15 (20)	1.0 NA 7.2 (1.3-39.6)	0.02
HIV RNA Undetectable Detectable	0/53 (0) 6/37 (16)	NA	0.002
CD4+ T-cell count <200 cells/mm ³ ≥200 cells/mm ³	1/36 (3) 5/68 (7)	1.0 2.8 (0.3-24.7)	0.4
HBV co-infection No Yes	0/39 (0) 7/74 (10)	NA	0.04
ART Never 3TC without TDF TDF	2/19 (11) 5/82 (6) 0/11 (0)	1.0 0.6 (0.1-3.1) NA	0.5

Abbreviations: ART, antiretroviral therapy; BMI, body mass index; HBV, hepatitis B virus; HIV, human immunodeficiency virus; RNA, ribonucleic acid; TDF, Tenofovir Disoproxil Fumarate; 3TC, Lamivudine

HBV molecular virology in 3TC exposed patients

Of 54 co-infected patients who were treated with 3TC without TDF, after a median duration of 28 months (IQR 12-60) on 3TC, HBV DNA and HIV RNA were detectable in 52/53 (98.1%, 95% CI 87.1-99.8) and 15/38 (39.5%, 95% CI 24.8-56.3) respectively. Among them, 14 of 53 (26.4%, 95% CI 16.0-40.3) had HBV DNA >20,000 IU/mL. I successfully sequenced the region of the polymerase gene containing the YMDD motif in 12 of these 14 patients and found 10 (83.3%) had M204V/I mutations. Additional compensatory mutations at rtL180M were detected in 8 (66.7%) patients and mutations at both rtL180M and rtV173L were detected 3 (25.0%) patients. Phylogenetic analysis of the polymerase gene sequence showed all but one patient (HBV genotype A) were infected with HBV genotype E.

I assessed the joint effect of HBV co-infection and 3TC resistance mutations on significant fibrosis (APRI >1.5) in HIV-infected patients. Compared to HIV mono-infected, the prevalence of significant fibrosis was higher in HIV-HBV co-infected patients, and especially in those with 3TC resistance mutations. While no HIV mono-infected patients (0/39) had significant fibrosis, 6/66 (9%) of patients co-infected with wild type HBV and 1/8 (13%) co-infected with resistant HBV strain had significant fibrosis (p for trend 0.05).

5.5. Discussion

This study provides two key messages: 1) compliance with the WHO hepatitis B testing and treatment guidelines [163] is poor with only 21.5% of HIV patients systematically screened for HBV infection and less than 15% on TDF-based ART, which puts HIV patients at risk of liver complications; and 2) testing and linkage to care for HBV infection in HIV patients is accepted and feasible in HIV care programme in The Gambia.

These messages are very important for The Gambia where the prevalence of HIV and HBV are high (2% and 8.5% respectively) with poor knowledge of hepatitis B and limited access to screening and treatment for HBV infection [193]. The Gambia also has one of the highest prevalence of HCC in West Africa with two-thirds of these cases attributable to HBV infection [12, 53, 206].

The compliance to WHO guidelines on the management of HBV and HIV co-infection in Africa, a region with high HIV-HBV coinfection prevalence [41, 152], has been poorly

documented. I found that in 2016, despite strong international recommendations and increasing global commitment to HBV elimination, HBsAg testing and effective HBV treatment was poor in the largest Gambian HIV facility, predisposing co-infected patients to severe clinical and virological outcomes, increased risk of liver-related morbidity, and diminished benefits of ART.

In this study, none of the 870 HIV-infected patients refused to be screened for HBsAg and almost 85% of HBsAg positive participants accepted to undergo liver assessment. This result is important since it suggests that HBV screening and linkage to care may be widely accepted in HIV-infected patients and feasible in HIV care facilities in Africa. This needs to be confirmed on a larger scale.

Whilst some West African countries had successfully adopted a TDF-based first-line ART regimen in HIV reference centres many year prior [237], the indication to treat and choice of ART regimen in Gambian HIV clinics was primarily determined by the type and stage of HIV infection. As a result, 3TC, a cheap and well-tolerated nucleoside analogue has been the backbone of first line ART in The Gambia since its introduction over a decade ago [238]. Although 3TC is an effective antiviral drug, it is associated with an increased risk of HBV resistance mutations following 12 to 24 months of monotherapy in patients with HBV infection [239]. Unsurprisingly, HBV DNA was detectable in almost all HIV-HBV co-infected patients in this study (98.1%) who received prolonged 3TC without TDF.

I found significantly higher prevalence (83.3%) of 3TC-associated HBV resistance mutations compared to the 14.2% reported in a similar Gambian HIV-HBV cohort six year earlier [240], as well as the 29.3% and 8.7% reported in a Ghanaian [241] and Ivorian [242] co-infected patients respectively. My findings confirm that widespread prolonged usage of 3TC monotherapy in ART poses diagnostic and management problems to individual patients as well as a potential public health risk.

The high proportion of patients with detectable HIV viral load and low CD4 count after many months of ART, as well as the detectable HBV DNA among almost all co-infected patients receiving TDF in this study could be the result of unsatisfactory adherence to therapy, which might contribute to higher levels of HBV viremia and consequently to higher fibrosis and HBV resistance. Another explanation could be the deteriorating HIV services in The Gambia since 2007 when its former president claimed to have found herbal cure for HIV infection. This claim disrupted decades of HIV campaign and negatively impacted vital HIV services and research partnerships resulting in

significant reduction in competent personnel and services in HIV facilities and suboptimal HIV care with frequent medication stock outs and several patients having their ARV regimens switched often. Since 2016, many of these lapses in HIV care have been resolved and overall quality of HIV care has improved. New HIV treatment guidelines were developed in 2020 [243] which recommends TDF-based ART as first line regimen in all HIV patients.

HIV-HBV co-infection is associated with increased liver fibrosis [241, 244-248] and mortality (both all-cause mortality and liver related mortality) [154, 249]. Although my study was not statistically powered to definitively show a positive relationship between 3TC resistance mutation and increase in liver fibrosis, I found a trend towards such relationship ($p = 0.05$). Prompt initiation of TDF-based ART has been shown to reduce HBV viral replication and mortality [250], further strengthening the need for compliance to the WHO HBV testing and treatment guidelines in Africa. It is worth noting that the uptake of TDF in first-line ART regimens in sSA is poorly documented. This study shows that until 2016, only a minority of HIV patients in The Gambia received HBV testing and an even lower proportion (7/78) of HIV-HBV co-infected patients received TDF.

None of the patients in my study had any form of liver assessment, including liver transaminases. Indeed, the local laboratory at this HIV facility, and almost all other HIV facilities in The Gambia, do not have the capacity to assess liver biochemistry. The 2016 consolidated HIV guidelines [163] recommending TDF-based ART for all HIV patients irrespective of degree of liver disease are thus well adapted to resource limited African settings, and African HIV facilities need to be supported to implement these guidelines.

This study has a few limitations: first, I analysed a limited number of HIV-HBV co-infected patients, therefore the study was not powered to show association between 3TC exposure or HBV resistance and risk of liver disease. Second, I assessed liver fibrosis using APRI score as recommended by the WHO for resource-limited settings without access to Fibroscan. The reliability and usefulness of platelet-based biomarkers like APRI for assessing liver fibrosis in HIV patients in Africa has been questioned [251], mainly because thrombocytopenia is commonly associated with advanced HIV infection. Third, this study did not assess hepatitis C virus (HCV) and hepatitis D virus (HDV) co-infection. However, previous studies in The Gambia confirmed very low prevalence rates of both HCV and HDV infections [193]. Finally,

this study suggests that adherence to ART was not satisfactory but I did not directly assess adherence to ART.

5.6. Conclusion

Compliance to WHO HBV testing and treatment guidelines for HIV infected patients is poor in this Gambian HIV facility, resulting in low coverage of HBV testing, prolonged treatment with 3TC without TDF for co-infected patients, and poor HBV viral suppression with increased risk of HBV resistance and liver complications. However, interventions to improve HBV screening and linkage to care for HBV infection in HIV facilities are feasible.

This chapter has been published in PLoS One.

5.7. Appendix

5.7.1. Ethics approval letter

The Gambia Government/MRC Joint

ETHICS COMMITTEE

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1 August 2014

Dr Gibril Ndow
Disease Control and Elimination Theme
MRC Unit, The Gambia
Fajara

Dear Dr Ndow

SCC 1388v2, Assessment of the burden of liver disease in adults with Hepatitis B and HIV co-infection in The Gambia

Thank you for submitting your proposal dated 16 July 2014 for consideration by The Gambia Government/MRC Joint Ethics Committee at its meeting held on 26 July 2014.

We are pleased to approve your proposed study.

With best wishes

Yours sincerely



Mr Malamin Sonko
Chairman, Gambia Government/MRC Joint Ethics Committee

Documents submitted for review:-

- Revised SCC application form – 16 July 2014
- Response letter – 16 July 2014
- Informed Consent Document, version 1.0 – 14 July 2014
- Clinic appointment form, version 1.0 – 18 May 2014 Enrolment Clinical Form, version 1.0 – 18 May 2014
- Global Health Clinical Fellowship Scheme – award letter – 2 June 2014
- Questionnaire
- CV – Gibril Ndow

The Gambia Government/MRC Joint Ethics Committee:

Mr Malamin Sonko, Chairman
Professor Ousman Nyan, Scientific Advisor
Ms Naffie Jobe, Secretary
Mrs Tulai Jawara-Ceesay
Dr Ahmadou Lamin Samateh
Dr Roddie Cole

Prof. Umberto D'Alessandro
Dr Stephen Howie
Dr Kalifa Bojang
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6. CHAPTER 6: Trend of timely hepatitis B birth dose vaccine coverage in The Gambia, and impact of the COVID-19 pandemic

6.1. Abstract

Background and aims

Africa has the lowest coverage of timely hepatitis B birth dose (HepB-BD) vaccination (17%) worldwide. In The Gambia, the first African country to introduce HepB-BD, I assessed (i) the coverage of timely HepB-BD vaccination; (ii) the factors associated with delayed HepB-BD vaccination; and (iii) the impact of the COVID-19 pandemic on HepB-BD coverage.

Methods

Using data from the Health and Demographic Surveillance System, I computed the rate of timely (day 0-1) and delayed (days 2-7 and 8-28) HepB-BD and assessed for change points in the average rate. Then I ran logistic regression to determine the factors associated with delayed HepB-BD and assess the impact of COVID-19 on timely HepB-BD.

Results

Out of 70,888 live births between 1st January 2015 and 31st December 2021, 4,513 (6.4%; 95% CI 6.2-6.5) received a timely HepB-BD. Timely HepB-BD coverage increased from 1.8% in January 2015 to 20.7% in December 2021, peaking at 30.8% in October 2021.

Delayed HepB-BD, administered between days 2-7 and days 8-28, was observed in 3,946/70,888 (5.6%; 95% CI 5.4-5.7) and 36,103/70,888 (50.9%; 95% CI 50.6-51.3) babies, respectively. In total, 40,616 (57.3%; CI 56.9-57.7) babies received HepB-BD (timely or delayed) by day 28. The median age at first dose of HepB vaccination was 20 days (IQR: 11-31 days).

Being born in a health facility [OR 0.76 (95% CI 0.68-0.84), p value <0.0001], born after July 2018 [OR 0.47 (95% CI 0.41-0.53), p<0.0001] or born to mother with Junior Secondary education [OR 0.50 (95% CI 0.34-0.77), p=0.001] increased chance of

timely HepB-BD. Delayed HepB-BD vaccine was associated with being born on Friday [OR 3.27 (95% CI 2.74-3.92), $p<0.0001$] or Saturday [OR 6.14 (95% CI 4.89-7.81), $p<0.0001$]; born in Basse [OR 1.96 (95% CI 1.75-2.20), $p<0.001$] or Farafenni [OR 1.51 (95% CI 1.33-1.73), $p<0.001$]; or born during rainy season [OR 1.12 (95% CI 1.03-1.22), $p=0.008$]. Increasing maternal age increased risk risk of delayed HepB-BD [OR 1.008 (95% CI 1.002-1.014), $p=0.007$] for every additional year.

Timely HepB-BD declined from 10.1% (CI 9.5-10.6) pre-COVID (from June 2019) to 5.4% (CI 4.5-6.3) during the first COVID-19 wave. After accounting for all other factors, the odds ratio of delayed HepB-BD among 3,187 babies born during the first COVID-19 wave was OR:1.4 (1.2-1.6), $p=0.0002$. Timely HepB-BD coverage improved immediately after the COVID-19 wave, reaching pre-COVID rates within 4-6 months.

Conclusion

Timely HepB-BD coverage improved marginally but remains low 25 years after its introduction in The Gambia. The negative impact of the COVID-19 pandemic on timely HepB-BD was transient. Strategies are urgently needed to achieve 90% HepB-BD coverage required to eliminate HBV by 2030.

6.2. Introduction

Infection with hepatitis B virus (HBV) is a major global health issue with an estimated 296 million people living with chronic HBV infection (CHB) worldwide and 820,000 annual deaths attributable to HBV-related complications, mainly cirrhosis and hepatocellular carcinoma (HCC) [41]. The 2019 global estimates reported 1.5 million new cases of HBV infection mainly in low-and-middle income countries (LMICs).

Sub-Saharan Africa (sSA) is one of the most affected regions with HBV worldwide. Approximately 82 million people, of whom 4.5 million are children younger than 5 years, live with CHB in sSA [41, 42, 44], and HBV-related HCC is a common cause of premature death in the region [41, 52, 64]. Africa accounts for 70% of the global HBV incidence with 990,000 new cases reported in 2019. A recent systematic review estimates that 350,000 new HBV infections are acquired annually in Africa from mother-to-child transmission [30].

In 2015, the World Health Organization (WHO) developed an HBV elimination strategy which targets a 90% decrease in new HBV cases and a 65% decrease in HBV-related mortality by 2030 [167]. One of the key recommendations to accomplish these targets is the administration of the timely hepatitis B birth dose (HepB-BD) vaccine, to be administered within 24 hours of birth to all neonates, followed by 3 additional infant hepatitis B (HepB3) vaccine doses given as part of the Expanded Programme on Immunization (EPI).

So far, only 14 African countries have adopted HBV vaccination at birth, and the current coverage of timely HepB-BD vaccine is estimated at only 17% in Africa (WHO Immunization Coverage, 14th July 2022. <https://www.who.int/news-room/fact-sheets/detail/immunization-coverage> Accessed 25th October 2022). Reasons for poor HepB-BD coverage have been inadequately documented and reviewed [252]. Yet, improving HepB-BD coverage is critical to prevent perinatal HBV infection which is associated with more severe liver disease and higher risk of HCC in adulthood compared to contamination later in life [12]. A recent modelling study also showed that scaling up timely HepB-BD coverage in Africa to 90% could avert 550,000 in the 2020-2030 birth cohort [131].

The Gambia was the first African country to adopt timely HepB-BD in 1997. However, in 2014, the estimated coverage of timely HepB-BD was only 1.1% in The Gambia [192] and the prevalence of HBV infection in the adult population was 8% [193].

Since the release of the 2016 WHO elimination plans, The Gambia has deployed major efforts to control and eliminate HBV infection: (i) a National Hepatitis programme was created in 2016, integrated within the existing National AIDS Control Program (NACP); (ii) a Viral Hepatitis Focal Person was appointed in the Ministry of Health; (iii) routine screening of blood products in transfusion centres have been implemented and strengthened; (iv) National Viral Hepatitis Prevention and Control Policy (2018-2022) and Strategic Plan (2018-2022) were developed and launched by the Ministry of Health in 2017 [195, 196]; and (v) National Viral Hepatitis guidelines are being developed. All these initiatives prioritise prevention of HBV mother-to-child transmission, and place emphasis on antenatal hepatitis B surface antigen (HBsAg) screening and administration of timely HepB-BD.

Given the high rates of HBV related morbidity and mortality found in Chapter 3–5 and the high risk of advanced liver disease associated with HBV MTCT [12], I assessed the trend of timely HepB-BD coverage during the 2015-2021 period, since the release of the WHO HBV elimination strategy and the introduction of the above viral hepatitis control measures in The Gambia. I also assessed the factors associated with delayed HepB-BD administration and the impact of the COVID-19 pandemic (and associated control measures) on timely HepB-BD.

6.3. Methods

The MRC Unit The Gambia at LSHTM (MRCG) set up Health and Demographic Surveillance Systems (HDSS) in 4 regions in The Gambia: Basse, Bansang, Farafenni and West Kiang (Keneba) – figure 6.1. Since 1981, the HDSS conducts surveys every 4 months to collect data on vital events: pregnancy, births, deaths, causes of death (through verbal autopsies), migration, vaccination status, education level, ethnicity and socio-economic status. HDSS data is used to monitor demographic trends and longitudinal health indicators in the population, to inform population-based research, and to assess impact of interventions [192, 253-255].

