Hepatitis B and C in Malawi: Epidemiology, Disease Burden and Opportunities for a Public Health Treatment Programme

Thesis submitted in accordance with the requirements of the

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Declaration of Authorship

This thesis is the result of my own work except where explicitly stated otherwise in the text. The contributions of my co-workers and collaborators in this project are described below.

Professor Anna Maria Geretti, University of Liverpool, United Kingdom (UK) Professor Melita A Gordon, University of Liverpool and Malawi-Liverpool-Wellcome Trust Clinical Research Programme (MLW), Malawi Professor Dean Everett, University of Edinburgh, UK	Doctor of Philosophy supervisors. Provided advice on the design, conduct, analysis and interpretation of the study.
The STRATAA (Strategic Typhoid alliance across Africa and Asia) study team in Malawi including: Prof Melita Gordon, Dr James Meiring, Maurice Mbewe, Deus Thindwa, Dr Pratiksha Patel & Dr Priyanka Patel (MLW)	Conducted the demographic census of Ndirande, and the community serological survey, allowing testing for markers of hepatitis B and C infection in serosurvey participants.
Dr Marc Henrion, MLW, Liverpool School of Tropical Medicine, Liverpool, UK Dr Jonathan Read, University of Lancaster, UK Dr Naor Bar-Zeev, Johns-Hopkins Bloomberg School of Public Health, Baltimore, MD, USA	Provided advice on statistical and epidemiological analyses.
Nyasha Gutukunuwha & Rose Sikwese (Study Nurses, MLW) Blessings Mbale, Ivyn Kusakala & Pamela Kamanga (Study Fieldworkers, MLW)	Responsible for screening and recruitment of patients, administration of study questionnaires and study procedures, collection of clinical samples and follow up of participants.
Maurice Mbewe, Yohane Kadzuwa Diness, Happy Chimphako Banda & Mike Mautanga (Laboratory Technicians, MLW)	Responsible for the preparation and storage of clinical samples for this study.
Dr Isaac Shawa, College of Medicine, Malawi Niza Silungwe, MLW	Assistance with laboratory work including enzyme immunosorbent assays (ELISAs) and polymerase chain reaction (PCR).
Dr Collins Mitambo & Dr Rabson Kachala Malawi Ministry of Health, Lilongwe, Malawi	Assistance with the conduct of the systematic review, quality assessment of the included articles (CM) and provision of epidemiological data
Dr Karen Chetcuti, MLW Dr Elizabeth Joekes, Royal Liverpool Hospital, Liverpool, UK	Training on ultrasound and assistance with advice on all aspects of ultrasound image interpretation and development of radiological protocols.
Professor Emma Thomson, Dr Ana Filipe da Silva, Dr Chris Davis, Dr Rajiv Shah, Dr Lily Tong and Dr Josh Singer, Centre for Virus Research, University of Glasgow, UK	Conducted next generation sequencing of hepatitis C from participants in this study and provided advice on bioinformatic analysis.
Dr Benno Kreuels (College of Medicine, Malawi and University of Hamburg, Germany)	Provided advice on the conduct of the inpatient study and analyses for the aetiology of cirrhosis and HCC.

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Kupha mkango ndi kusamala

To kill a lion you have to be gentle and cunning

Chichewa Proverb

Publications, Presentations and Impact Arising from Thesis

Peer reviewed journal articles

Stockdale AJ, Mitambo C, Everett D, Geretti AM, Gordon MA. Epidemiology of hepatitis B, C and D in Malawi: systematic review. **BMC Infect Dis**. 2018 Oct 12;18(1):516

Oral Presentations

"Epidemiology of hepatitis B and evaluation of vaccine efficacy in a census-based community serosurvey of Ndirande township in Blantyre, Malawi"
1st Conference on Liver Disease in Africa (COLDA), Nairobi, Kenya. 12th September 2018

Invited Speaker: "Global Epidemiology of Hepatitis D 1998-2018"
Hepatitis Delta International Network, European Association for the Study of the Liver Meeting, Vienna, Austria. 10th April 2019

Invited Speaker: "Forgotten, not gone: Hepatitis Delta in the World"
Viruses, Vaccines and Eradication, QEII Centre, London. 6th June 2019

"Aetiology and outcomes of cirrhosis and hepatocellular carcinoma (HCC) in Queen Elizabeth Central Hospital (QECH), Blantyre, Malawi"

"Performance of hepatitis B e antigen (HBeAg) rapid diagnostic tests (RDTs) in Malawi: A comparative evaluation in community and inpatient populations with hepatitis B virus (HBV) infection"

"Seroprevalence of hepatitis C in Malawi: A cross-sectional community census-based serological survey of Ndriande, Blantyre"

Research Dissemination Conference, Malawi College of Medicine, Blantyre, Malawi 8th November 2019

Poster

"Epidemiology of hepatitis B and evaluation of vaccine efficacy in a census-based community serosurvey of Ndirande township in Blantyre, Malawi"

11th European Congress on Tropical Medicine and International Health. Liverpool, United Kingdom 16th September 2019

Policy Contributions

Epidemiological data gathered as part of this PhD project were cited in the National Strategic Plan for Viral Hepatitis from the Ministry of Health of Malawi including the systematic review of existing evidence, and results from the community serological survey and inpatient study.

I assisted the Viral Hepatitis Strategy Group at the Malawi Ministry of Health with development of the National Strategic Plan for Viral Hepatitis, contributing to epidemiological reports and to formulation of treatment eligibility criteria.

Science Communication

Results of this study were communicated to community leaders and chiefs of Ndirande in December 2019.

The 1st Conference on Liver Disease in Malawi was held in Blantyre in November 2018. The results of the hepatitis B community seroprevalence study were disseminated at the conference to delegates from across Malawi and reported in national radio and national newspapers including the Nation, Malawi Broadcasting Corporation Radio and Zodiak Radio.

On 19th November 2019, we organised the 2nd Annual Conference on Liver Disease in Malawi, in Lilongwe. The conference was attended by 100 delegates including representatives from the Ministry of Health Viral Hepatitis Strategy Unit, Non-Governmental Organisations, representatives of the charitable and Private Sector health service providers and colleagues from throughout Malawi interested in viral hepatitis. At the conference, I presented the results of this thesis relating to the epidemiology of viral hepatitis in Malawi. These conferences were funded by the British Society of Gastroenterology and the World Gastroenterology Organisation.