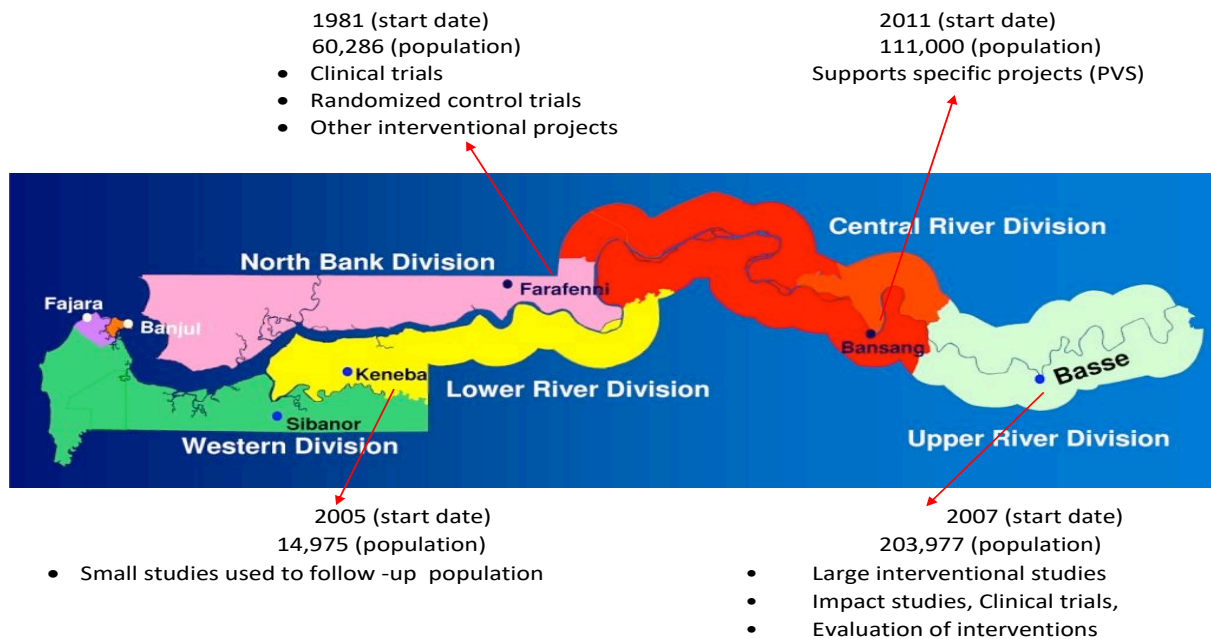


Figure 6.1 – The four HDSS regions in The Gambia

For this study, I extracted data from the Basse, Bansang and Farafenni HDSS for the period 2015 to 2021. Data analysis was conducted in R [256] using RStudio [257].

I computed the rate of timely (day 0-1) and delayed (days 2-7 and 8-28) HepB-BD for each month from January 2015 to December 2021. Then I looked for change points, first by visually observing for salient breaks in the time series and then applying change point detection algorithms [258-263] to corroborate visual observations. Change points are reported in magenta vertical dashed lines on the plots. For each time window between two change points, I compute the average timely HepB-BD rate – these averages are shown in blue thick horizontal solid lines on the plots.

Next, I used the glm function in R [264, 265] to run standard logistic regression, restricting the analysis to the period from January 2015 to July 2021 as the last 5 months of the dataset (August-December 2021) were not consolidated and had several missing variables. Among the variables available in the HDSS dataset, I kept the following: region, place of birth, day of birth, mother's age, mother's level of education, birth to a primiparous mother, birth during the rainy season, and birth during the first COVID-19 period. I also carried out segmented regression [266] with either time segmentation (i.e. restricting the dataset to each specific time window between change points) or geographical segmentation (restricting the dataset to each region).

6.4. Results

Between 1st January 2015 and 31st December 2021, 71,284 births were recorded for the three regions: Basse (42,933 births), Bansang (14,961) and Farafenni (13,390). Of these, 396 births were excluded from this analysis: 394 had date of first hepatitis B vaccine earlier than the date of birth, and 2 were stillbirths. In total, 70,888 live births were analysed. The number of births per month is shown in figure 6.2. An increasing proportion of babies, over the period, were born in health facilities (figure 6.3).

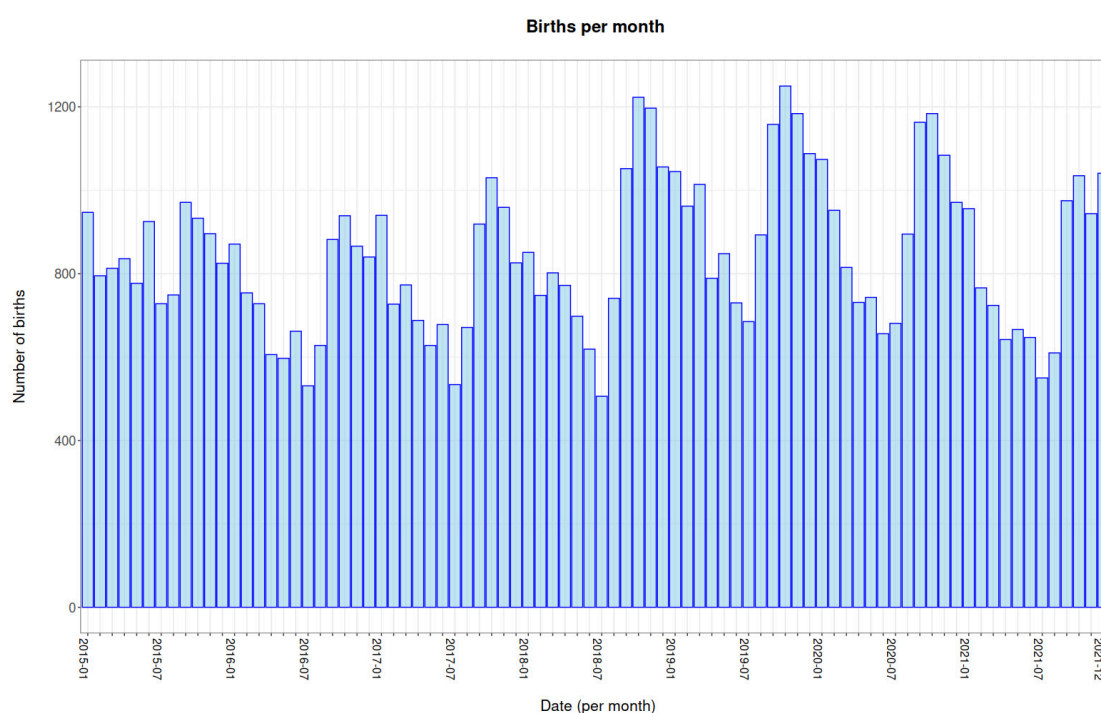


Figure 6.2. Number of births per month between January 2015 to December 2021

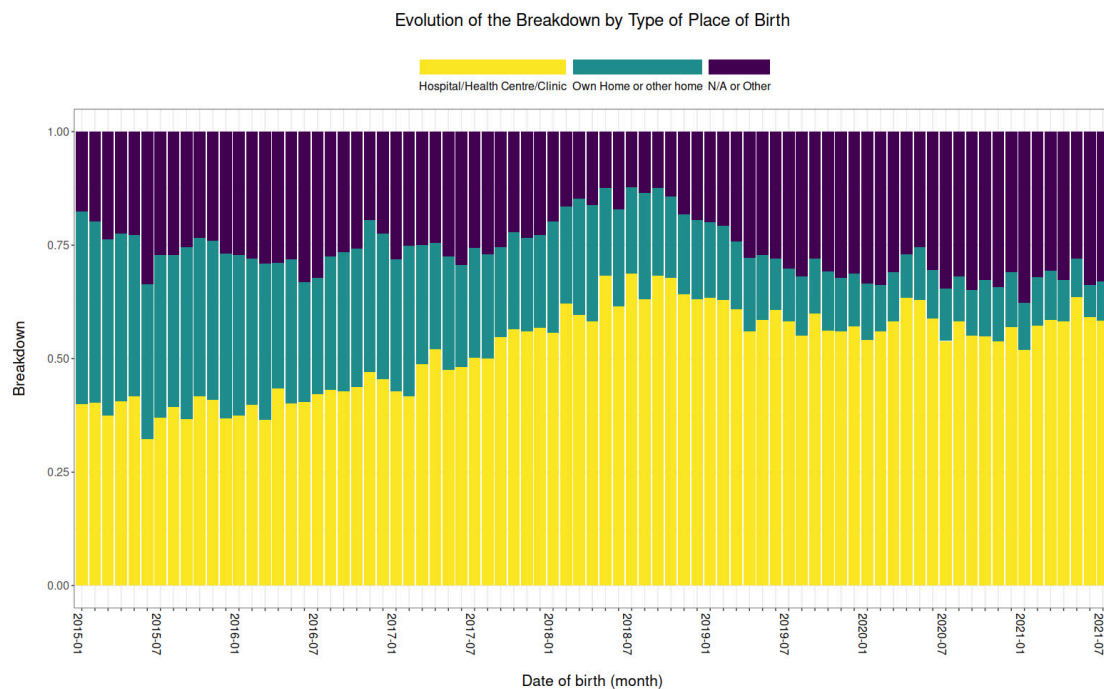


Figure 6.3. Place of birth between January 2015 to July 2021

Timely HepB-BD

In total, 4,513/70,888 (6.4%; 95% CI 6.2-6.5) babies received timely HepB-BD vaccine within days 0-1 of birth. Timely HepB-BD coverage in the three regions increased from 1.8% in January 2015 to 20.7% in December 2021, with a peak at 30.8% in October 2021 (Figure 6.4a). The trend of timely HepB-BD coverage over time was similar across the three regions (figure 6.4b).

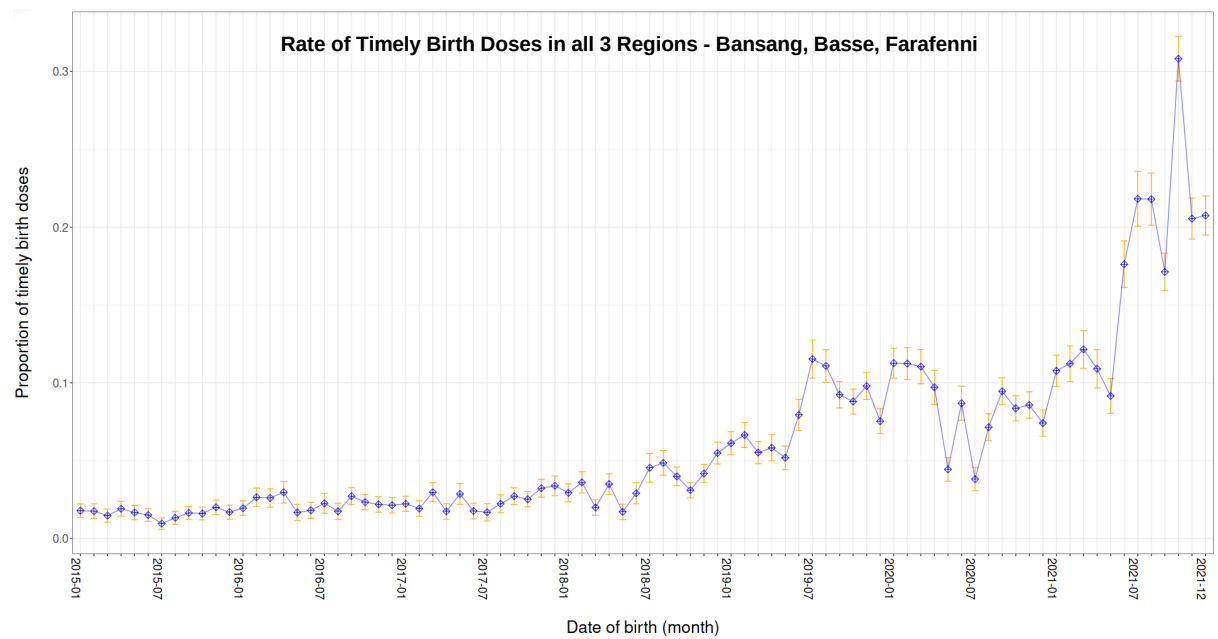


Figure 6.4a. Rate of timely HepB-BD in all three regions, January 2015 to December 2021

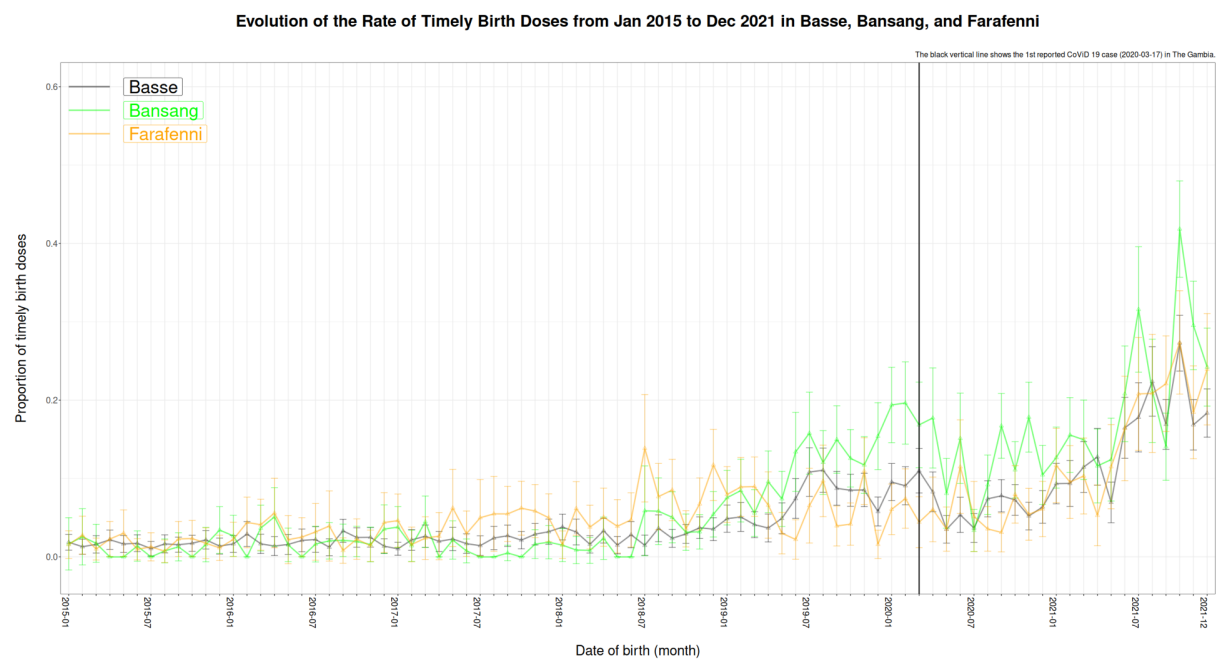


Figure 6.4b. Rate of timely HepB-BD by region, January 2015 to December 2021

Evolution of timely HepB-BD

Change points (called break points) in the average rate of timely HepB-BD were detected in the time series at the following times: July 2018, July 2019, March 2020, September 2020, January 2021 and July 2021 (figure 6.5a). No significant difference

in change points of the average rate of timely HepB-BD coverage was detected between the three regions (figure 6.5b).

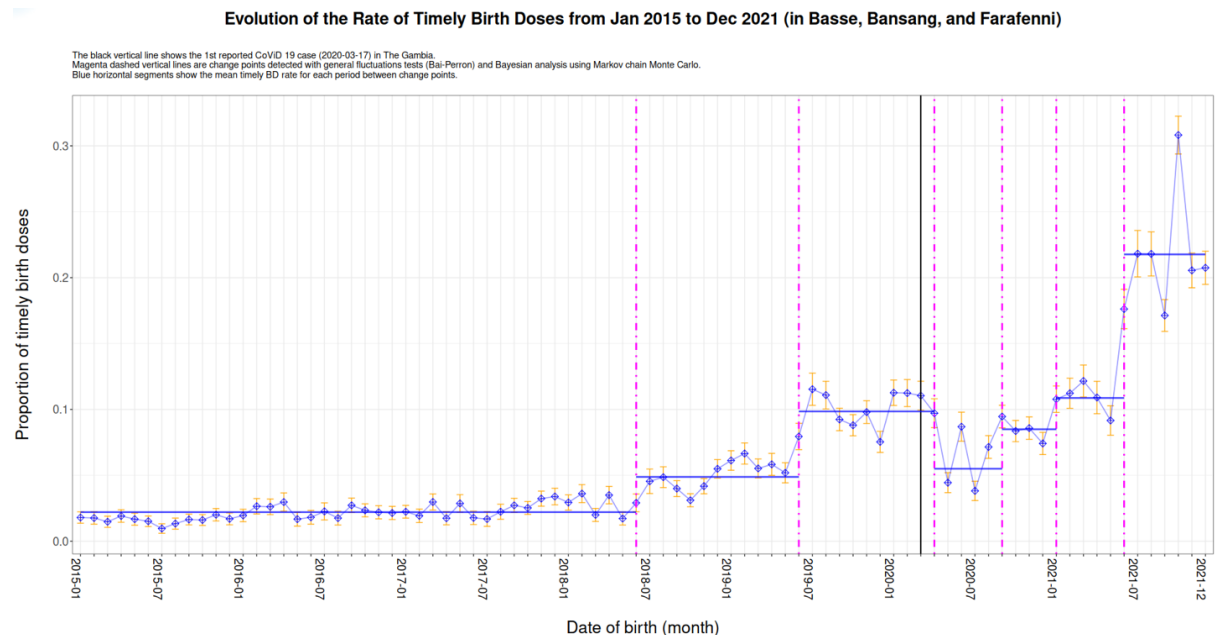


Figure 6.5a. Evolution and change points in the average rate of timely HepB-BD between 1st January 2015 to 31st December 2021 across all regions

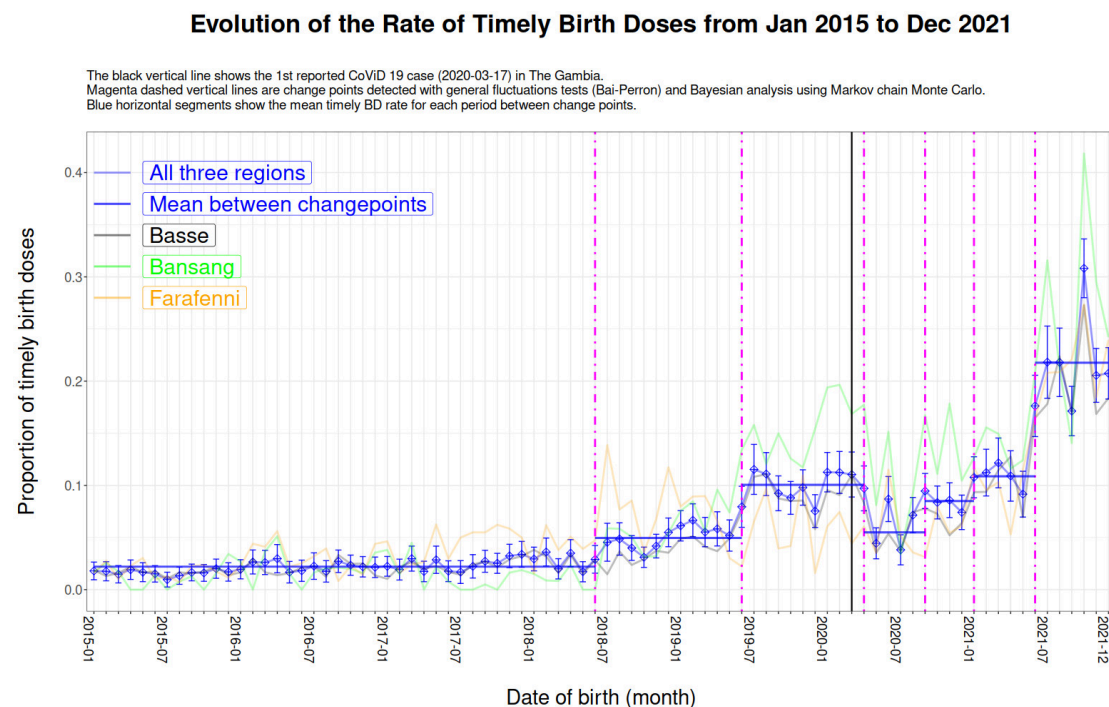


Figure 6.5b. Evolution and change points in the average rate of timely HepB-BD between 1st January 2015 to 31st December 2021 by region

Delayed HepB-BD vaccination

Delayed HepB-BD vaccine, administered between days 2-7 and days 8-28, was observed in 3,946/70,888 (5.6%; 95% CI 5.4-5.7) and 36,103/70,888 (50.9%; 95% CI 50.6-51.3) babies, respectively. In total, 40,616 (57.3%; CI 56.9-57.7) babies received HepB-BD (timely or delayed) by day 28. The remaining either received a HepB-BD vaccine with a delay longer than 28 days (17,485 babies, including 5,969 babies with a delay longer than 42 days) or they had no record of receiving a HepB-BD vaccine (12,787 babies, 18.0%). The median age at first dose of hepatitis B vaccination was 20 days (IQR: 11-31 days). A breakdown of the time of HepB-BD administration is shown in figure 6.6.

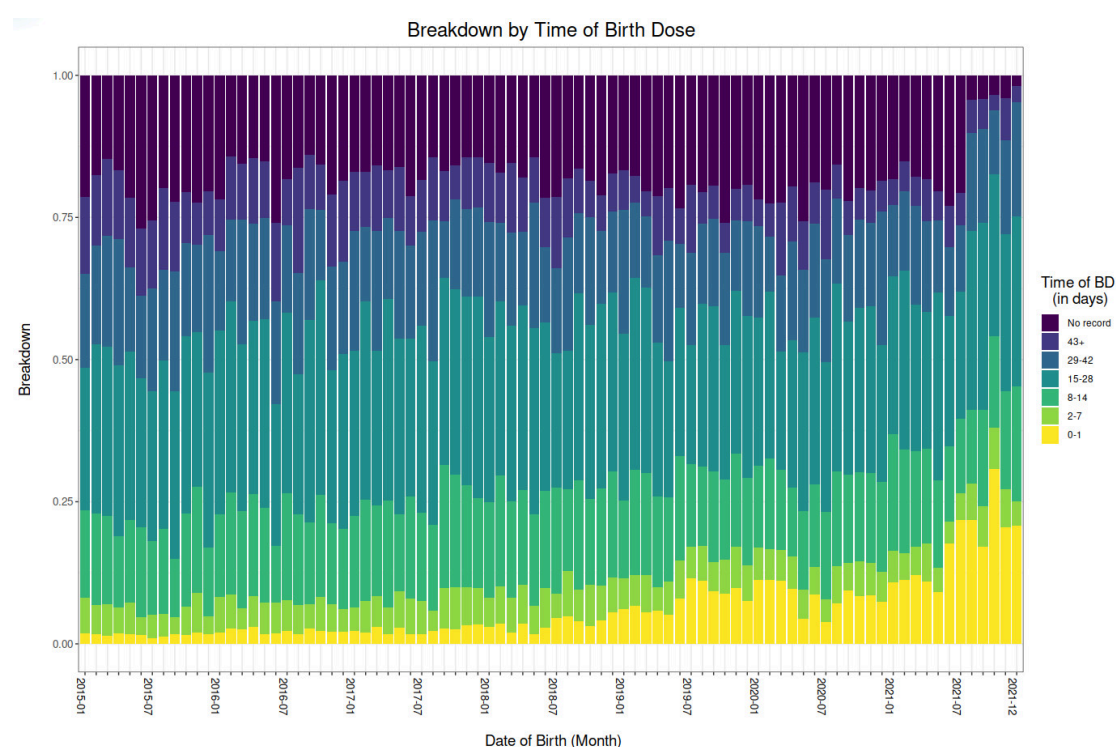


Figure 6.6. Breakdown by time of HepB-BD, January 2015 to July 2021

Between January 2015 and July 2021, the rate of timely HepB-BD was lower among babies born on Friday (3.3%, 95% CI 3.0-3.7) or Saturday (2.3%, 2.0-2.6), compared to other days of the week (5.8-9.7%) (figure 7.7). Compared to babies born on Monday, being born on Friday [OR 3.27 (95% CI 2.74-3.92), $p < 0.0001$] or Saturday [OR 6.14 (95% CI 4.89-7.81), $p < 0.0001$] were strongly associated with delayed HepB-BD. Being born on a Tuesday, on the other hand, reduced risk of delayed HepB-BD [OR 0.85 (95% CI 0.75-0.97), $p = 0.02$].

Rate of timely HepB-BD among babies born either in a health facility (hospital, clinic or health centre) or home were 6.3% and 2.8% respectively. Risk of delayed HepB-BD was reduced among babies born in a health facility [OR 0.76 (95% CI 0.68-0.84), p value <0.0001] compared to being born at home or elsewhere. Increasing maternal age slightly increased risk of delayed HepB-BD [OR 1.008 (95% CI 1.002-1.014), p=0.007] for every additional year.

Bansang had the best coverage of timely HepB-BD. Risk of delayed HepB-BD was [OR 1.96 (95% CI 1.75-2.20), p<0.001] and [OR 1.51 (95% CI 1.33-1.73), p<0.001] among babies born in Basse and Farafenni, respectively.

Compared to mother with no education, babies born to mothers with Junior Secondary education had reduced risk of delayed HepB-BD [OR 0.50 (95% CI 0.34-0.77), p=0.001]. However, risk of delayed HepB-BD increased among babies born to mothers with College/University education [OR 1.49 (95% CI 1.19-1.87), p=0.0005], Senegalese education [OR 2.67 (95% CI 1.42-5.70), p=0.005] or Madrasa education [OR 1.23 (95% CI 1.04-1.46), p=0.02].

Birth during the rainy season increased risk of delayed HepB-BD [OR 1.12 (95% CI 1.03-1.22), p=0.008]. Babies born after July 2018 better chance of receiving timely HepB-BD [OR 0.47 (95% CI 0.41-0.53), p<0.0001] compared to babies born before June 2018 and outside the rainy season.

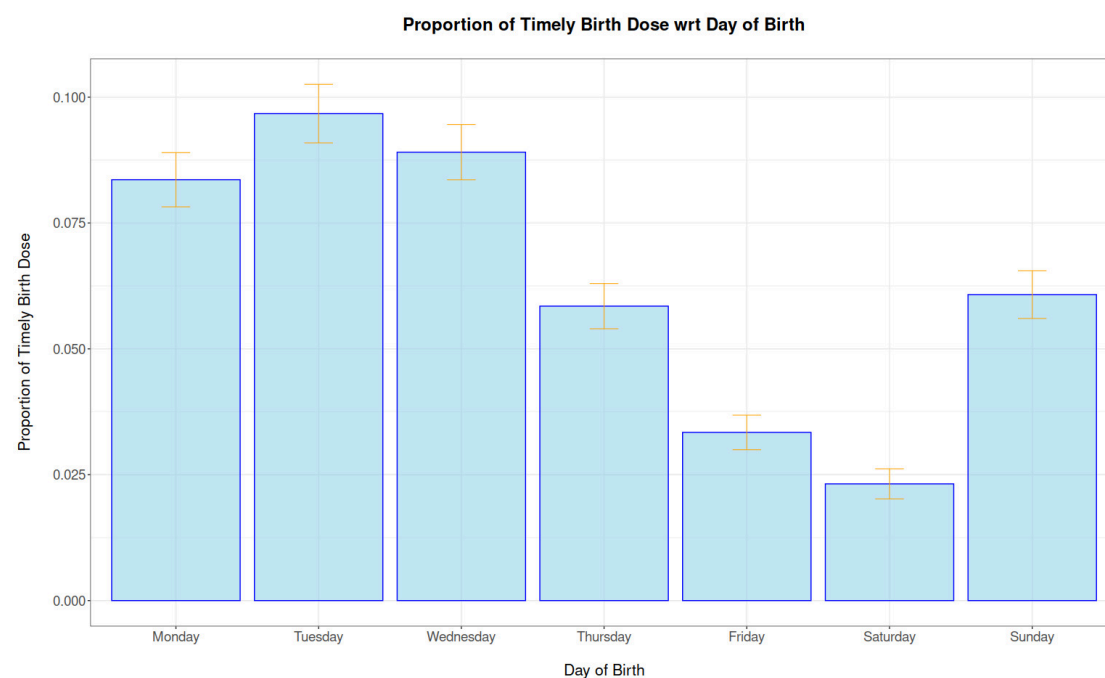


Figure 6.7 Proportion of timely HepB-BD by day of birth, January 2015 to July 2021

Impact of the COVID-19 outbreak

The first case of COVID-19 infection was registered in The Gambia on 17th March 2020 and the first COVID-19 wave, along with intense lockdown and other control measure measures, were between March and August 2020. There were 3,187 live births registered in the three HDSS regions during the first COVID wave.

The average rate of timely HepB-BD increased in June 2019 and remained stable until March 2020 at the start of the COVID-19 wave in The Gambia. Therefore, taking the period between 15th June 2019 to 15th March 2020 as reference period, I observed that the average rate of timely HepB-BD declined from 10.1% (95% CI 9.5-10.6) between June 2019-March 2020 to 5.4% (95% CI 4.5-6.3) in April-August 2020. This immediate impact of the COVID-19 wave on timely HepB-BD was observed in all three regions (figures 6.5a and 6.5b).

Compared to babies born between 15th June 2019 – 15th March 2020, being born during the first COVID-19 wave significantly increased risk of delayed HepB-BD [OR1.37 (1.16-1.62), $p=0.0002$]. Babies born in a health facility during the COVID-19 wave had higher chance of receiving a timely HepB-BD vaccine [OR 0.56 (0.5-0.6), $p<0.001$] compared to babies born at home. Timely HepB-BD coverage improved immediately after the first COVID-19 wave, surpassing pre-COVID rates by January 2021 and reaching new record of coverage rate in all regions by July 2021.

6.5. Discussion

This study reports moderate improvement in the rate of timely HepB-BD coverage in The Gambia from 1.8% in January 2015 to 20.7% by December 2021. This suggests that hepatitis related interventions and activities in The Gambia could have positively impacted timely HepB-BD coverage.

The first change point in the average rate of timely HepB-BD coverage was observed in July 2018, with HepB-BD increasing from 2.2% (95% CI: 2.0-2.4) to 5.0% (95% CI: 4.5-5.4). This increase followed the launch of the National Viral Hepatitis Control Policy and Strategic Plan 2018-2022 [195, 196] and series of activities to commemorate World Hepatitis Day (WHD). More importantly, the average rate of timely HepB-BD increased from 5.0% (95% CI: 4.5-5.4) to 10.1% (95% CI: 9.5-10.6) in July 2019. In

May-June 2019, the National Hepatitis Program convened a Viral Hepatitis Technical Working group tasked with planning the 2019 WHD celebrations and subsequently developing draft guidelines for national viral hepatitis screening, care, and treatment. The technical working group comprised members from other programs/departments in the Ministry of Health, including Department of Planning & Information, EPI program, and Reproductive & Child Health program. The focus of discussions in 2019 was around increasing awareness, diagnosis, and timely HepB-BD.

Despite some improvement, the rate of timely HepB-BD coverage remained significantly lower than the WHO targets of 50% and 90% coverage by 2020 and 2030 respectively [167]. Health facility delivery significantly increases odds of receiving a timely HepB-BD. However, only 6.3% of babies born in a health facility received a timely HepB-BD. The 2019-2020 Demographic and Health Survey of The Gambia reports that more than 82% of all births in The Gambia take place in a health facility [267]. In my dataset, 38,831/70,888(50.5%) babies were born in a health facility (hospital, clinic or health centre). Births at home were 14,293 (20.2%) and the remaining 20,764 (29.3%) either had place of birth missing or recorded as “other”. Nonetheless, closing the gap in timely HepB-BD in health facility deliveries can increase coverage rates to reach WHO targets. It is therefore urgent to develop strategies that ensure all babies born in health facility receive a timely HepB-BD vaccine.

The Gambia uses the multi-dose hepatitis B monovalent vaccine for HepB-BD, and all vaccines including the HepB-BD are managed and administered by Public Health officers (PHOs). Officially, PHOs work Monday to Friday, 8am to 4pm, with limited cover in evenings, weekends and public holidays. In rural areas, the limited PHOs allocate specific days of the week/month for trekking clinics to cover remote areas. As a result, babies born in health facility outside working hours or during weekends/public holidays may not immediately receive a timely HepB-BD. The multi-dose monovalent hepatitis B vaccine can be stored at room temperature for up to 28 days [268-272], therefore reducing need for cold chain and concerns over vaccine wastage. This presents a unique opportunity to increase access to timely HepB-BD, for example by providing the vaccine in labour wards or with community health nurses who work shift and attend to most deliveries. Community education and out-of-reach programs such as out-of-cold chain vaccine were effective in increasing HepB-BD coverage in remote areas of China where facility birth rate was low [273], as well as in Solomon Islands [274], Lao PDR [275] and Vietnam [270].

The annual increase in rate of timely HepB-BD observed in July 2018, 2019 and 2021 suggest that WHD celebrations, which often involve increased hepatitis awareness among healthcare workers, policy makers and the general public, suggest that increasing disease knowledge is critical to scale up HepB-BD coverage and eliminate HBV [197].

In my study, 36,103 babies (50.9%) received their first hepatitis B vaccine between days 8-28, with a median age at first dose of vaccine being 20 days. This is similar to the previous report of 58.4% by Miyahara et al [192]. The delayed HepB-BD beyond day 8 may, in part, be driven by cultural norms where new-borns and their mother stay home for the first seven days [276]. Being born on Friday or Saturday had very strong association with delayed HepB-BD, further highlighting the gaps in the current system of hepatitis B vaccine administration.

Interestingly, the effect of maternal education was not linear. Babies born to mothers with junior secondary education had the highest chance of receiving a timely HepB-BD vaccine. In contrast, maternal College/University education reduced chance of timely HepB-BD. The reason for the lower HepB-BD coverage among babies born to mothers with College/University education is not clear.

The PROLIFICA group previously reported decreased access to and use of viral hepatitis services in Africa during the COVID-19 outbreak [277]. In this study, I report an immediate effect of COVID-19 on timely HepB-BD coverage after the declaration of the outbreak and diagnosis of the first case in The Gambia. Despite the relatively low incidence of COVID-19 infection and mortality in The Gambia [278, 279], timely HepB-BD coverage reduced by almost half, from 10.1% to 5.5% during the peak of the first COVID-19 wave. Babies born during the COVID-19 wave were 37% more likely to receive a delayed HepB-BD or no HepB-BD at all compared to babies born during the 10-month period immediately before the COVID-19 wave. Arsenault et al report similar immediate disruptions in healthcare service delivery in 10 other countries [280, 281].

Although the COVID-19 pandemic continued beyond 2020, it was expected that healthcare systems would have adjusted and adapted service delivery by late 2020. In my study, I found that the impact of COVID-19 on timely HepB-BD coverage was transient, with rates returning to pre-COVID levels by end of 2020. This could suggest a degree of resilience in the EPI and early neonatal care services, and improved efforts to maintain vaccination services. In Ghana, childhood vaccinations (BCG, pentavalent,

pneumococcal, rotavirus and measles) and postnatal care services increased by the last quarter of 2020 [281]. Arsenault et al found that COVID-19 case numbers and the stringency of control policies had low to moderate correlations with the magnitude of health service disruptions [281]. The mild COVID-19 wave in The Gambia with relatively few case numbers, low mortality [278, 279] and weak enforcement of control policies could favour the transient impact of COVID-19 on HepB-BD.

The higher rate of HepB-BD in the second half of 2021, exceed pre-COVID rates, could indicate positive collateral effect of the pandemic, with increased attention to health and vaccination issues among the population and enhanced vaccination programs.

6.6. Conclusion

Despite moderate increase over time, timely HepB-BD coverage remains low 25 years after its introduction in The Gambia putting at risk the hepatitis elimination goals. Initial response to the COVID-19 pandemic negatively impacted HepB-BD, but effect was transient. Strategies are urgently needed to achieve the 90% HepB-BD coverage required to eliminate HBV by 2030.