Hepatitis B and C in Malawi: Epidemiology, Disease Burden and Opportunities for a Public Health Treatment Programme

Alexander Stockdale

Abstract

In sub-Saharan Africa, hepatitis B virus (HBV) infection is the principal cause of liver cirrhosis and hepatocellular carcinoma (HCC). Mortality from cirrhosis and HCC is projected to rise beyond 2030 unless adult HBV treatment programmes are implemented. Infant HBV vaccination was introduced across sub-Saharan Africa between 1994-2014, and in Malawi in 2002 where it is given at 6, 10 and 14 weeks of life. Hepatitis C virus (HCV) is an important contributor to liver disease globally, with an estimated population prevalence of 1% in sub-Saharan Africa. In Southern Africa there is a paucity of HCV prevalence data, with no previous random probability-sampling community studies.

A hospital-based study of cirrhosis and HCC in a tertiary hospital, and seroprevalence studies in an urban township, were conducted in Blantyre, Malawi, to determine HBV and HCV prevalence and HBV vaccine impact. Of 97,386 censused individuals, single stage non-replacement age-stratified probability sampling was used to select 6,073 individuals who were tested for hepatitis B surface antigen (HBsAg) in a community serosurvey. HBsAg-positive individuals aged ≥16 were recruited to assess treatment eligibility. Among individuals aged ≥16 in the serosurvey, 1661 (51%) were randomly selected for HCV antigen/antibody (Ag/Ab) testing with confirmatory HCV RNA PCR. Prevalence estimates were standardised to census age and sex distribution using post-stratification proportional fitting.

In the hospital study, the population attributable fraction (PAF) of HBV to cirrhosis and HCC was 23.1% (95% CI 15.7- 29.8) and 71.5% (59.3- 80.1) respectively among 250 consecutively recruited patients. For HCV the PAF was 1.6% (95% CI -0.4 – 3.6) for cirrhosis and 4.8% (-0.1, 9.5) for HCC. Patients with HCC were diagnosed at an advanced stage with a median tumour size of 12.6cm and a median survival of 1.3 months. Six-month survival was 67% (59.0- 73.8) among patients with cirrhosis.

Standardised HBsAg prevalence in serosurvey participants born prior to, and after HBV vaccine introduction, was 5.1% (95% CI 4.3- 6.1) and 0.3% (95% CI 0.1- 0.6) respectively. Three-dose vaccination coverage was 97.4% (1141/1171) among 1171/2085 children aged ≤10 years with known vaccine status. By comparison of participants born 5 years before and after vaccine introduction, vaccine impact was 95.9% (95% CI 70.6- 99.4). Treatment eligibility was assessed in 94/150 HBsAg

positive people aged ≥16 years from the serosurvey, of whom 24/93 (26%) were HIV positive, and 16/24 (67%) were receiving antiretroviral therapy containing tenofovir, with HBV DNA suppression. Among 69 HIV-negative HBsAg positive individuals, 3,6 and 9% were eligible for HBV treatment by WHO, EASL and AASLD criteria respectively.

Standardised HCV Ag/Ab prevalence was 0.78% (95% CI 0.46- 1.33) and HCV RNA prevalence was 0.18% (95% CI 0.06- 0.53). HCV Ag/Ab positive individuals were older than the general population but no differences in sex, educational, employment or marital status were observed.

In an urban township in Malawi, HBV prevalence was intermediate at 5.1% among unvaccinated adults. Infant HBV vaccination was associated with a vaccine impact of 96%. Among HBsAg-positive adults, one quarter were HIV-positive and 3-9% of HIV-negative adults were eligible for antiviral therapy. Estimated population HCV RNA prevalence was 0.2%. Future prevalence studies should sample rural communities and specific risk groups. HCC is diagnosed at an advanced stage with a poor prognosis in Malawi, and HBV is an important cause. The burden of HBV and HCV associated liver disease represents both a challenge, and an opportunity to implement public health treatment programmes to reverse rising liver-related mortality in Southern Africa.

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Abbreviations

AASLD	American Association for the Study of Liver Disease
AFB_1	Aflatoxin B1
AFP	Alfa fetoprotein
Ag/Ab	Antigen/antibody
Anti-HBc	Antibody against hepatitis B core antigen
Anti-HBe	Antibody against hepatitis B e antigen
Anti-HCV	Antibody against hepatitis C
Anti-HDV	Antibody against hepatitis D
ALT	Alanine aminotransferase
ALP	Alkaline phosphatase
APRI	Aspartate aminotransferase (AST) to platelet ratio index
ART	Antiretroviral therapy
AST	Aspartate aminotransferase
AUROC	Area under the receiver operating curve
CCA	Circulating cathodic antigen (schistosomiasis)
cccDNA	Covalently closed circular deoxyribonucleic acid
CD	Cluster of differentiation
CE	Conformité Européenne
CI	Confidence interval
CLD	Chronic liver disease
CLIA	Chemiluminescent immunoassay
CSW	Commercial sex worker
CT	Computed tomography
CTC	Controlled temperature chain
DAA	Directly acting antiviral
DBS	Dried blood spot
DNA	Deoxyribonucleic acid
EASL	European Association for Study of the Liver
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme linked immunosorbent assay
FBC	Full blood count
FDA	Food and drug administration

GAVI	Global Vaccine Alliance
GGT	Gamma-glutamyl transferase
GPR	Gamma-glutamyl transferase to platelet ratio
GPS	Global positioning satellite
HBcAg	Hepatitis B core protein (antigen)
HBsAg	Hepatitis B surface antigen
HBeAg	Hepatitis B e antigen
HBIG	Hepatitis B immune globulin
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HCVcAg	Hepatitis C core antigen
HDV	Hepatitis D virus
HEV	Hepatitis E virus
HIV	Human immunodeficiency virus
HSPG	Heparin sulphate proteoglycans
IC	Immunochromographic
IL	Interleukin
IQR	Interquartile range
IT	Immune tolerant
IVC	Inferior vena cava
IVMD	In-vitro medical device
JAK-STAT	Janus Kinase-signal transducers and activators of transcription pathways
LFT	Liver function test
LLD	Lower limit of detection
LLQ	Lower limit of quantification
LSM	Liver stiffness measurement
MAVS	Mitochondrial antiviral signalling protein
MoH	Ministry of Health
MLW	Malawi-Liverpool-Wellcome Trust Clinical Research Programme
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
MSM	Men who have sex with men
MTCT	Mother-to-child transmission