A manuscript of this chapter has been submitted to the Lancet Global Health.

7. CHAPTER 7. General discussion and conclusion

7.1. Key findings and potential implications

The main findings of this PhD show that (i) hepatitis B related mortality, primarily from cirrhosis and HCC, continues to be high in The Gambia; (ii) OBI is an independent risk factor of mortality, further compounding the mortality associated with overt HBsAg infection; (iii) treatment with TDF improves survival among CHB patients with cirrhosis; and (iv) coverage of timely hepatitis B birth dose vaccination remains low and was impacted by the COVID-19 pandemic.

The impact of CHB infection on morbidity and mortality has been well demonstrated globally and in West Africa [42, 49]. For the first time, my results demonstrate that occult hepatitis B infection is an independent risk factor of advanced liver disease (OR 2.8, 95% CI 1.3-6.0) and accounts for 12.9% of cases in HBsAg negative adults. In addition, 75.0% of patients with cirrhosis and 65.8% with HCC in my study population had positive HBsAg. The median survival for cirrhosis (17.1 months) and HCC (1.5 months) are extremely poor in The Gambia, similar to reports from other African countries [71]. Therefore, three decades after the introduction of routine infant hepatitis B vaccination in The Gambia, CHB infection (overt HBsAg or OBI) continues to be a significant cause of death in young adults.

Late presentation is common among patients with advanced liver disease in Africa [71]. In my cohort, two-thirds of patients with cirrhosis (67.9%) or HCC (63.9%) presented late with decompensated cirrhosis or multifocal tumour respectively. With the limited access to treatment for decompensated cirrhosis and HCC in Africa [64], active screening and surveillance, and expanded HBV treatment eligibility criteria targeting non-cirrhotic patients, could prevent morbidity and mortality in The Gambia. My study found that treatment with TDF improved survival among HBV positive patients with cirrhosis. However, the delay between initial diagnosis of cirrhosis and start of TDF was 4.9 months. In addition, the current WHO treatment cut-offs and criteria have been shown to miss half of the African patients in need of antiviral therapy [104] and results from a network of hepatitis B cohorts in Africa (HEPSANET) show that lower APRI cut-offs of 0.65 had 56.2% specificity and 90.0% sensitivity to rule-in cirrhosis in African patients [282]. Therefore, there may be indication for revised WHO guidelines adapted to the African setting. Expanding the WHO treatment criteria to initiate therapy at lower APRI cut-offs, without need for viral load, could extend treatment to more at risk patients in Africa and avert deaths.

Although a treat all strategy, where all HBsAg positive patients are offered treatment, could significantly expand treatment access, more data is needed to demonstrate the benefits (health outcomes, cost effectiveness and feasibility) of this strategy.

OBI screening should be considered in HBsAg negative patients with advanced liver disease. According to current international guidelines, cirrhotic patients with detectable HBV DNA viral load are eligible for antiviral therapy [33, 80]. However, the impact of treatment in OBI is not well demonstrated and additional OBI screening will increase the cost implications of hepatitis elimination in HBV endemic areas where resource for hepatitis control are already limited.

Given the limited resources for screening and management of hepatitis B and related complications, prevention of infection must remain the cornerstone of hepatitis elimination efforts. Timely hepatitis B birth dose (HepB-BD) vaccination remains a major challenge in many African countries, with coverage in the WHO Afro region being significantly below the 2020 targets (WHO Immunization Coverage, 14th July 2022. <https://www.who.int/news-room/fact-sheets/detail/immunization-coverage> Accessed 25th October 2022). I found that in The Gambia, the first African country to adopt the HepB-BD vaccine, the coverage of timely HepB-BD improved only marginally following national efforts towards elimination, but remain below the WHO targets. In my results, there seems to be a strong correlation between annual viral hepatitis activities such as World Hepatitis Day commemoration and increase in average rate of timely HepB-BD. In the absence of a control group, it is difficult to truly demonstrate the impact of such activities on HepB-BD. Nonetheless, increasing awareness is central in increasing HepB-BD coverage and eliminating viral hepatitis in Africa [197].

Further, my results show that being born during the week and/or in a healthcare facility improved chance of timely hepatitis B birth dose vaccination. This implies that providing the vaccine at the place of delivery could be a feasible strategy to scale up timely HepB-BD. More than 80% of babies in The Gambia are born in a healthcare facility [267]. Access to timely HepB-BD among these facility births will immediately improve coverage. Therefore, adaptive strategies such as transferring the HepB-BD vaccine to midwives, and possibly use of out-of-cold chain hepatitis B vaccine in remote areas without constant access to cold chain [273, 274], should be considered. These strategies should be supported by strong policies, access to training and raising awareness among healthcare workers, policy makers and women/general public.

Indeed such strategies leave a critical gap among out-of-facility births. The use of pre-filled injection devices such as Uniject have improved HepB-BD coverage rates in Asia [271, 273], and could be trialled in carefully selected African settings to demonstrate their the impact of timely HepB-BD. In addition, the impact of antiviral prophylaxis alone without timely Hep-BD is unknown. If prophylaxis plus delayed HepB-BD are effective in preventing HBV mother-to-child transmission, then antenatal screening and prophylaxis could be a scalable strategy for Africa given the high antenatal coverage and relative access to HBsAg testing.

7.2. General conclusion

In The Gambia, there remain significant gaps in meeting the WHO targets of reducing new HBV infections and HBV related mortality by 2030. Adaptive strategies and simple interventions, integrated within existing services and using existing resources, could improve access and coverage, and drive towards meeting elimination targets.

8. Perspectives and Future work

8.1. Perspectives

I thoroughly enjoyed doing this PhD and writing up my findings. Most importantly, during this PhD, I learnt to successfully manage, maintain and expand a longitudinal cohort in Africa. The PROLIFICA cohort, now followed up for 11 years, is one of the largest and well characterised CHB cohorts in the continent. I took over the leadership of the PROLIFICA group in The Gambia in 2019, and have since then:

- Successfully reassessed the entire original PROLIFICA cohort enrolled in 2011-2014, accounting for every death and loss to follow-up, and assessing treatment eligibility in previously untreated patients
- Set up additional cohorts of (i) adults with CHB infection referred from blood banks and various health facilities (n=698), (ii) HBsAg-positive pregnant women (n=437), and (iii) infants born to HBsAg-positive mothers.
- Transformed the Liver Clinic originally set up by the PROLIFICA group at the MRC Unit The Gambia into a more efficient facility, with strong electronic medical record systems to capture and manage clinical and research data
- Successfully set up a nurse-led HBV clinic at the Liver Clinic, able to assess newly diagnosed patients, conduct investigations, assess treatment eligibility and monitor treated and untreated patients
- Consolidated and catalogued serial biological samples collected from the original PROLIFICA and additional cohorts
- Set up a successful PMTCT baseline study to assess the proportion of pregnant women at high risk of HBV MTCT (defined as HBV DNA >200,000IU/mL and/or positive HBeAg) and estimate the rate and risk factors of HBV MTCT in The Gambia
- Further expanded the basic science research scope within the PROLIFICA Gambia to study mechanisms of HBV persistence and clearance using metabolomics, immunology and in vitro (Liver-on-chip technology) platforms.

To support these additional clinical and research activities, I have had to attract significant funding and expand the team. Personally, I have been awarded grants and fellowships from Gilead US (\$100,000), MRC West Africa platform (£10,000), AREF-EDCTP (€57,095) and Wellcome Trust (£310,950). In addition, I am co-investigator on two recently awarded larger grants, both building on my baseline PMTCT study and extending the research scope to study the impact of timely HepB-BD on MTCT

(IMPACT-B study) and the feasibility and impact of triple elimination of HBV, HIV and syphilis (TRI-MOM study), respectively.

In these perspectives, I will discuss the results and progress from some of the key research projects that I have led alongside my main PhD projects. All the projects discussed here fit within the scope of HBV elimination in Africa, but the work is either not fully completed or is not sufficient to make up a standalone chapter in this thesis. Some of these projects have provided vital pilot data for larger grants and prestigious fellowships for my post-doctoral training.

8.1.1. Retention in care and mortality during long term follow up of the PROLIFICA hepatitis B cohort in The Gambia

Background and methods

There is limited data on disease progression and follow up outcomes in treated and untreated CHB infected patients in Africa. The PROLIFICA longitudinal cohort of CHB infected adults in The Gambia provides a unique opportunity to study these.

Between December 2011 to February 2014, the PROLIFICA program in The Gambia enrolled 943 HBsAg positive adults: 402 from the community-based screening, 300 from blood bank screening and 241 from the historical Keneba-Manduar cohort [193]. The baseline characteristics of this cohort are presented in Table 8.1. Among 943 patients, 70 (7.4%) were on treatment.

As part of my PhD, I re-invited all HBsAg positive adults in this cohort for reassessment. I retrieved telephone numbers and home addresses and used a standardised follow-up approach comprising three telephone calls followed by a home visit if necessary. Patients unreachable after at least three telephone calls and 1 home visit were regarded as lost to follow up. Among patients who died, verbal autopsy was done to determine likely cause of death (liver related or non-liver related, place of death and date of death). Patients who were alive and successfully reached were invited to the MRCG Liver Clinic for reassessment, comprising physical examination, fasting transient elastography, bedside ultrasound scan, liver biochemistry, repeat HBsAg, HBeAg and HDV serology, and HBV DNA viral load.

Table 8.1. Baseline characteristics of study participants

	Community screening (n=402)	Blood bank screening (n=300)	Keneba & Manduar (n=241)	p value
Age (years)	38 (33-47)	31 (27-35)	36 (32-44)	<0.001
Male sex	193 (48.0%)	291 (97.0%)	113 (46.9%)	<0.001
Attended primary school	179/386 (46.4%)	185/223 (83.0%)	91/208 (43.8%)	<0.001
BMI (kg/m ²)	22.5 (20.0-26.5)	22.4 (20.4-25.2)	22.0 (19.9-25.5)	0.611
Ever drank alcohol	28/397 (7.1%)	25/297 (8.4%)	9/231 (3.9%)	0.112
Family history of HCC	16 (4.0%)	7 (2.3%)	11 (4.6%)	0.333
Liver stiffness (kPa)*	4.7 (3.9-5.7)	5.9 (4.9-7.5)	4.7 (4.0-5.8)	<0.001
METAVIR score				
F0-1	358 (93.5%)	237 (82.8%)	219 (94.4%)	<0.001
F2-3	16 (4.2%)	32 (11.2%)	12 (5.2%)	
F4	9 (2.3%)	17 (6.0%)	1 (0.4%)	
ALT (IU/L)	23 (18-30)	27 (21-35)	24 (19-30)	<0.001
ALT ≥ 40 IU/L	48/394 (12.2%)	55/297 (18.5%)	23/234 (9.8%)	0.008
AST (IU/L)	29 (24-34)	31 (27-39)	29 (24-34)	<0.001
GGT (IU/L)	25 (19-34)	28 (22-39)	25 (19-33)	<0.001
ALP (IU/L)	90 (76-112)	81 (68-96)	92 (75-112)	<0.001
Platelets (10 ⁹ cells per L)	203 (159-253)	170 (142-205)	215 (169-260)	<0.001
HBeAg positive	13/395 (3.3%)	23/291 (7.9%)	11/235 (4.7%)	0.024
HBV DNA (IU/mL)				
Undetectable	188 (49.2%)	107 (40.5%)	95 (40.2%)	0.16
50-1999	153 (40.1%)	119 (45.1%)	111 (47.0%)	
2000-19 999	20 (5.2%)	12 (4.6%)	11 (4.7%)	
20 000-199 999	7 (1.8%)	8 (3.0%)	3 (1.3%)	
≥ 200 000	14 (3.7%)	18 (6.8%)	16 (6.8%)	
HBV genotype				
A	27 (16.1%)	14 (13.5%)	2 (4.3%)	0.111
E	141 (83.9%)	90 (86.5%)	45 (95.7%)	
HIV positive	13/398 (3.3%)	6 (2.0%)	7/233 (3.0%)	0.588
HCV positive	4/395 (1.0%)	5 (1.7%)	2/230 (0.9%)	0.642
HDV positive	8/395 (2.0%)	1/293 (0.3%)	0/224 (0%)	0.02
Phase of natural history				
HBeAg-pos chronic infection	6 (1.5%)	6 (2.1%)	8 (3.4%)	0.021

HBeAg-pos chronic hepatitis	6 (1.5%)	9 (3.1%)	3 (1.3%)	
HBeAg-neg chronic infection	313 (79.6%)	214 (73.5%)	190 (80.9%)	
HBeAg-neg chronic hepatitis	9 (2.3%)	5 (1.7%)	5 (2.1%)	
HBeAg-neg grey zone	50 (12.7%)	40 (13.8%)	28 (11.9%)	
Cirrhosis	9 (2.3%)	17 (5.8%)	1 (0.4%)	
Eligible for EASL 2012 criteria	18 (4.5%)	29 (9.7%)	11 (4.6%)	0.009
Eligible for AASLD 2009 criteria	14 (3.5%)	21 (7.0%)	8 (3.3%)	0.049

*Excludes 34 participants without measurement and 8 with unreliable measurements

Preliminary analysis

The full analysis of this study is still ongoing.

Between October 2018 and November 2019, I followed up all 943 HBsAg positive patients previously enrolled in the PROLIFICA study. Of these, 783 (83.03%) were alive, 35 (3.72%) died and 125 (13.26%) were lost to follow up. Among patients who were alive, 607/783 (77.52%) accepted the invitation and were successfully reassessed. Figure 8.1 shows a breakdown of the follow-up outcomes of this cohort.

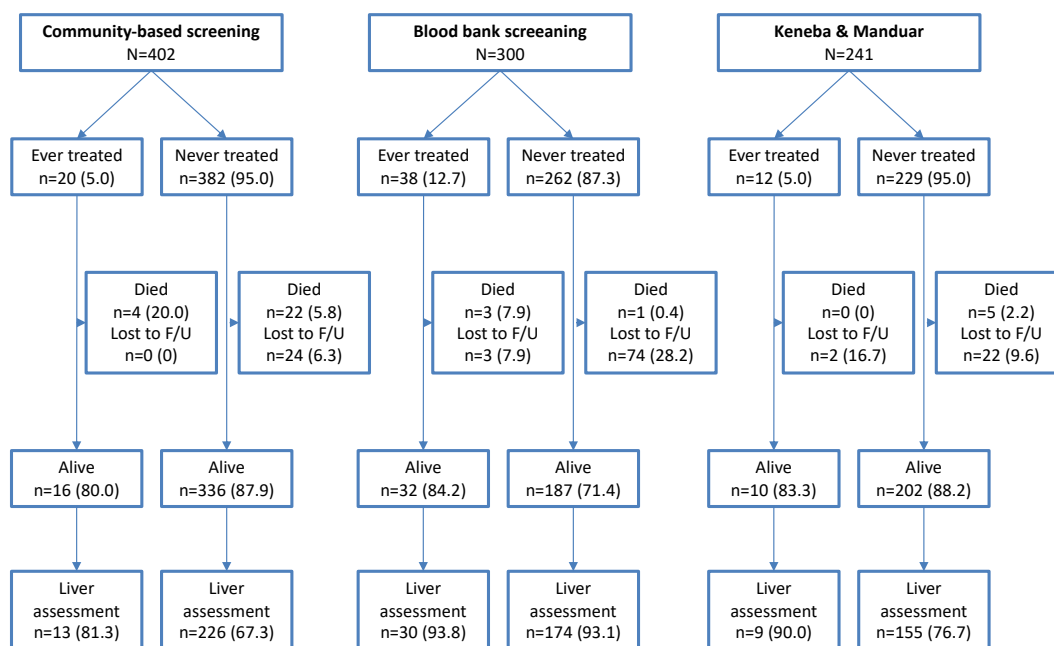


Figure 8.1. Flow chart of study participants

After 5,119 person-years of follow up, the incidence of all-cause mortality in the entire cohort was 684 (95% CI 491-952) per 100,000 person years. Older age was associated with all-cause mortality (adjusted OR 10.45, 95% CI 1.14-95.91 for patients aged >50 years). Table 8.2 shows the factors associated with all-cause mortality.

The incidence of liver related mortality after 5,119 person-years of follow up was 195 (105-363) per 100,000 person years (Table 8.3). Cirrhosis was strongly associated with liver related death compared to HBeAg-negative chronic infection ($p=0.002$) (figure 8.3).