NA	Nucleos(t)ide analogue
NAFLD	Non-alcohol fatty liver disease
NS	Non structural protein (hepatitis C)
NTCP	Sodium taurocholate co-transporting polypeptide
ODK	Open Data Kit
OR	Odds ratio
OP	Outpatient
Peg-IFNα	Pegylated interferon alpha
pgRNA	Pre-genomic ribonucleic acid
PCR	Polymerase chain reaction
PWID	People who inject drugs
qPCR	Quantitative polymerase chain reaction
QECH	Queen Elizabeth Central Hospital
RAS	Resistance associated substitution
rc-DNA	Relaxed circular DNA
RCT	Randomised controlled trial
RDT	Rapid diagnostic test
RIG	Retinoic acid inducible gene-I
RNA	Ribonucleic acid
RR	Relative risk
RT	Reverse transcriptase
RUQ	Right upper quadrant
S/CO	Sample to cut-off ratio
SES	Socioeconomic status
siRNA	Small interfering ribonucleic acid
SMC	Structural maintenance of chromosome
STRATAA	Strategic Typhoid alliance across Africa and Asia
SVP	Sub-viral particle
SVR(12)	Sustained virological response (12 weeks after completion of treatment)
TAF	Tenofovir alafenamide
TB	Tuberculosis
TDF	Tenofovir disoproxil fumarate
Th	T helper cell
TE	Transient elastography

TLR	Toll like receptor
TRIF	Toll-interluekin-1 receptor domain containing adaptor inducing IFN-β
UGIB	Upper gastrointestinal bleeding
ULN	Upper limit of normal
UNAIDS	Joint United Nations Programme on HIV/AIDS
UNICEF	United National Children's Fund
US	Ultrasound
USD	United States Dollars
VCT	Voluntary counselling and testing (for HIV)
WHO	World Health Organisation

Chapter 1. General Introduction

Summary

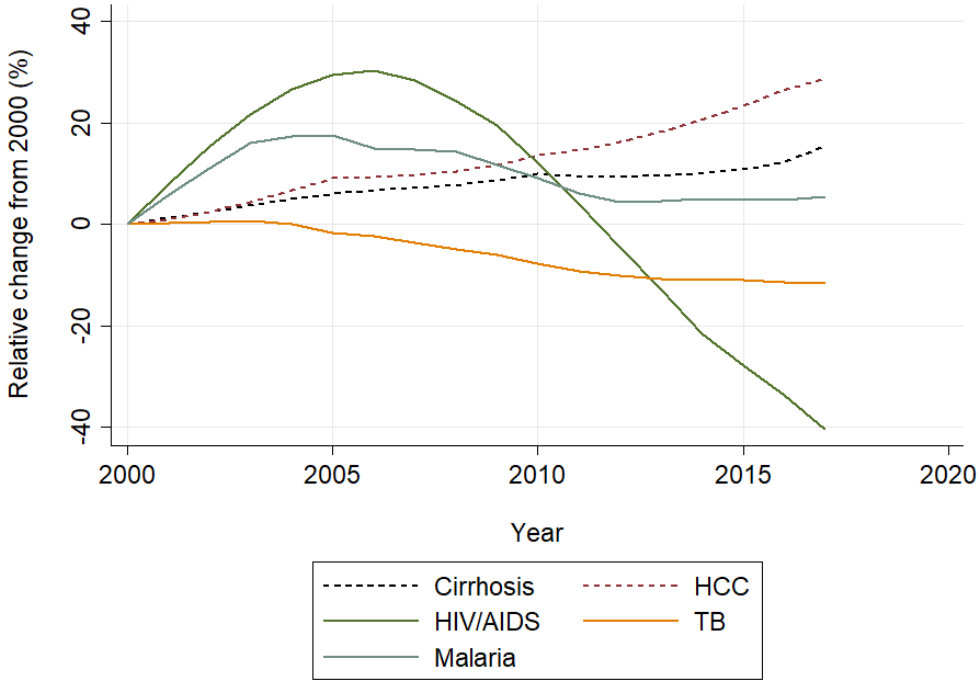
Hepatitis B is the principal cause of cirrhosis and liver cancer in sub-Saharan Africa. The World Health Organisation (WHO) has established ambitious targets for a reduction of 80% of incident cases and 65% reduction in mortality from viral hepatitis, yet deaths from HBV are rising in sub-Saharan Africa and projected to increase beyond 2030 without the implementation of treatment programmes for adults. Hepatitis B is a non-cytopathic virus and disease is a function of chronic intrahepatic inflammation leading to cirrhosis and specific oncogenic mechanisms that cause liver cancer including epigenetic modification of cellular regulation and the carcinogenic effects of HBV DNA integration and oxidative stress. The HBV life cycle involves assembly of intranuclear circular covalently closed DNA (cccDNA) which acts as a transcriptional template for replication and is the basis of HBV persistence. Treatment eligibility is assessed based on evidence of established liver disease or a profile suggestive of active liver inflammation. Current treatment is based on lifelong use of nucleos(t)ide analogues which suppress HBV polymerase but are unable to target cccDNA or prevent cccDNA replenishment. Hepatitis C virus (HCV) represents an important cause of liver disease globally and major transmission routes include nosocomial healthcare associated infection from reuse of injection equipment, injecting drug use and sexual transmission particularly among men who have sex with men. Estimated population prevalence of HCV for sub-Saharan Africa is 1% but there is a paucity of data on prevalence in low bias community samples or among key population groups. In Africa, there is limited availability of diagnosis and treatment services and there is an estimated relative increase in prevalence of 2% per year. There are several important research priorities that could facilitate viral hepatitis disease control in sub-Saharan Africa. These include: improving epidemiological data including enhancing understanding of HBV and HCV transmission, provision of data on efficacy of the HBV vaccine from the region and optimisation of HBV patient treatment selection.

1.1. Introduction

Viral hepatitis was estimated to be responsible for 1.34 million deaths in 2015, exceeding mortality from HIV (1.3 million), malaria (0.9 million) or tuberculosis (1.3 million) (Spearman et al., 2017, World Health Organisation, 2017a). In sub-Saharan Africa, mortality from viral hepatitis is increasing (Figure 1-1). The hepatitis B virus (HBV) is one of the most widely distributed infectious diseases with an

estimated 2 billion people having been infected, and 257 million people chronically infected, amounting to 3.5% of the global population (World Health Organisation, 2017a, Trepo et al., 2014). An estimated 60 million people are living with chronic HBV in the WHO Africa region, a prevalence of 6.1% (95% confidence interval (CI) 4.6-8.5) (World Health Organisation, 2017a). Hepatitis C is less widely distributed in the WHO Africa region, with an estimated 11 million people infected, amounting to 1.0% (95% CI 0.7, 1.6).

Figure 1-1: Cause of adult mortality in sub-Saharan Africa, relative change since 2000



Data source: (Global Burden of Disease Collaborative Network, 2018) Adult mortality defined as deaths among individuals aged ≥15 years.

In 2016, the World Health Assembly agreed a strategic plan for viral hepatitis control with ambitious targets to reduce the incidence of chronic hepatitis B and C by 90% and mortality by 65% by 2030 (World Health Organisation, 2016a). Yet deaths from cirrhosis and hepatocellular carcinoma will continue to increase beyond 2030 in the absence of additional disease control interventions (Nayagam et al., 2016b). A mathematical modelling study estimated that use of strategies for the prevention of infant infection, comprising HBV vaccination or antiviral therapy for infected mothers, without introducing HBV treatment programmes for adults, deaths from HBV were estimated to continue to

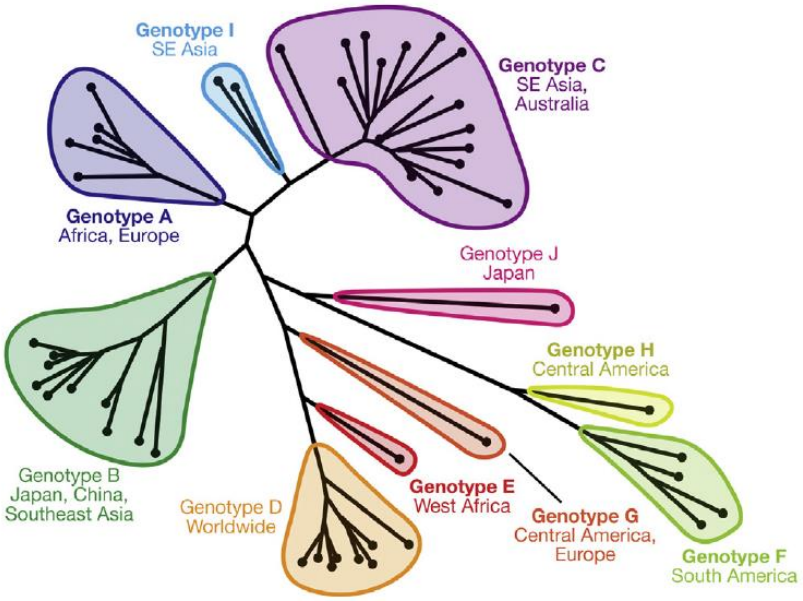
rise to a peak of 2040 and would not be eliminated in sub-Saharan Africa by 2100 (Nayagam et al., 2016b).

1.2. Hepatitis B

1.2.1 Structure and genome

The hepatitis B virus (HBV), a member of the family *hepadnaviridae*, is a small spherical virus of 42nm with icosahedral symmetry (Trepo et al., 2014). It has a compact partially double stranded circular DNA genome of approximately 3200 nucleotides, with length varying by genotype (McNaughton et al., 2019). The 5' negative-sense complete DNA strand is covalently bound to viral polymerase. HBV is divided into nine genotypes A-I, and one additional putative genotype J identified in a single individual based on intergroup sequence diversity of >7.5% (Kramvis, 2018, Rajoriya et al., 2017) (Figure 1-2). There are 35 subgroups defined by numerical subscripts defined by divergence of 4-7.5%, and with distinct geographical distribution (Sunbul, 2014, Tatematsu et al., 2009). Clinical implications of HBV genotype have been observed, manifesting as variation in response to antiviral therapy, progression of disease and risk of development of HCC (Sunbul, 2014).

Figure 1-2: Phylogenetic relationship of HBV genotypes



Reproduced from McNaughton et al., (2019). Colours denote separate genotypes.

It has been postulated that HBV phylogeny mirrors major human migration events, based on evidence from phylogeographic and phylodynamic approaches using genetic and archaeological data from human migration events and molecular clock analyses of diverse HBV genotypes (Paraskevis et al., 2013). Isolated indigenous populations frequently have distinct HBV subgenotypes and estimates of subgenotype divergence match major human migration events such as movement from Africa to Haiti coinciding with the emergence of subgenotype A5, distinct settlements of Polynesian islands (forming subgenotypes C3 and D4) and an ancient emergence of genotype F in South America among the Amerindian indigenous population (Paraskevis et al., 2013). Thus major HBV genotype cladogenesis may mirror human migration, reflecting the close relationship between host and pathogen.

HBV genotypes have a distinct geographical distribution with Genotype A1 in Southern and Eastern Africa and India, genotype E in West Africa, genotype A2 in Europe and North America, B and C in Asia and Oceania, D in North Africa, Europe, the Middle East and Oceania, F in South America and G and H in South and Central America (Figure 1-3) (Rajoriya et al., 2017).