Table 8.2 Overall mortality rates

	No. of participants	Person-years of follow-up	No. of deaths	Incidence rates per 100 000 person-years	95% CI	Crude rate ratio	95% CI	p-value	Adjusted rate ratio	95% CI	p-value
Overall	943	5119	35	684	491-952	N/A	N/A	N/A	N/A	N/A	N/A
Age at baseline (years)											
<30	167	720	1	139	20-987	1	Reference	<0.001	1	Reference	<0.001
30-39	464	2585	10	387	208-719	2.78	0.36-21.74		1.6	0.18-14.38	
40-49	173	1018	2	197	49-786	1.41	0.13-15.59		0.87	0.07-11.37	
≥50	139	797	22	2760	1817-4192	19.86	2.68-147.32		10.45	1.14-95.91	
Sex											
Female	346	2079	14	673	399-1137	1	Reference	0.94			
Male	597	3040	21	690	450-1060	1.03	0.52-2.02				
Origin											
Community-based screening	402	2445	26	1064	724-1562	1	Reference	0.006	1	Reference	0.075
Blood bank screening	300	1237	4	323	121-862	0.3	0.11-0.87		0.4	0.11-1.41	
Keneba & Manduar	241	1437	5	348	145-836	0.33	0.13-0.85		0.38	0.14-1.00	

Phase of natural history											
HBeAg-neg chronic infection	717	3891	22	565	372-859	1	Reference	0.018	1	Reference	0.122
HBeAg-pos chronic infection	20	115	1	867	122-6153	1.53	0.21-11.37		1.35	0.14-13.09	
HBeAg-pos chronic hepatitis	18	104	0	0	N/A	<0.01	N/A		<0.01	N/A	
HBeAg-neg chronic hepatitis	19	110	2	1812	453-7244	3.2	0.75-13.62		1.51	0.22-10.13	
HBeAg-neg grey zone	118	648	3	463	149-1436	0.82	0.25-2.74		0.86	0.25-2.99	
Cirrhosis	27	125	5	4003	1666-9616	7.08	2.68-18.69		5.48	1.59-18.88	
EASL criteria											
Ineligible	885	4816	30	623	436-891	1	Reference	0.044	1	Reference	0.587
Eligible	58	303	5	1647	685-3956	2.64	1.03-6.81		1.48	0.36-6.00	
Ever treated											
No	873	4729	28	592	409-858	1	Reference	0.009	1	Reference	0.286
Yes	70	390	7	1795	856-3766	3.03	1.32-6.94		1.88	0.59-6.04	

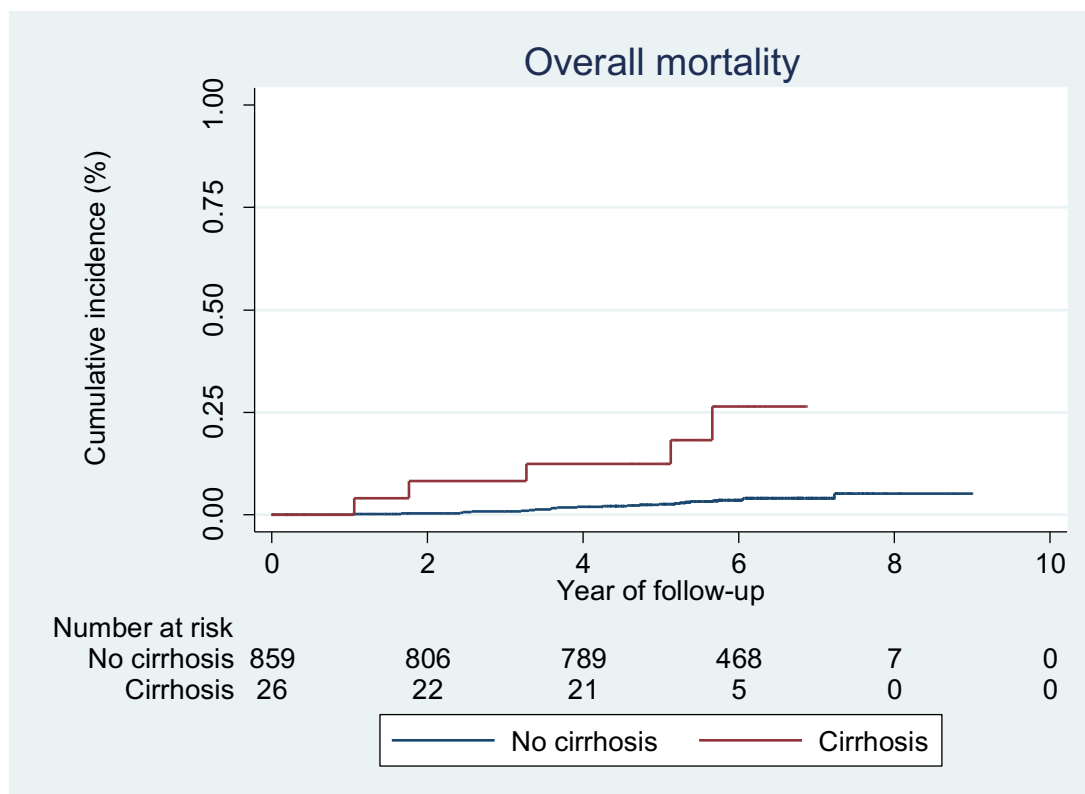


Figure 8.2. Overall all-cause by disease stage (cirrhosis or no cirrhosis) in the CHB cohort

Table 8.3. Liver related mortality

		No. of participants	Person-years of follow-up	No. of deaths	Incidence rates per 100 000 person-years	95% CI	Crude rate ratio	95% CI	p-value	Adjusted rate ratio	95% CI	p-value
Overall		943	5119	10	195	105-363	N/A	N/A	N/A	N/A	N/A	N/A
Age at baseline (years)												
<30		167	720	1	139	20-987	1	Reference	0.288			
30-39		464	2585	3	116	37-360	0.84	0.09-8.03				
40-49		173	1018	2	197	49-786	1.41	0.13-15.59				
≥50		139	797	4	502	188-1337	3.61	0.40-32.30				
Sex												
Female		346	2079	2	96	24-385	1	Reference	0.164			
Male		597	3040	8	263	132-526	2.74	0.58-12.89				
Origin												
Community-based screening		402	2445	8	327	164-654	1	Reference	0.024	1	Reference	0.235
Blood bank screening		300	1237	2	162	40-646	0.49	0.10-2.33		0.24	0.05-1.24	
Keneba & Manduar		241	1437	0	0	N/A	<0.01	N/A		<0.01	N/A	

Phase of natural history												
HBeAg-neg chronic infection		717	3891	4	103	38-274	1	Reference	0.003	1	Reference	0.059
HBeAg-pos chronic infection		20	115	0	0	N/A	<0.01	N/A		<0.01	N/A	
HBeAg-pos chronic hepatitis		18	104	0	0	N/A	<0.01	N/A		<0.01	N/A	
HBeAg-neg chronic hepatitis		19	110	2	1812	453-7244	17.63	3.23-96.23		15.04	1.64-138.09	
HBeAg-neg grey zone		118	648	1	154	22-1096	1.5	0.17-13.44		1.25	0.14-11.51	
Cirrhosis		27	125	3	2402	775-7446	23.36	5.23-104.38		19.36	2.89-129.48	
EASL criteria												
Ineligible		885	4816	7	145	69-305	1	Reference	0.005	1	Reference	0.562
Eligible		58	303	3	988	319-3064	6.79	1.76-26.29		0.57	0.09-3.77	
Ever treated												
No		873	4729	6	127	57-282	1	Reference	0.004	1	Reference	0.145
Yes		70	390	4	1026	385-2734	8.09	2.28-28.66		3.46	0.65-18.39	

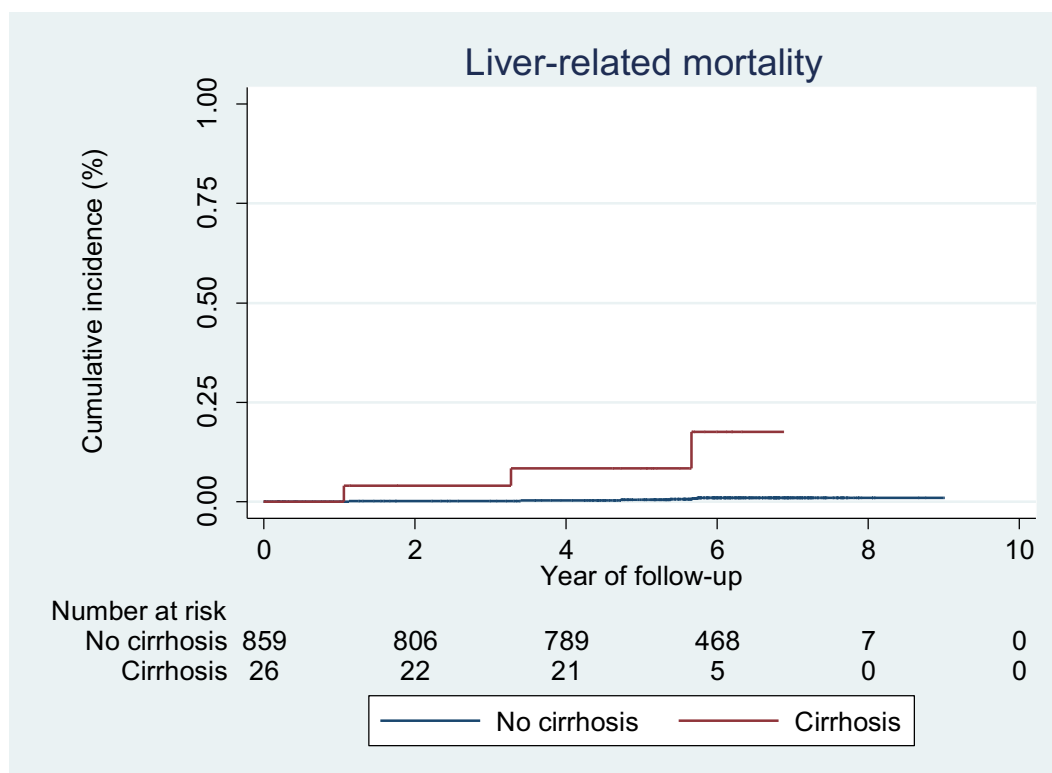


Figure 7.3. Liver related mortality in the CHB cohort

Conclusion

Long term follow up of patients with CHB infection using telephone calls and home visit is feasible in Africa, and rate of retention in care is high. Cirrhosis and older age are risk factors of mortality.

8.1.2. Decentralising HBV care in Africa: assessing the field effectiveness of the Xpert HBV viral load in CHB management

Background and methods

In low- and middle-income countries (LMICs), access to routine and effective HBV molecular diagnostics to determine treatment eligibility and monitor treatment response is often scarce, only available in capital cities of most countries. Where available, HBV DNA measurement is expensive, with some commercial assays costing up to €150 per test [283]. Consequently, scaling up and decentralising HBV treatment programs at national levels has continued to be a major challenge in almost all HBV endemic countries. It is therefore urgent to find reliable, cost-effective alternatives to HBV molecular diagnosis for Africa, a region that has been identified as a priority region for HBV research due to the high burden of hepatitis B and the absence of guidelines adapted to the local setting [104, 130].

The automated GeneXpert system – a rapid nucleic acid testing platform – has offered alternative access to high quality molecular diagnostics for several other diseases including tuberculosis and hepatitis C [284, 285]. The GeneXpert technology revolutionised the management of TB in LMICs where it has been used with great success in test-and-treat TB strategies. As a result, GeneXpert machines have become ubiquitous in HBV endemic countries in Africa and Asia (which are also endemic for TB). The newly launched Xpert HBV Viral Load test to detect and quantify HBV DNA viral load using the GeneXpert system therefore has the potential to significantly improve the scale up of hepatitis B diagnosis and monitoring in LMICs using existing resources.

However, its field effectiveness and validity against the gold standard quantitative PCR (qPCR) in Africa is unknown. In addition, the Xpert HBV test is licensed for use with plasma and serum samples collected within 6 weeks. Decentralised HBV program may rely on alternative samples such as dried blood spots or plasma/serum stored over longer periods prior to testing. The validity of the Xpert HBV test to accurately quantify viral load in these “off-label” samples is not determined.

In this project, nested within the longitudinal follow-up of the PROLIFICA cohort in my PhD, I determined the specificity and sensitivity of the Xpert HBV viral load test as compared to the gold standard qPCR in detecting HBV, and the agreement in quantified HBV DNA level, in African patients using off-label samples. Analysing 200

viremic samples was required to demonstrate that sensitivity is at least higher than 95% at one-sided significance level of 2.5%, with a power of 90% when the true sensitivity was 98%. In addition, 100 non-viremic control samples (HBsAg-positive with undetectable HBV DNA by qPCR) were needed to show that the specificity is at least higher than 95% at one-sided significance level of 2.5% with a power of 90%, when the true specificity was 99%.

Therefore, for this study, I tested plasma and DBS samples collected from HBsAg-positive patients enrolled in PROLIFICA and followed up as part of my PhD. Plasma was extracted from EDTA whole blood within 4 hours of collection and stored in -80C for at least 12 months prior to testing. DBS samples were collected from capillary blood and left to air dry at room temperature for 2 hours before stored in -80C. All samples were tested for HBV DNA in parallel using Xpert (according to manufacturer instructions) and qPCR [187].

Preliminary results

In total, 306 plasma and 91 DBS samples were tested using Xpert HBV assay. After excluding 23 plasma samples that were not tested for PCR (insufficient sample volume), there were a total of 283 samples included in this analysis. The general characteristics of the patients included in this study are shown in table 8.4

Table 8.4. General characteristics

Variables		Number (%)
Sex	Men	202 (71%)
	Women	81 (29%)
Age (years)	18-29	23 (8%)
	30-39	120 (43%)
	40-49	83 (29%)
	≥50	57 (20%)
Disease status (by US)	No apparent liver disease	261 (92%)
	Cirrhosis	14 (5%)
	HCC	1 (1%)
	Fatty liver disease	7 (2%)

Concordance between Xpert HBV and qPCR

A total of 110 stored samples had detectable HBV DNA results using both Xpert HBV and qPCR. Scatter plot of the Xpert versus qPCR plasma viral load results is shown in figure 8.4

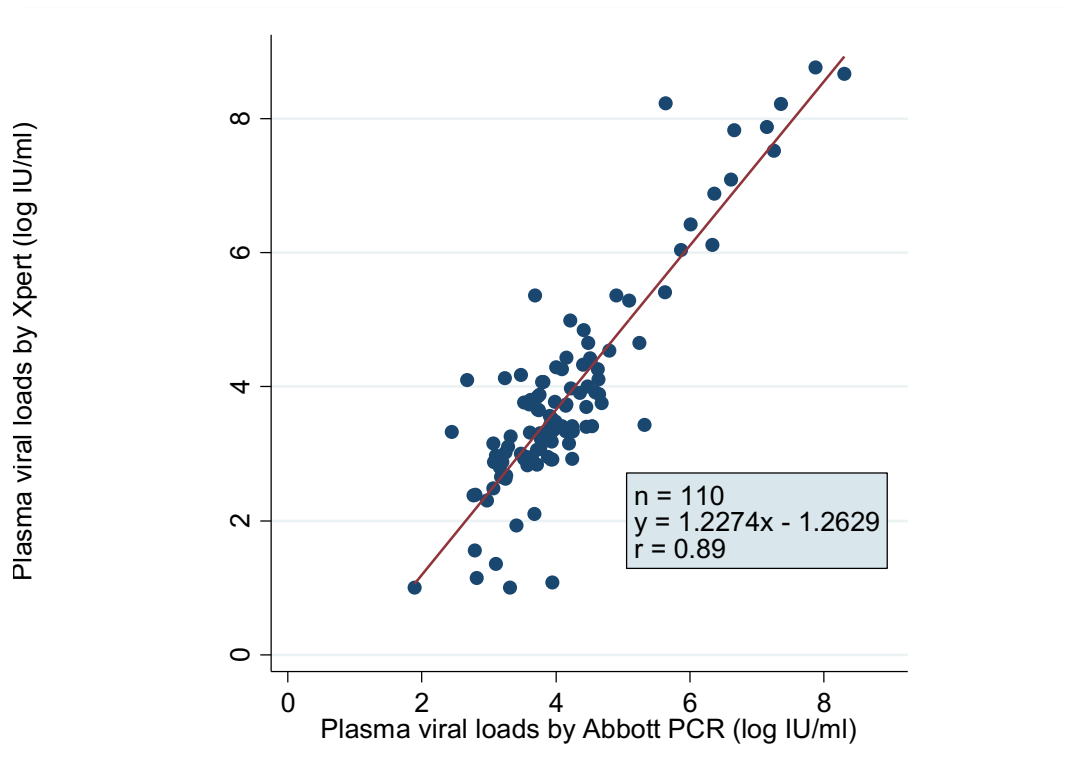


Figure 8.4. Scatter plot of Xpert vs qPCR for plasma viral load for samples with numerical results by both methods

The mean difference between Xpert HBV and qPCR was 0.316 log IU/mL (95% limits of agreement -1.189, 1.820). Only 6/110 results (5.45%) were outside the limits of agreement (figure 8.5). These results indicate good agreement between Xpert HBV and qPCR

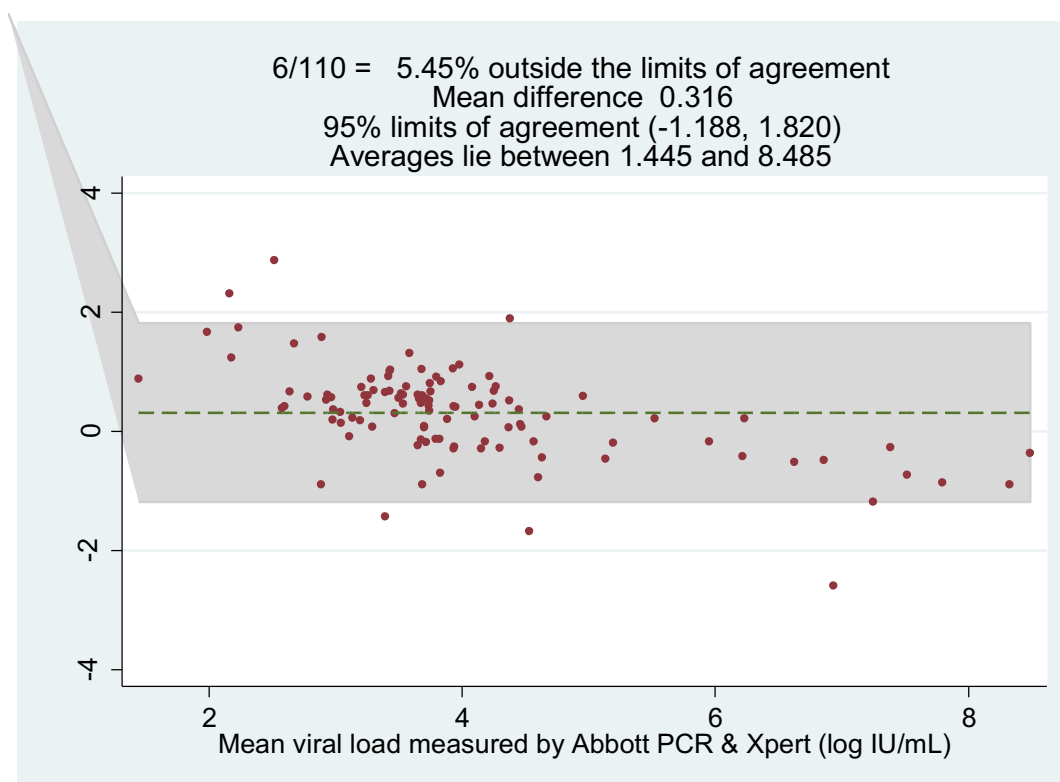


Figure 8.5. Bland-Altman plot of Xpert minus qPCR plasma viral loads (vertical axis) against mean of Xpert and qPCR plasma viral load (horizontal axis)

Agreement between plasma and DBS using Xpert HBV test

In 91 paired plasma and DBS samples analyses in parallel using the Xpert HBV viral load test, 34 had detectable HBV viral load in both samples (Table 8.5).