Figure 1-3: Geographic distribution of HBV genotypes

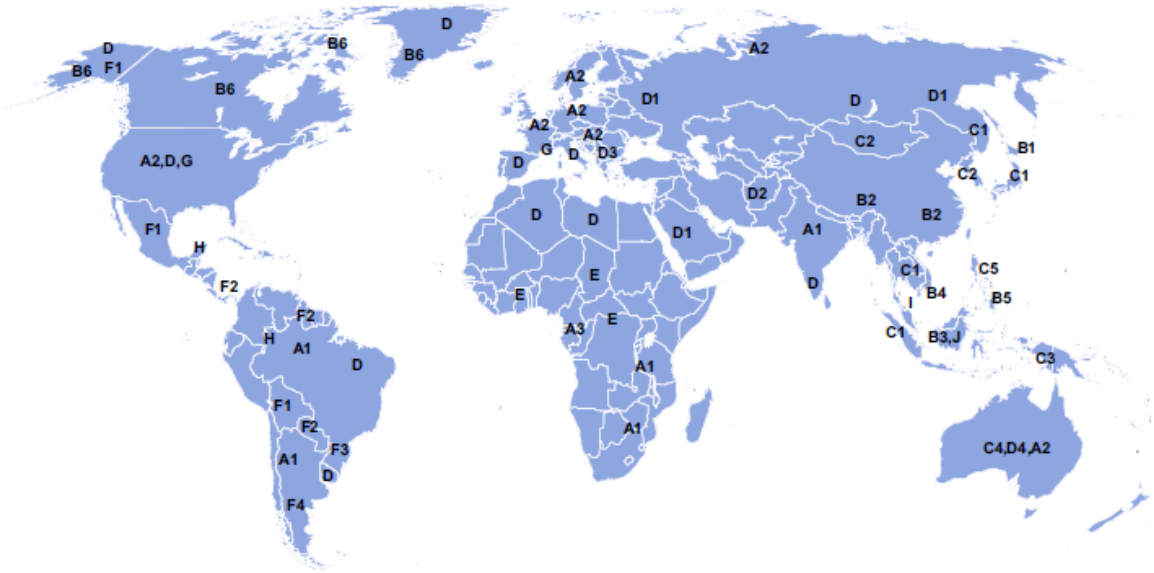
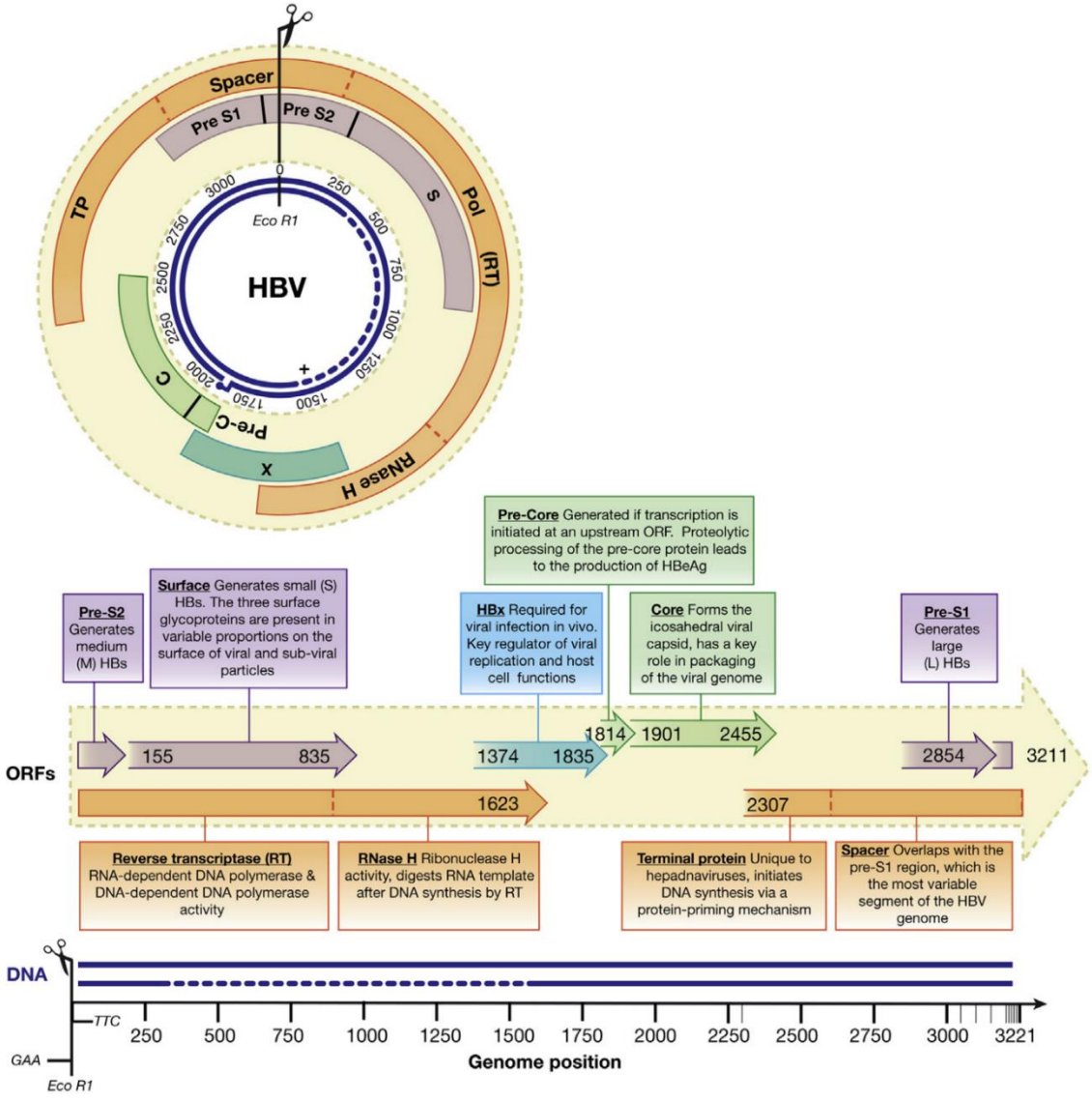


Figure reproduced from Rajoriya et al., (2017)

The HBV genome has four overlapping reading frames (ORFs) that comprise four genes encoding seven proteins: polymerase (P), surface (S/preS1/preS2), X gene and core (C) (Figure 1-4) (Seeger and Mason, 2015). The structure results in a highly information-dense genome, with almost two thirds of the genome responsible for encoding more than one functional element (McNaughton et al., 2019, Pavesi,

2015). The polymerase gene encodes for reverse transcriptase which is a virus-derived enzyme responsible for transcription from pregenomic RNA (pgRNA) into DNA, a critical element of the viral life cycle. The surface gene encodes three proteins of variable length, the small, medium and large surface antigens, respectively encoded by S, S/preS2 and S/preS1/preS2 (Urban et al., 2010). These three proteins, together with lipids, form the viral envelope (Figure 1-5).