Table 8.5. Test results by type of sample using Xpert HBV assay

		Plasma samples			
		Undetectable (n=14)	Detectable (n=73)	Error (n=2)	Invalid (n=2)
DBS samples	Undetectable (n=18)	5 (36%)	11 (15%)	1 (50%)	1 (50%)
	Detectable (n=37)	1 (7%)	34 (47%)	1 (50%)	1 (50%)
	Error (n=3)	0 (0%)	3 (4%)	0 (0%)	0 (0%)
	Invalid (n=33)	8 (57%)	25 (34%)	0 (0%)	0 (0%)

In these 34 paired samples, the mean bias between plasma and DBS was +1.831 log IU/mL (95% limits of agreement: 0.660=3.001). Bias was smaller at lower viral load levels. The kappa statistic (κ) was 0.10, indicating very poor agreement between the Xpert HBV results for plasma and DBS samples. After excluding samples having error or invalid results by either type of samples (n=51), the kappa statistics (κ) was 0.34, indicating poor agreement (figures 8.6 and 8.7).

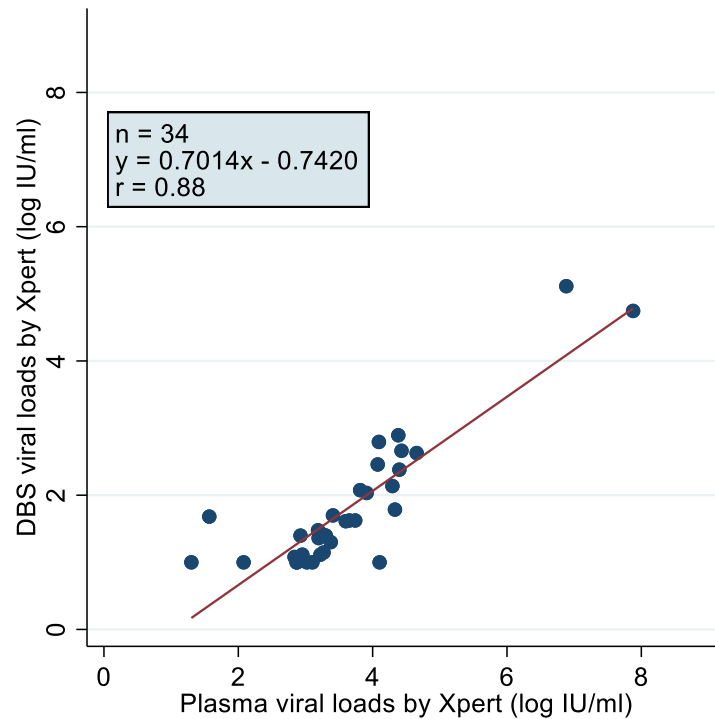


Figure 8.6. Scatter plot of Xpert using DBS vs Xpert using plasma for samples that had numerical results by both methods (n=34)

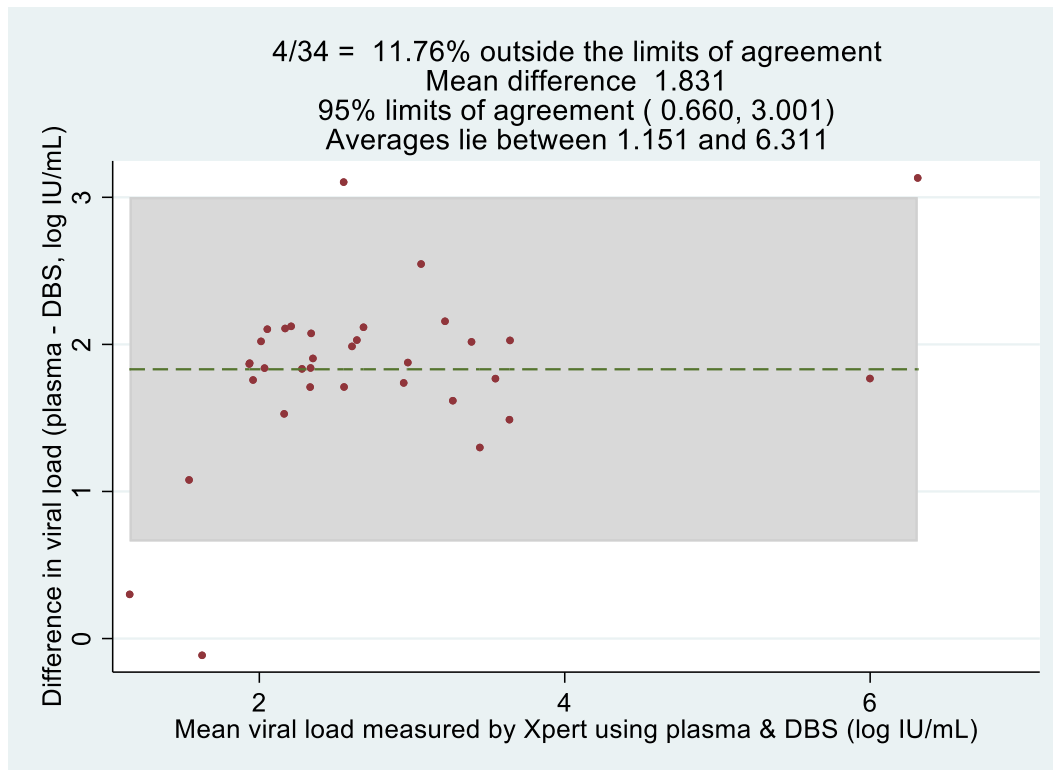


Figure 8.7. Bland-Altman plot of Xpert HBV using DBS minus Xpert HBV using plasma (vertical axis) against the mean of Xpert using DBS and Xpert using plasma (horizontal axis) for samples with numerical results by both methods (n=34).

Conclusion

The Xpert HBV viral load test has good correlation with qPCR to detect and quantify HBV DNA using plasma samples stored over extended periods in Africa. The assay had poor performance in testing DBS samples in our study.

8.1.3. Understanding the mechanisms underlying CHB disease progression in Africa

There are still significant gaps in our understanding of the mechanisms underlying CHB disease evolution and outcomes in Africans. As a result, current guidelines have poor performance to correctly identify Africans in need of CHB treatment [104], and available antiviral treatment does not clear CHB infection or eliminate risk of HCC [286].

In vitro and in vivo mouse model studies have used metabolomics to demonstrate that hepatitis B virus (HBV) induces upregulation of lactate and hexosamine to escape innate immune responses and promote viral replication [287, 288]. Previous work by our group and others also showed that the triglyceride, phospholipid, free fatty acid and sphingomyelin metabolic pathways are affected during CHB infection and metabolites including inosine, indole-3-acetate and lactate can distinguish between CHB-infected patients with cirrhosis and HCC [205, 289-291]. These human studies have primarily focused on CHB-related cirrhosis and HCC, did not investigate metabolic changes over CHB progression, did not include African subjects or HBV genotypes present in sSA, and/or were insufficiently powered to detect small effect sizes. Therefore, the role of these and other metabolites and metabolic pathways in CHB disease progression and HBsAg loss in Africans living in sSA is unknown.

During my PhD, I had stratified the HBV positive patients in the PROLIFICA cohort into sub-groups by disease stage based on the EASL nomenclature [33]. In this project, funded through a preparatory fellowship award by AREF (African Research Excellence Fund) and EDCTP (European and Developing Countries Clinical Trials Partnership), I profiled the untargeted serum metabolome of 134 carefully selected HBV-positive patients (14 with HBsAg loss, 30 chronic infection, 30 chronic hepatitis, 30 grey zone, 30 on treatment), and 30 matched HBsAg-negative health controls. I used liquid chromatography mass spectrometry (LC-MS) to run reverse positive (RPOS), reverse negative (RNEG) and HILIC data acquisition at the National Phenome Centre in Imperial College London.

I applied a supervised partial least-squares discriminant analysis (sPLS-DA) method to stratify between chronic infection (inactive carriers), grey zone and chronic hepatitis patients based on their metabolic signature. Preliminary on the LC-MS reverse phase (positive and negative mode) data show that the sPLS-DA model retains a good fit

($R^2Y=0.571$) and significantly predicts a discriminatory metabolic variation between the three groups ($Q^2Y=0.172$; $pQ^2=0.001$) (Figure 8.8).

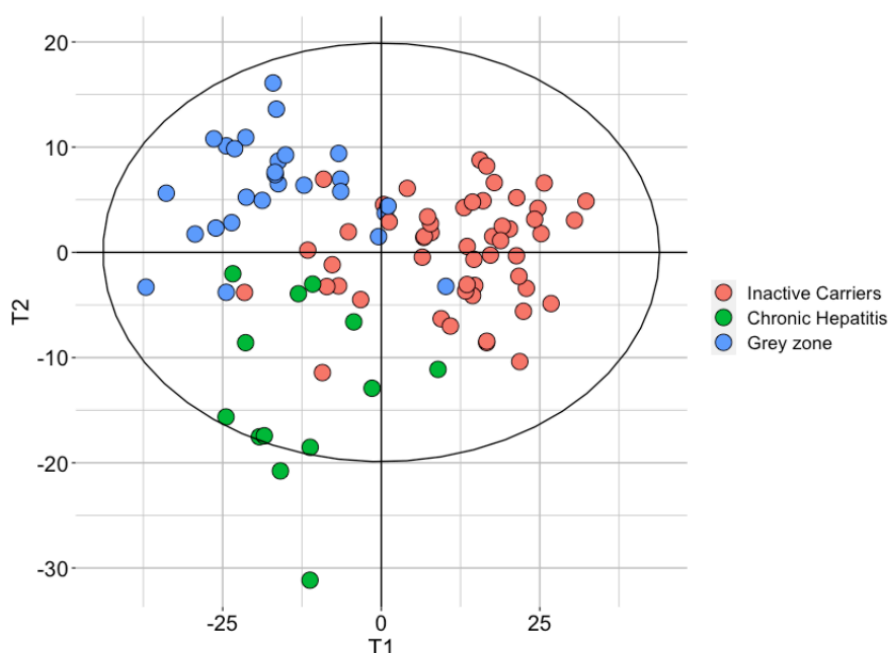


Figure 8.8. PLS-DA plot showing separation between inactive carriers (red), grey zone (blue) and chronic active hepatitis (green)

Analysis of the HILIC dataset and identification of metabolites will be completed in the coming months. Pilot results from this project supported a successful Wellcome Trust fellowship.

8.1.4. Prevention of HBV mother-to-child transmission in Africa

In July 2020, the WHO recommended antiviral prophylaxis in highly viremic or HBeAg-positive pregnant women to prevent HBV MTCT [112]. Implementing these recommendations relies on routine HBsAg testing in pregnant women, and quantification of HBV DNA or testing HBeAg during pregnancy. Routine HBsAg testing is not implemented in antenatal services in Africa, and access to HBV DNA and HBeAg remains scarce.

In this baseline study, I assessed the feasibility of incorporating HBsAg screening into routine antenatal services in public facilities in The Gambia, and assessed the proportion of pregnant women at high risk of HBV MTCT define by HBV DNA

>200,000IU/mL and/or positive HBeAg. I also administered questionnaires to assess pregnant women's knowledge on HBV and HepB-BD, and their perception on training mother to administer timely HepB-BD using prefilled syringes (such as the Uniject device).

Finally, I followed up babies born to HBsAg-positive mother to estimate the rate of HBV MTCT, stratified by maternal viral load, HBeAg status and time of HepB-BD administration.

Preliminary results:

Between December 2020 and February 2022, 9,695 women attending antenatal clinic in urban and rural Gambia were offered HBsAg testing. All women accepted HBsAg screening. Among 4,309 women interviewed prior to HBsAg testing, only 149 (3.46%) had a prior HBsAg testing, and 3,094 (71.80%) women were willing to vaccinate their babies for HepB-BD using prefilled device if trained.

Out of 9,682 women tested for HBsAg who had a valid result, 409 (4.2%) were HBsAg positive. I analysed HBV viral load in 320 HBsAg-positive women, of whom 19 (5.94%) and 30 (9.38%) had HBV DNA viral load >200,000IU/mL and >20,000IU/mL respectively. HBeAg was tested using rapid point-of-care test kits. Among 223 HBsAg-positive women tested for HBeAg, 13 (5.83%) were HBeAg positive.

So far, 97 babies born to HBsAg-positive women have been assessed at age 6 months. Among them, 32 (32.99%) received a timely HepB-BD vaccine. Two babies (2.06%) were HBsAg positive, both babies were born to HBeAg positive mothers with HBV DNA viral load >200,000IU/mL.

Over the coming months, I will continue the follow up of infants, complete HBeAg serology tests, and run comprehensive analysis of the dataset.

8.2. Future work and directions

8.2.1. Rates and risk factors of CHB disease progression

The natural history of CHB infection in Africa is not well understood, and best practice for managing and monitoring Africans with CHB have not been established. During my PhD, I reassessed all patients previously enrolled in the PROLIFICA study, 7-10 years after initial enrolment.

In this analysis, I will assess the rates and risk factors of disease progression, HBsAg seroclearance and HCC incidence in CHB patients followed up during my PhD. I will assess the proportion of patients who were not previously eligible for antiviral therapy who have become eligible for treatment or had disease progression. Among patients on treatment with TDF, I will assess the safety (renal and bone) and efficacy (viral suppression) of treatment, and estimate the impact of treatment on regressing liver fibrosis and preventing HCC incidence and death.

Findings from this analysis are expected to inform the national and WHO hepatitis B response and guidelines. The data from this analysis will complement data from other cohorts in Africa (within the HEPSANET consortium) to determine cut-off levels for treatment initiation and best practice for following up untreated patients at various disease stages.

8.2.2. Metabolic phenotypes, cellular pathways and mechanisms of persistence in CHB infection

Building on the pilot study completed during my PhD, I will analyse serial biological samples collected from PROLIFICA patients at various stages of CHB infection [33] to answer four primary questions:

- Identify specific metabolites associated with HBV disease progression and HBsAg loss
- Quantitatively measure the expression of candidate metabolites at various time points in CHB infection
- Directly measure the influence of candidate metabolites on viral replication and macrophage response using a liver-on-chip microphysiological system (CN Bio Innovations Ltd) for HBV infection [292].
- Identify cellular pathways of disease progression and potential targets for cure

This project is funded by the Wellcome Trust through an International Training fellowship awarded in December 2021. The design of the study is summarised in figure 8.9.

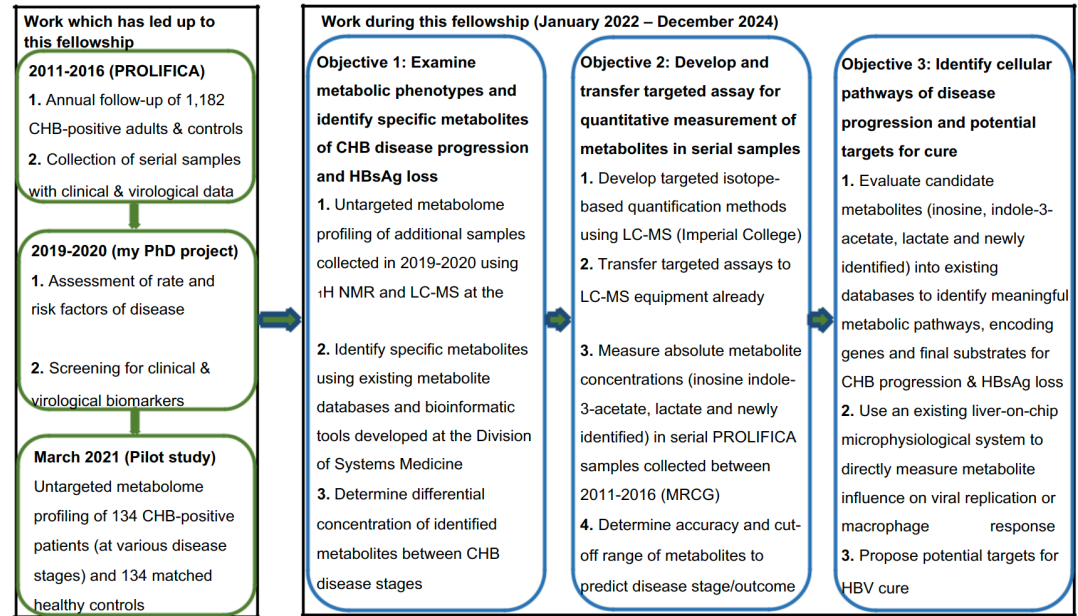


Figure 8.9. Study design

I expect that results from this project will improve understanding of the mechanisms underlying CHB disease progression in Africa, potentially simplify CHB treatment and monitoring by identifying biomarkers to predict CHB outcomes and/or need for antiviral therapy, and open new research questions for HBV cure in Africa.