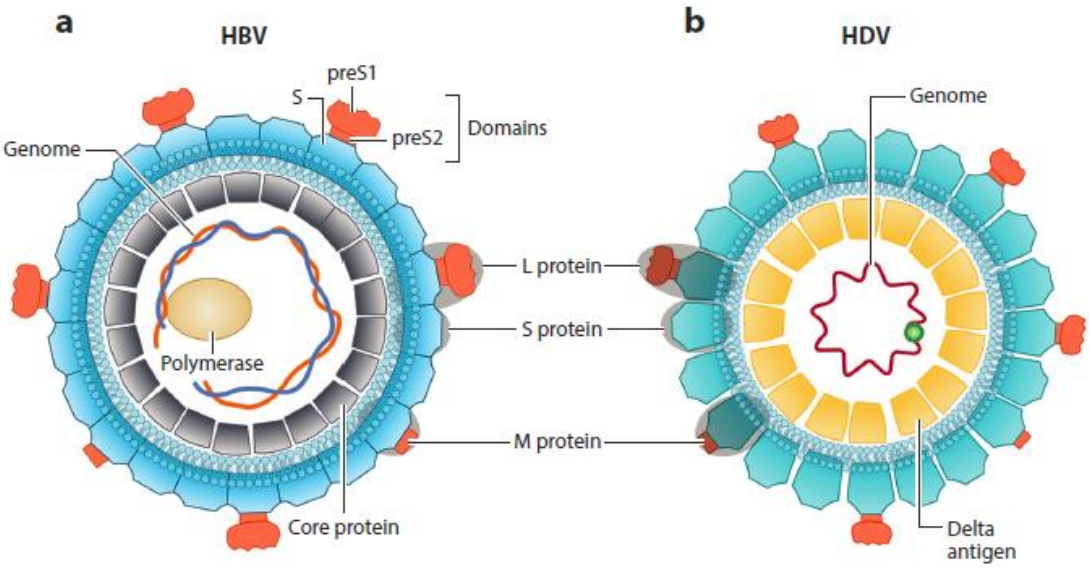
Figure 1-4: Structure and function of HBV genome



Reproduced from McNaughton et al 2019

The presence of surface antigen (HBsAg) is the hallmark of HBV infection, the principal target for diagnostic assays and the basis of the HBV vaccine. HBV polymerase and surface genes overlap by over 1000 nucleotides, which is the largest known overlap of any animal virus (Pavesi, 2015). The core gene is responsible for the production of the viral nucleocapsid subunit (the core protein) and for e antigen (HBeAg). The nucleocapsid is an icosahedral shell comprised of 120 dimers of the core protein (HBcAg) (Nassal, 2015). The HBeAg, a 25kDa soluble protein, is an immunomodulatory protein, translated from pre-core/C open reading frame and converted to HBeAg by post-translational modification (Kramvis et al., 2018). It causes downregulation of the immune response, acting as a T cell tolerogen, attenuating the adaptive response, favouring Th2 over Th1 activation (Milich and Liang, 2003) and downregulating toll-like receptor expression (Visvanathan et al., 2007). The regulatory X protein is responsible for activation of gene expression from cccDNA, through degradation of structural maintenance of chromosome (SMC) complex proteins which restrict HBV cccDNA replication (Murphy et al., 2016, Decorsiere et al., 2016, van Breugel et al., 2012).

Figure 1-5: Diagram of hepatitis B (panel a) and D (panel b) viral structure



HBsAg can exist in several different forms. Aside from infectious virions (termed “Dane particles”), non-infectious sub-viral particles (SVPs) measuring 22nm are also produced with long filamentous or spherical forms (Cao et al., 2019). SVPs are produced in great excess over infectious virions of a magnitude exceeding 10,000 times, with the spherical form exceeding filamentous SVPs (Cornberg et al., 2017). Spherical SVPs, consisting of S and M HBsAg proteins, are excreted by the Golgi secretory

pathway whereas filamentous SVPs, comprised of S and L proteins, and infectious virions are excreted via the multivesicular body complex (Caballero et al., 2018). The role of SVPs is postulated to be to induce immune tolerance and persistence of chronic infection or to reduce non-productive binding of virions to HSPGs (Cao et al., 2019, Leistner et al., 2008).

1.2.2 Replication

HBV is an hepatotropic virus and, like the satellite virus hepatitis D (HDV) , enters hepatocytes via the systemic circulation through a specialised hepatocyte receptor, sodium taurocholate cotransporting polypeptide (NTCP) (Eller et al., 2018). NTCP acts as a transporter for bile acids, steroid sulfate conjugates and drug conjugates (Li, 2015). The preS1 domain of HBsAg binds to NTCP. Heparin sulphate proteoglycans (HSPG) are essential co-factors for viral entry causing low-affinity binding. HSPGs are present in peri-sinusoical space of the liver and facilitate attachment to NTCP through enrichment of virions at the hepatocyte surface (Eller et al., 2018). The epidermal growth factor receptor (EGFR) has recently been described as an host cofactor that is crucial in HBV-NTCP host cell internalisation (Iwamoto et al., 2019b). After binding to NTCP, the virus enters hepatocytes with a relaxed circular DNA (rc-DNA) confirmation, with the DNA genome bound to viral protease.

After entering the hepatocyte nucleus, the viral genome can be integrated into the host genome, or repaired by host DNA repair mechanisms to complete the partial strand, resulting in circular covalently closed (cccDNA) following a covalent ligation of completed DNA strands (Seeger and Mason, 2015). CccDNA is packaged as a minichromosome together with host histones, in a stable chromatin-like structure, thus promoting indefinite persistence within the nucleus (Urban et al., 2010). cccDNA acts as a transcriptional template for viral replication within the nucleus and as a reservoir of viral infection and is the basis for HBV persistence (Tang et al., 2018). CccDNA transcription is epigenetically regulated.

Host RNA polymersase II transcribes the pre-genomic RNA (pgRNA) intermediate and produces four functional mRNA transcripts: a 3.5kb transcript endcoing precore RNA which includes the full genome length, two 2.4 and 2.1kb subgenomic transcripts which encode for pre-S and S proteins, and a 0.7 kb m transcript encoding the X protein (Seeger and Mason, 2015). PgRNA is also incorporated into a nucleocapsid where it undergoes reverse transcription using virus-derived polymerase to produce DNA containing nucelocapsids. First pgRNA is converted into single stranded minus stand DNA, then following cleavage by RNAse H, into rc-DNA (Hu et al., 2019). Nucleocapsids containing rc-DNA may be re-imported into the nucleus to provide replenishment of cccDNA or enveloped and exported for

secretion through the endoplasmic reticulum and Golgi (Karayiannis, 2017). Hepatitis B surface proteins may be exported as infectious replication-competent virions (Dane particles measuring 42nm) or as smaller sub-viral particles (20nm). Sub-viral particles, which lack a nucleocapsid, outnumber infectious virions by a magnitude of 10^3- 10^6 (Urban et al., 2010). The HBV viral population comprises both infectious particles, empty viral envelopes without genomes and virion like particles including HBV pgRNA (Butler et al., 2018).