8.2.3. Triple elimination of HIV, syphilis and HBV: integrated antenatal screening and treatment in The Gambia and Burkina Faso (the TRI-MOM study)

In 2016, WHO Member States adopted three aligned global health sector strategies on HIV, viral hepatitis and sexually transmitted infections (STIs) to guide global elimination, with priority to end mother-to-child transmission (MTCT) [41]. The 2022-2030 WHO Global Health Sector Strategy on HIV, viral hepatitis and sexually transmitted infections (STIs) further reinforces the call for integrated strategies to end these three infections by 2030 [293].

All three infections are endemic in Africa and can be transmitted vertically from infected mother during pregnancy. Significant progress has been made in HIV prevention of

mother-to-child transmission (PMTCT) in Africa. However, PMTCT of HBV and STIs is not well defined and developed, and there are currently no clear guidelines on best practice to integrate these services into routine antenatal services.

Between 2023-2025, we will build up on the experience from the INFANT-B study to integrate HBV and syphilis testing and treatment within antenatal services in The Gambia and Burkina Faso. The study will align antenatal HBsAg and syphilis testing alongside the routine HIV antenatal screening and provide HBeAg and/or HBV DNA viral load testing in real-time to all HBsAg positive mothers using the GeneXpert platform. Women with HBV DNA >200,000IU/mL and/or HBeAg will be offered antiviral prophylaxis from the second trimester until 4 weeks after delivery. Women who test positive for syphilis will be provided treatment with antibiotics and offered repeat and partner testing. HIV positive women will be managed according to existing national protocols in the two countries. All babies of HIV, HBV and syphilis positive mothers will be assessed to estimate the rates of MTCT. We will compare rate of HBV MTCT in this study against the rates determined in the INFANT-B study, to quantify the additional benefit of HBV antiviral prophylaxis in preventing HBV MTCT in West Africa.

In parallel, we will train peer mediators and social workers who will provide counselling and support pregnant women and will collaborate with medical anthropologists to assess the perspectives of the pregnant women, their partners, communities, healthcare workers and policy makers on best practice and approach toward triple elimination.

This study is funded by Expertise France and will be done in collaboration with the Ministries of Health and Civil Societies in The Gambia and Burkina Faso, as well as experts in epidemiology, PMTCT and health systems research at Imperial College, INSERM and Institut Pasteur. Findings from the study will guide and inform PMTCT and elimination strategies in Africa.

8.3. Appendix

8.3.1. Ethics approval – PROLIFICA longitudinal follow up

The Gambia Government/MRC Joint

ETHICS COMMITTEE

C/o MRC Unit: The Gambia @ LSHTM, Fajara
P.O. Box 273, Banjul
The Gambia, West Africa
Fax: +220 – 4495919 or 4496513
Tel: +220 – 4495442-6 Ext. 2308
Email: ethics@mrc.gm

10 May 2018

Professor Mark Thursz
Imperial College London

Dear Professor Thursz

SCC 1579v1.1, The PROLIFICA programme in The Gambia: 5 year longitudinal follow-up

Thank you for submitting your proposal dated 15 February 2018 for consideration by The Gambia Government/MRC Joint Ethics Committee at its meeting held on 27 April 2018.

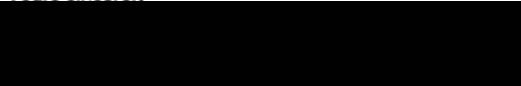
The Ethics Committee is happy to approve your proposal with the proviso that you submit annual progress report which should include an agreed transition plan to show the sustainability of the project post 5 years.

The committee also strongly urged you (investigators) to continue the advocacy and networking initiative for this project.

Finally, you are requested to list down Drs Ramou Njie and Sheikh Omar Bittaye as co-investigators in the appropriate section of the SCC application form.

With best wishes

Yours sincerely



Mr Malamin Sonko
Chairman, Gambia Government/MRC Joint Ethics Committee

Cc: Professor Umberto D'Alessandro

Documents submitted for review:

- SCC approval letter – 13 March 2018
- SCC application form, version 1.1 – 15 February 2018
- Response letter
- Protocol, version 0.2 – 22 January 2018
- ICD, version 1.1 – 15 February 2018
- Enrollment visit – clinical and epidemiologic forms, version 1.1 – 15 February 2018
- Award letter from MRC UK – 19 December 2018
- Participant invitation – home visit and telephone, version 1.1 – 15 February 2018
- Letter re roles and responsibilities – 6 January 2018

The Gambia Government/MRC Joint Ethics Committee:

Mr Malamin Sonko, Chairman
Prof Ousman Nyan, Scientific Advisor
Ms Naffie Jobe, Secretary
Dr Roddie Cole
Dr Ahmadou Lamin Samateh
Mrs Tulai Jewara-Ceesay

Prof. Umberto D'Alessandro
Dr Mamady Cham
Dr Ramatoulie Njie
Dr Jane Achen
Prof Martin Antonio

8.3.2. Gilead Public Health Award letter

GRANT LETTER OF AGREEMENT
12/2/2019

London School of Hygiene and Tropical Medicine whose administrative offices are at Keppel Street, London, WC1E 7HT and operating as a branch office as the MRC Unit The Gambia at London School of Hygiene and Tropical Medicine, Atlantic Boulevard, Fajara, P.O. Box 273, Banjul, The Gambia

Dear London School of Hygiene and Tropical Medicine,

On behalf of the Gilead Sciences, Inc. ("Gilead"), I am pleased to inform the Medical Research Council Unit The Gambia at the London School of Hygiene and Tropical Medicine ("Grantee") that Gibril Ndow's request for a grant to support "Management of chronic hepatitis B in Africa: is a one-stop assessment of liver disease enough?" ("Project"), as set forth in greater detail in the Grant Request Proposal 05235, has been approved in the amount of 100,000.00 USD ("Grant Funds"), payable as set forth in this letter agreement below ("Letter Agreement"). Grant Funds must be used solely to support the Project.

Gibril Ndow's application for the GPHA program has been approved and awarded USD 100,000 for a period of two years. The disbursement of grant funds will be made directly to Medical Research Council Unit The Gambia at the London School of Hygiene and Tropical Medicine. The grant award will be divided into two payments, one initial payment for year one of the award period (USD 50,000) and a second payment (USD 50,000) made after the progress report is deemed satisfactory by the Scientific Review Committee for year two of the award.

In accepting the Grant Funds, Grantee hereby agrees to the following terms and conditions:

1. Approved Use of Grant Funds. Grantee shall only use Grant Funds for the Project and as listed out in the Grant Request Proposal 05235. Any material or substantive change in the Project must be approved by Gilead. The Grantee must submit the Gilead in writing a detailed Change of Scope through the online grants portal, before Grant Funds may be spent for such material or substantive Project change. *Please note that the Grant Funds may not be used to subsidize patient purchases of prescription medications.*

2. Reports. Within 60 days of the completion of the Project,

Grantee shall submit a final report along with a reconciled budget concerning the Project to Gilead through the online grants portal.

If applicable, a Disclosure spreadsheet is due within 60 days of completion of the Project. This completed spreadsheet must be returned to Gilead at

266407.1

8.3.3. Longitudinal follow up: participant information sheet and consent form



Identification code: SOP-CTS-015 *Management of Chronic Hepatitis B in Africa (MATCH-B)*

Version : 1.1 16.05.2018

PARTICIPANT INFORMATION SHEET

Version 1.1 Date 16th May 2018

Study Title: **Management of Chronic Hepatitis B in Africa (MATCH-B)**

SCC: 1579 Protocol: 1.1

Sponsor: Imperial College London

Funder: MRC UK and Gilead Pharmaceuticals

Participant Identification: EG_____

What is informed consent?

You are invited to take part in a research study. Participating in a research study is not the same as getting regular medical care. The purpose of normal medical care is to improve one's health. The purpose of a research study is to gather information that may be useful in the future for the whole population. Your choice to participate or not will be respected. It is your decision and you can stop any time without giving any reason.

Before you decide you need to understand all about the study and what will happen in it. Please take time to read the following information or get the information explained to you in your language. Listen carefully. You can ask questions if there is anything that you do not understand. Ask for it to be explained until you understand it. You may also wish to discuss with your husband/wife, family members or others before deciding to take part in the study.

If you decide to join the study, you will need to sign or thumbprint a consent form saying you agree to be in the study. You will receive a copy of the consent form.

Why is this study being done?

Hepatitis B virus (HBV) is very common in West Africa. In The Gambia, approximately 1 in every 10 adults is chronically infected with the virus. This virus can be passed in blood, through unprotected sex with an infected partner, from mother to child at birth, or in early life from child to child with close contact. For the last 25 years, all children born in The Gambia are offered vaccination against HBV. However, adults over 30 years of age were born before the vaccine was introduced and may carry the virus. People with HBV may have no symptoms. However, in some people, the virus may silently damage the liver and eventually cause liver cancer and liver failure.

Version 1.1 Date 16th May 2018

Page 1 of 6

Identification code: SOP-CTS-015

MRC Unit The Gambia

Management of Chronic Hepatitis B in Africa (MATCH-B)

Version : 1.1 16.05.2018

SCC: 1579

Because not everyone who is infected with HBV will develop symptoms and diseases, current international guidelines recommend treatment for those HBV infected patients with or at risk of advanced liver disease or liver cancer. It is however difficult to identify chronic HBV infected patients who will or will not progress to develop liver disease. This is especially challenging in West Africa where the current international guidelines may not be optimal in identifying patients in need of treatment given the different strains of HBV and human genetics.

In this follow-up study, we will determine the rate of progression of clinical and viral markers of disease severity among patients who did not meet the criteria for HBV treatment. This will help us formulate new criteria (where necessary) for determining who is most likely to benefit from HBV treatment in the sub-region. We will also investigate the efficacy of Tenofovir in controlling HBV and reversing liver disease among HBV infected patients receiving treatment in The Gambia.

What does this study involve?

In this study, we will re-invite all HBV infected participants previously enrolled in the PROLIFICA WATCH study for re-assessment. Participation in this study will involve one visit to the liver clinic at MRC Fajara or MRC Keneba field station.

During this visit, you will complete a questionnaire and undergo a clinical examination including a FibroScan and a liver ultrasound scan similar to the ones you had when you were initially enrolled into the PROLIFICA study a few years ago. Neither of these scans is painful or harmful to you. Following the clinical examination, we will collect 24mL of blood from your arm, approximately the volume of one tablespoon. We will also collect 20mL of urine from you.

If you are already receiving treatment for HBV, you will continue to receive your treatment and attend your routine follow-up visits. If you do not currently receive treatment, this reassessment will determine whether you need to start treatment. If you need treatment, you may be re-invited for a second assessment to do a few more tests and / or to start treatment.

What will happen to the samples taken in this study?

During your assessment visit, we will collect approximately one tablespoon of blood from your arm. We will use some of this blood to check the health of your liver and kidneys, measure your haemoglobin and platelets, and the amount of hepatitis B virus in your blood. We will do additional tests to detect how your natural immune system is fighting against the hepatitis B virus, and to test if you have been exposed to a fungus called *Aflatoxin* that can sometimes cause liver disease. In some participants, we may do additional hepatitis tests to detect certain hepatitis antigens (HBsAg and HBeAg) in your blood. If you need hepatitis B treatment, we will offer you an HIV test.

The remainder of the blood, and the urine samples will be stored at the MRC Unit The Gambia and later sent to England where additional tests will be carried out to analyse viral variations and to develop biomarkers that will ease the identification of patients in need of future treatment.

Version 1.1 Date 16.05.2018

Page 2 of 6

Identification code: SOP-CTS-015

MRC Unit The Gambia

Management of Chronic Hepatitis B in Africa (MATCH-B)

Version : 1.1 16.05.2018

SCC: 1579

What harm or discomfort can you expect in the study?

The clinic visit is expected to last approximately 1 hour. You may, however, have to wait to be seen by the study nurse and clinician, and your total clinic visit could be longer. The study coordinators will endeavour to make your visit as short as possible. In order to collect blood sample, we will make a small injection in your arm, which may be painful. In a few individuals, bleeding from this injection site may continue for a while. There is almost no risk of severe bleeding.

You will undergo an ultrasound scan of your liver, similar to the one you had when enrolled into PROLIFICA. This scan, and the blood tests we will do, may identify certain health problems that you were not aware of. In such instances, we will speak with you about our findings and refer you to the best place for the management of your condition.

What benefits can you expect in the study?

You will undergo a thorough clinical assessment to check the status of your liver and your hepatitis B infection. This assessment will include some clinical and laboratory tests that are otherwise not available in routine clinics in The Gambia.

If we find that your hepatitis B infection is progressing and that you will benefit from treatment, we will provide you with treatment for your hepatitis B infection. The study team will discuss this with you.

Will you be compensated for participating in the study?

You will not get paid by the study, but MRC will provide transport or give you back the money for your transport.

Are there other products or treatment?

No.

What happens if you refuse to participate in the study or change your mind later?

You are free to join the study or not and you have the right to stop being in the study at any time without giving a reason. You will still get the normal medical care.

If you do not want to continue in the study, we will use only the samples and information already collected from you.

If we find any new information during the study that may change if you can be in the study, we will tell you as soon as possible.

If you are injured in the study what compensation will be available?

If you have an unwanted reaction or get any medical problems from the study, we will treat you or refer you as needed. If it is an emergency, please go to your nearest health centre or clinic and call immediately the research team or field worker who gave his/her telephone number to you or contact Saydiba Tamba on 7405060.

Version 1.1 Date 16.05.2018

Page 3 of 6

Identification code: SOP-CTS-015

MRC Unit The Gambia

Management of Chronic Hepatitis B in Africa (MATCH-B)

Version : 1.1 16.05.2018

SCC: 1579

How will personal records remain confidential and who will have access to it?

All information that is collected about you in the study will be kept strictly confidential. Your personal information will only be seen by the study team members, the sponsor and if necessary the Ethics Committee and Government authorities.

Who should you contact if you have questions?

If you have any questions or concern, you can call Saydiba Tamba on 7405060, and you can always call the personal numbers of the study staff given to you. If you have any concerns you can also call staff at your health centre or hospital.

Please feel free to ask any question you might have about the study.

Who has reviewed this study?

This study has been checked by scientists at the Medical Research Council and by the Gambia Government/MRC Joint Ethics Committee. The Ethics Committee protects your rights and wellbeing, and has given permission for this study to take place.

Version 1.1 Date 16.05.2018

Page 4 of 6



Scientific Coordinating Committee

Dr Gibril Ndow
MRCG at LSHTM
Fajara
19 June 2019

Dear Dr Ndow,

MRCG ethics ref: 17555: Assessing the field effectiveness and cost-effectiveness of the Xpert HBV Viral Load test in the management of chronic hepatitis B infection in West Africa: a validation study

Thank you for responding to the SCC's request for further information on the above research and submitting revised documentation.

The further information has been considered by the Chair.

Confirmation of favourable SCC opinion

The SCC is pleased to approve your proposed study which will be forwarded to the Ethics committee for further consideration at their next meeting on 27 June 2019.

Documents submitted for review are:

Document Type	File Name	Date	Version
Local Approval	L2018.23_Lemoine_Approved_7Jun18	07/06/2018	1
Local Approval	L2018.23_Lemoine_Resubmission_29 Jun18	29/06/2018	1
Protocol / Proposal	GeneXpert_HBV_Cepheid_Final	15/05/2019	V1.1
Consent form	Info_Consent_V1.1_Final	16/05/2019	1.1
Investigator CV	MRCG-PP-CV-GNdow	20/05/2019	V1.0
Investigator CV	Short CV May2019	20/05/2019	V1.0
Covering Letter	Cepheid Letter HBV project in Gambia	14/06/2019	1.0
Covering Letter	Cover%20Letter_SCC-Response_17555_V1.0_FINAL	14/06/2019	1.0
Covering Letter	GeneXpert_HBV_Cepheid_V2.0_14thJune2019_CLEAN	14/06/2019	2.0
Protocol / Proposal	GeneXpert_HBV_Cepheid_V2.0_14thJune2019_CLEAN	14/06/2019	2.0

With best wishes

Yours sincerely



Professor Beate Kampmann

Acting Chair, Scientific Coordinating Committee

8.3.5. Xpert HBV study: Cepheid award

June 13 2019

To: Dr. Maud Lemoine; Dr. Gibril Ndow

Cc: Stephen Opoku, Pierre Le Roux, Rujeko Tsomondo, Sophie Pease (Cepheid)

Ref: **Assessing the field effectiveness and cost-effectiveness of the Xpert HBV Viral Load test in the management of chronic hepatitis B infection in West Africa: a validation study**

Dear Maud, dear Gibril,

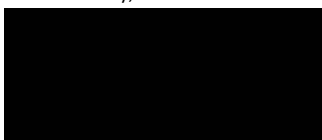
We have well received your request for technical assistance to support the study in reference for HBV Viral Load testing to be performed in Gambia, using the GeneXpert systems already in place.

We can confirm that we are ready to provide our assistance in terms of technical support, training and maintenance as follows :

- Online training sessions for the use of the HBV viral load kits
- Provision of Software updates when required
- Provision of Xpert Checks kits that may be due at the time the study is initiated
- Onsite training and support, upon request, from our local partner, Ecomed

Please feel free to come back to us to make sure all systems are functional and all personnel ready to operate at the moment the study starts, and at any time during the study.