Though HBV encodes polymerase acting as reverse transcriptase, HBV polymerase is distinct from retroviruses in several respects: there are no polyproteins, such that viral proteases are neither required nor encoded and all proteins have their own specific mRNA with the exception of core and pol, which comprise the preCore RNA (Seeger and Mason, 2015). Most of the steps involved in cccDNA synthesis are the result of host-derived DNA repair mechanisms and the DNA synthesis activity of viral polymerase is not essential for cccDNA formation (Hu et al., 2019). This aspect of the HBV replication life cycle has important implications for HBV therapy based on inhibition of viral polymerase since cccDNA persists even during long-term therapy.

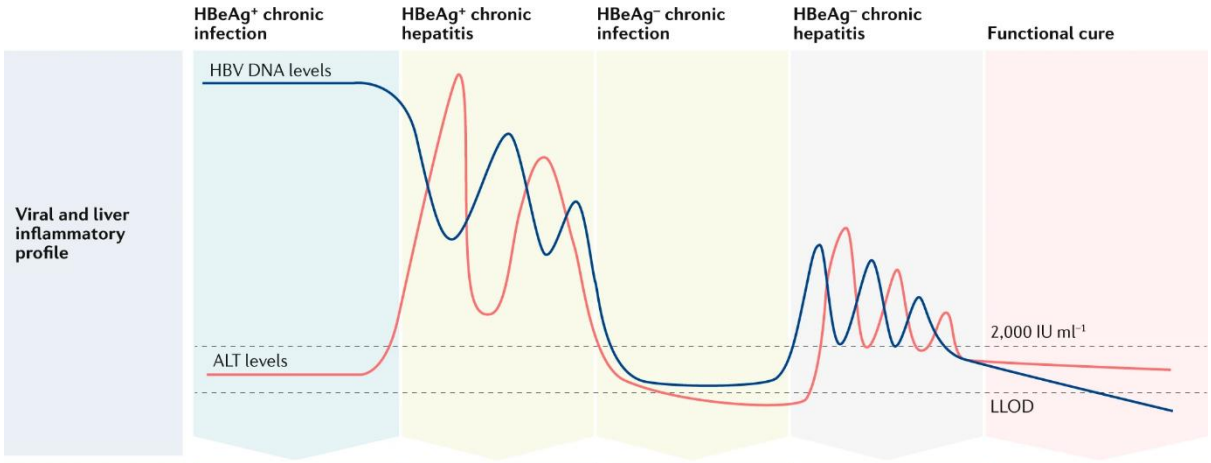
1.2.3 Immunopathogenesis and natural history

Hepatitis B is a non-cytopathic virus. As such, clinical manifestations of HBV-associated disease are effected by immunological interaction with HBV (Vanwolleghem et al., 2015, Chang et al., 2012). In immunocompetent adults exposed to HBV, an efficient humoral response occurs with development of neutralising anti-HBs accompanied by a robust T cell response from CD4 and CD8 cells. Neonates exposed to HBV in around 90% of cases will progress to chronic HBV with persistence of HBsAg, whereas chronic HBV infection occurs in 20-40% of young children and only 5-10% of adults (Chen and Yang, 2011). The reason for HBV persistence in neonatal infection has not been fully elucidated. Suggestions of immunotolerance among neonates exposed to HBV antigens in utero have been based on transgenic animal models among hosts that do not naturally support HBV infection (Hong and Bertoletti, 2017). In studies of HBV-exposed HBsAg-negative infants, functional T cell responses to HBV vaccination were observed, likely as a result of prior intrauterine or perinatal exposure to HBV antigens (Koumbi et al., 2010). HBV-exposed infants have been shown to have a Th1-like cytokine response, suggestive of immunological “training” from perinatal HBV exposure (Hong and Bertoletti, 2017).

Chronic HBV infection is considered to be established partly as a result of an immunologically tolerogenic liver environment with intrahepatic leukocyte populations continually exposed to large numbers of bacterial antigens (Boeijen et al., 2017). HBV replication also facilitates chronic infection

through tolerogenesis, with a large excess of HBV antigens, particularly HBeAg and subviral particles expressing HBsAg, leading to deletion and functional impairment of HBV-specific T and B cells (Fanning et al., 2019). During HBV replication, viral RNA or RNA-DNA hybrids are contained within the host nucleocapsid and do not elicit innate immune responses (Suslov et al., 2018). An analysis of intrahepatic gene expression among chronic HBV patients showed global impairment of interferon type I/III responses, antiviral effectors and Toll-like and pathogen recognition receptors relative to HBV negative control participants indicating innate immune suppressive effects of HBV infection (Lebosse et al., 2017).

Figure 1-6: Phases of HBV infection



Reproduced from Fanning et al 2019

Clinical manifestations of HBV disease vary considerably between individuals. Five main phases of HBV disease have been described in the natural history of HBV infection (Figure 1-6) (European Association for the Study of the Liver, 2017).

The first phase usually occurring after infection, referred to as immune tolerant (IT)/ chronic HBeAg infection, is characterised by high levels of HBV DNA, the expression of HBeAg and normal alanine transaminase (ALT). ALT is been used as a marker of hepatocyte inflammation as it is released by damaged hepatocytes into the extracellular compartment. Patients can remain in this phase for decades (Bertoletti and Kennedy, 2015). This is followed by the HBeAg positive immune reactive phase where HBV DNA is lower with fluctuations in ALT and HBV DNA. The immune active/HBeAg-positive chronic hepatitis phase has been associated with increased progression to fibrosis and increased risk of HCC (European Association for the Study of the Liver, 2017). This progresses to the immune control/inactive carrier (HBeAg negative chronic infection) phase, characterised by low levels of HBV DNA and minimal ALT elevation (Gill and Kennedy, 2014). This may be followed in a subset of patients by an immune escape phase (HBeAg negative chronic hepatitis) associated with mutations in the core or pre-core region leading to viral mutants that do not express HBeAg in a minority of patients. In this phase, moderate to high fluctuation of ALT and HBV DNA is associated with chronic liver inflammation and risk of cirrhosis.