Your sincerely,



Augustin Mortamet
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8.3.6. AREF award letter



EDCTP
The power of sharing science

P.O. Box 93015, 2509 AA
The Hague, The Netherlands
Anna van Saksenlaan 51, 2593 HW
The Hague, The Netherlands
T: +31 70 344 0880
www.edctp.org

Dr Gibril Ndow
Medical Research Council (MRC) Unit - The Gambia
United Kingdom
gndow@mrc.gm

Date: 1 April 2020
E-mail: singh@edctp.org

Ref. #: 121234

Subject: **EDCTP2 Call for Proposals TMA2019PF - Preparatory Fellowships – in collaboration with the Africa Research Excellence Fund (AREF)**

Proposal: TMA2019PF-2771 - DOMINANCE

Evaluation result letter — Grant preparation invitation letter

Dear Dr Gibril Ndow,

I am writing in connection with your proposal for the above-mentioned call. Thank you for submitting an application in response to the EDCTP-AREF Call for Proposals.

Having completed the evaluation, I am pleased to inform you that your proposal has been favourably evaluated and that AREF would now like to start grant preparation.

Please find enclosed the evaluation summary report (ESR). It reflects the comments and opinion of the experts that evaluated the proposal.

Invitation to grant preparation

Grant preparation will be based on the following:

1. **Proposal No:** TMA2019PF-2771 – DOMINANCE
2. **Title:** Developing metabonomics skills to understand chronic hepatitis B infection and identify new biomarkers [Ndow]
3. **Type of action:** Training & Mobility Action (TMA)
4. **AREF contact:** Dr Peter Dukes
Director of Programme
Africa Research Excellence Fund
peter.dukes@aref-africa.org.uk
5. **Maximum grant amount:**
Requested contribution (according to proposal): 57.095,00 EUR
Maximum grant amount (proposed amount, after evaluation): **57.095,00 EUR**
6. **Duration of the action:** 12 months

EDCTP-Association is registered in The Hague, The Netherlands. Chamber of Commerce file no. 60471700

European & Developing Countries Clinical Trials Partnership

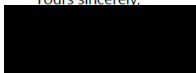
Supported by the
European Union



Upon receipt of this letter, please make contact with Dr Dukes at the Africa Research Excellence Fund, for further guidance regarding the grant negotiation process.

Please note that this letter does NOT constitute a **formal commitment for funding**. The final decision by AREF will only be taken at a later stage, since it depends on the finalisation of grant preparation and the rest of the selection procedure (implying further checks, for instance, of operational and financial capacity, non-exclusion, etc.).

Yours sincerely,

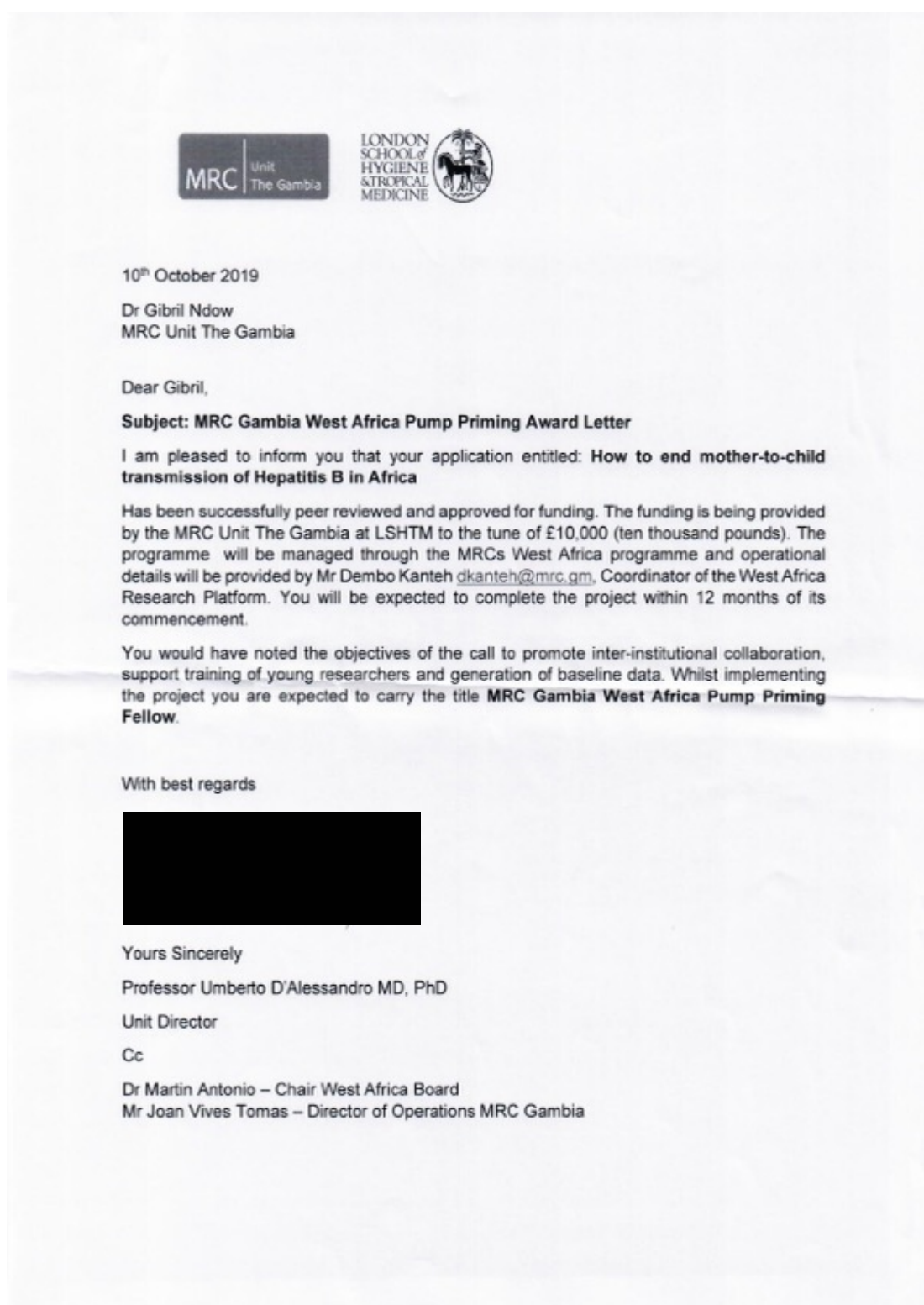


Dr Michael Makanga
Executive Director
EDCTP

cc: Dr Peter Dukes, Africa Research Excellence Fund

Enclosed: Evaluation summary report

8.3.7. INFANT-B: Pump-priming grant award letter



8.3.8. INFANT-B: SCC & Ethics approvals



Scientific Coordinating Committee (SCC)

Dr Gibril Ndow
C/O MRCG at LSHTM, Fajara

9 August 2020

Dear Dr Ndow

Study Title: How to end mother-to-child transmission of Hepatitis B - the Intervention For Avoidance of Neonatal Transmission of Hepatitis B in Africa (INFANT-B) study

Project ID/Ethics ref: 19099

Thank you for submitting your application which was considered by the SCC at its meeting held on 3 August 2020.

Confirmation of favourable SCC opinion

The SCC is pleased to approve the proposed amendment which will be forwarded to the Ethics Committee for further consideration at their next meeting.

Documents submitted for review are:

Document Type	File Name	Date	Version
Local Approval	Informed Consent Document_PROPEL_V3.0_14May2016	14/05/2016	3.0
Local Approval	Informed Consent Document_Men A_V1.1_16Feb2018	16/02/2018	1.1
Local Approval	GaPs Informed Consent Document V1.0_19Mar2018	19/03/2018	1.0
Other	INFANT B_Gambia_Final_V1.3_18Jul2020	18/07/2020	1.3
Other	INFANT B timelines v1.2	18/07/2020	1.2

With best wishes

Yours sincerely

Kalifa Bojang

Dr Kalifa Bojang

Acting Chair, Scientific Coordinating Committee

London School of Hygiene & Tropical Medicine
 Keppel Street, London WC1E 7HT
 United Kingdom
 Switchboard: +44 (0)20 7636 8636
www.lshtm.ac.uk



Observational / Interventions Research Ethics Committee

Dr Gibril Ndow
 LSHTM

29 July 2020

Dear Dr Ndow

Study Title: How to end mother-to-child transmission of Hepatitis B - the Intervention For Avoidance of Neonatal Transmission of Hepatitis B in Africa (INFANT-B) study

LSHTM Ethics Ref: 19099

Thank you for your application for the above research project which has now been considered by the Observational Committee via Chair's Action.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

Conditions of the favourable opinion

Approval is dependent on local ethical approval having been received, where relevant.

Approved documents

The final list of documents reviewed and approved is as follows:

Document Type	File Name	Date	Version
Investigator CV	GibrilNdow_CV_August2019	22/12/2019	1.0
Investigator CV	CV Maud LEMOINE Oct 2019	22/12/2019	1.0
Protocol / Proposal	PP Award Gibril	22/12/2019	1.0
Protocol / Proposal	Abbott LOA HBV PMTCT Grant fully executed	14/01/2020	1.0
Covering Letter	Cover%20Letter_SCC-Response_19099_V1.0	14/01/2020	1.0
Covering Letter	Cover%20Letter_SCC-Response_19099_V2.0	23/01/2020	2.0
Protocol / Proposal	INFANT B_EnrolmentForm_V1.2	23/01/2020	1.2
Protocol / Proposal	INFANT B_Gambia_Final_V1.2	23/01/2020	1.2
Other	INFANT B_InfoSheet_V1.2_23rdJan2020	23/01/2020	1.2
Information Sheet	INFANT B_InfoSheet_V1.2_23rdJan2020	23/01/2020	1.2
Information Sheet	INFANT B_Consent_V1.2_23rdJan2020	23/01/2020	1.2
Protocol / Proposal	INFANT B timelines	26/06/2020	2.0
Covering Letter	EthicsResponse_19099	19/07/2020	1.0

After ethical review

The Chief Investigator (CI) or delegate is responsible for informing the ethics committee of any subsequent changes to the application. These must be submitted to the committee for review using an Amendment form. Amendments must not be initiated before receipt of written favourable opinion from the committee.

The CI or delegate is also required to notify the ethics committee of any protocol violations and/or Suspected Unexpected Serious Adverse Reactions (SUSARs) which occur during the project by submitting a Serious Adverse Event form.

An annual report should be submitted to the committee using an Annual Report form on the anniversary of the approval of the study during the lifetime of the study.

At the end of the study, the CI or delegate must notify the committee using the End of Study form.

All aforementioned forms are available on the ethics online applications website and can only be submitted to the committee via the website at: <http://leo.lshtm.ac.uk>.

Further information is available at: www.lshtm.ac.uk/ethics.

Yours sincerely,

8.3.9. INFANT-B: Participant information sheet

PARTICIPANT INFORMATION SHEET

Version 1.2 Date 23rd January 2020

Study Title: **How to end mother-to-child transmission of hepatitis B – the Intervention For Avoidance of Neonatal Transmission of Hepatitis B in Africa (INFANT-B) study**

SCC/Protocol No:

Sponsor & Funder: MRCG at LSHTM (West Africa Platform) and Abbott Charity

What is informed consent?

You are invited to take part in a research study. Participating in a research study is not the same as getting regular medical care. The purpose of normal medical care is to improve one's health. The purpose of a research study is to gather information that may be useful in the future for the whole population. It is your decision to take part and you can stop at any time without giving any reason.

Before you decide you need to understand why the study is being done and what will happen in it. Please take time to read the following information or get the information explained to you in your language. Listen carefully. You can ask questions if there is anything that you do not understand. Ask for it to be explained until you are satisfied. You may also wish to speak your spouse, family members or others before deciding to take part in the study.

If you decide to join the study, you will need to sign or thumbprint a consent form saying you agree to be in the study. You will receive a copy of the consent form.

Why is this study being done?

Hepatitis B virus (HBV) is very common in West Africa. In The Gambia, approximately 1 in every 10 adults is chronically infected with the virus. This virus can be passed in blood, from mother to child at birth, in early life from child to child with close contact, or through unprotected sex with an infected partner. For the last 25 years, all children born in The Gambia are offered vaccination against HBV. Receiving this hepatitis B vaccine at birth, as recommended by the World Health Organisation, reduces risk of chronic hepatitis B infection by 70%.

Presently, pregnant women in The Gambia are not routinely tested for hepatitis B. In addition, the test to measure the amount of hepatitis B virus in a patient's blood is expensive and requires laboratories, equipment and expertise that is not routinely available. This study therefore is being conducted in order to assess whether hepatitis B testing can be easily added to the current package of care offered to pregnant women attending antenatal clinics in The Gambia. We will test the acceptability of hepatitis B testing by pregnant women and find out what proportion of women have very high hepatitis B viral load and are therefore at higher risk of transmitting the virus to their babies. We will also look for alternative tests that can identify pregnant women with high viral load without relying on the current test for viral load which is not widely available.

We will tell the results of this study to your community.

Version 1.2 Date 23rd January 2020

Page 1 of 3

What does this study involve?

In this study, we will enrol 2,000 pregnant women attending antenatal clinic in The Gambia. Eligible women will be counselled on hepatitis B infection, consented to participate in this study, and then tested for hepatitis B using a rapid hepatitis B surface antigen point-of-care test kit. This rapid test uses blood from a fingerpick, is done in the clinic, with results available within 15 minutes.

All women who test positive for hepatitis B will be advised to give birth at a health facility where their can receive the HBV vaccine soon after birth and will be linked to the MRCG Liver Clinic for further clinical assessment after delivery. We will also collect 2mL of blood, which is less than the volume of half a teaspoon, from HBSAg-positive mothers. This blood will be collected in a small tube as well as on a special type of paper called a dried blood spot card. The blood will be used to measure hepatitis B viral load and to test for simpler markers of hepatitis B.

HBSAg-positive women will also be invited come with their babies to the MRCG liver clinic 6 months after delivery. During this visit, we will review babies' vaccination records to assess the time of receipt of the first HBV birth dose vaccine and therefore the coverage of HBV birth dose vaccination in this cohort.

If we find out that you are sick and cannot join the study, you will receive the care routinely available in The Gambia. You may be treated at the study site and if necessary, you will be referred to a health facility that can manage the condition better.

If the research study needs to be stopped for any reason, we will tell you and you will have normal medical care if you need it.

What will happen to the samples taken in this study?

We will collect less than half a teaspoon (2mL) of blood from your arm. We will use this blood to measure the amount of hepatitis B virus in your blood, and to test for other simpler markers of hepatitis B called HBSAg and HBcAg. We anticipate that these markers may be accurate alternatives to hepatitis B viral load and can therefore be used in future to identify women with high viral load. All these tests, except HBcAg, will be conducted at the MRC Gambia laboratories in Fajara. The HBcAg test is not currently available in The Gambia, and therefore this test will be conducted at the Institut Pasteur laboratory in Dakar, Senegal.

What harm or discomfort can you expect in the study?

The HBSAg testing is expected to last approximately 15 minutes. If you are HBSAg positive, you will be asked to stay for additional counselling and blood draw. These will last an extra 30 minutes. The study coordinators will endeavour to make your visit as short as possible. In order to collect blood sample, we will make a small injection in your arm, which may be painful. In a few individuals, bleeding from this injection site may continue for a while. There is almost no risk of severe bleeding.

Version 1.2 Date 23rd January 2020

Page 2 of 3

What benefits can you expect in the study?

You will be tested for HBSAg and if positive will receive an assessment to check the status of your liver and your hepatitis B infection. Some of the laboratory tests that we will do for you in this study are not otherwise available in routine clinics in The Gambia.

Will you be compensated for participating in the study?

You will not get paid by the study, but MRC will provide transport or give you back the money for your transport.

What happens if you refuse to participate in the study or change your mind later?

You are free to join the study or not and you are free to stop being in the study any time without giving a reason. You will still get the normal medical care.

If you do not want to continue in the study, we will use only the samples and information already collected from you.

If we find new information during the study that may change if you can still be in the study, we will inform you as soon as possible.

How will personal records remain confidential and who will have access to it?

All information that is collected about you in the study will be kept strictly confidential. Your personal information will only be seen by the study team members, the sponsor and if necessary, the Ethics Committee and Government authorities.

Who should you contact if you have questions?

If you have any questions or are worried you can call Dr Gibril Ndow or Rohey Bangura on 2717102 and you can always call the personal numbers of the MRC workers given to you.

Please feel free to ask any question you might have about the study.

Who has reviewed this study?

This study has been checked by scientists at the Medical Research Council and by the Gambia Government/MRC Joint Ethics Committee. The Ethics Committee protects your rights and wellbeing and has given permission for it to take place.

8.3.10. Wellcome Trust fellowship award letter



E r.brooks@wellcome.org
Our Ref: 224366/Z/21/Z
22nd December 2021

Private and Confidential

Prof Umberto D'Alessandro
London School of Hygiene and Tropical Medicine
Medical Research Council Unit The Gambia
Atlantic Boulevard
Fajara
Gambia

Dear Prof D'Alessandro

International Training Fellowship, 'Metabolic phenotypes, cellular pathways and mechanisms of persistence in chronic hepatitis B infection'

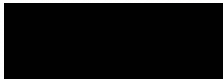
I am writing to confirm that Wellcome has agreed to award London School of Hygiene & Tropical Medicine a grant of up to £310,290 over 36 months to provide a Wellcome International Training Fellowship for Dr Gibril Ndow. The proposed start date is 01 January 2022.

Further details of how to manage your award are attached, including

- the financial composition of the award
- how to start the award
- how to claim the funds
- the grant conditions
- what we expect of Wellcome award holders.

If you need any further information or assistance, please do not hesitate to contact me.

Yours sincerely



Rosie Brooks
Funding Manager
Research Funding

cc: Dr Gibril Ndow

9. References

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