The nomenclature of "immune tolerant" has been challenged by emerging evidence. This includes the existence of substantial histological inflammation despite normal ALT; that HBV T-cell responses are preserved and that HBV DNA integration events and clonal hepatocyte expansion persist during the IT phase (Mason et al., 2016, Seto and Yuen, 2016). A cohort study of perinatally infected children showed that a third and 7% of patients had evidence of moderate and severe hepatitis, respectively among patients with a median duration of infection of 7 years (Iorio et al., 2007). A cross-sectional study of 319 treatment naïve HBV patients undergoing liver biopsy showed that elevations in ALT did not predict significant liver inflammation or fibrosis (Seto et al., 2012). Established stage 2 to 3 Ishak fibrosis, continued HBV integration and clonal hepatocyte expansion were observed among an analysis of patients classified as immunotolerant, indicating hepatocarcinogenesis may be underway even in early HBV infection (Mason et al., 2016). Analysis of blood transcriptome signatures during the IT phase showed a high level of activity of innate interferon and B cell responses comparable to the immune active phase (Vanwolleghem et al., 2015). An analysis of cytokine profiles among young patients with clinically defined immunotolerant HBV had a cytokine profile consistent with T-cell exhaustion rather than tolerance, with evidence of HBV-specific T cells with a capacity to proliferate and secrete cytokines that was superior to older patients (Kennedy et al., 2012). It has been suggested that traditional reference ranges for ALT (with an upper limit of 40 IU/l) are insensitive for detecting hepatic immunological activity and that revised, lower criteria should be used (30 IU/l for males; 19 IU/l for females) and these lower limits have been adopted in the UK guidelines (Gill and Kennedy,

2014, Sarri et al., 2013). In the AASLD guidelines an ULN of 35 U/L for males and 25 U/L for females are recommended whereas EASL guidelines use the traditional ULN of 40 U/L (Terrault et al., 2018b, European Association for the Study of the Liver, 2017).

HBsAg loss can occur naturally and is termed spontaneous seroclearance. Longitudinal quantification of the kinetics of HBsAg concentration can predict HBsAg seroclearance (Chevaliez et al., 2013). In HBeAg positive individuals, the immune tolerant phase is associated with both high HBV DNA levels and HBsAg levels, with HBsAg concentration considerably higher than those in the immune reactive or HBeAg positive hepatitis phase (Cornberg et al., 2017). HBsAg seroclearance is described further in section 1.15.

1.2.4 Oncogenesis

HBV is one of the leading causes of HCC globally, with one third of cases attributable to HBV (Global Burden of Disease Liver Cancer et al., 2017). In Southern sub-Saharan Africa it is estimated that 29% of HCC cases are due to HBV and 20% to HCV, while 40% of cases are attributable to excess alcohol consumption (Global Burden of Disease Liver Cancer et al., 2017). Evidence from Taiwan was among the earliest reports elucidating the importance of HBV as a risk factor for HCC. In a cohort study of 22,707 men, a relative risk of 223 for HCC was observed among HBsAg carriers, while HCC and cirrhosis accounted for 54% of deaths observed among HBsAg carriers and 1.5% of deaths in non-carriers (Beasley et al., 1981).

Carcinogenesis occurs via two main mechanisms: first prolonged intrahepatic inflammation causes apoptosis, necrosis and regeneration leading to genetic mutations and epigenetic changes (Fallot et al., 2012). Secondly, HBV DNA integration into the host genome causes genomic instability due to repeated integration and repair events (Hu et al., 2019). Finally there is evidence that the HBx protein has a role through alteration of epigenetic pathways by increasing DNA methyltransferase expression (Gao et al.).

Persistent HBV-associated inflammation causes hepatic fibrosis, progressing to cirrhosis (Kim et al., 2015b). Cirrhosis has been described as a pre-cancerous state due to frequent chromosomal mutations and epigenetic modification events such as DNA methylation which cause changes to tumour suppressor gene function. A stepwise accumulation of DNA methylation events have been shown among CpG islands, increasing among a continuum from cirrhosis to dysplastic nodules to early and progressive HCC (Um et al., 2011). Persistent inflammation and viral surface proteins

accumulating in the endoplasmic reticulum have been associated with oxidative stress with an increase in intracellular reactive oxygen species. This can lead to genome instability, and activation of intracellular signalling pathways associated with oncogenesis (Fallot et al., 2012).

HBV integration does not contribute to viral replication; the template for replication is cccDNA and HBV DNA integration is usually incomplete with insertion occurring throughout the host genome. Analysis of viral-host junctions has shown an increase in targeting of genes involved in cellular signalling and growth control (Murakami et al., 2005). DNA integration events may thus contribute to hepatocarcinogenesis.

1.3. Role of aflatoxin exposure

Aflatoxins are mycotoxins that contaminate stable crops, and are classified as carcinogens by the International Agency for Research on Cancer (IARC 2012). They are formed by several fungus species of the genus *Aspergilii,* primarily *Aspergillus flavus* and *A. parasiticus* (Aydin et al., 2015, Humans, 2012). Aflatoxin B1 (AFB_1) is among the most potent naturally occurring carcinogens and aflatoxins have been particularly implicated in the pathogenesis of hepatocellular carcinoma. Aflatoxin M_1 (AFM_1) is a metabolite of AFB_1 which, following ingestion by animals of AFB_1-contaminated feeds is excreted in the milk of lactating animals (Sirma et al., 2019). Metabolism by cytochrome P450 isoforms of AFB_1 producing highly reactive metabolites such as AFB_1-8,9,*exo*-epoxide which reacts with DNA, leading to mutational effects (FAO/World Health Organization, 2017).

The major hosts for aflatoxin producing fungi are maize, groundnuts and cottonseed (IARC 2012). Aflatoxin exposure is therefore a considerable problem in low and middle income countries in sub-Saharan Africa where consumption of domestically produced maize and groundnuts accounts for the main source of nutrition (Ekpa et al., 2019). A study of foetal aflatoxin exposure in Ghana, Kenya, Sierra Leone and Nigeria showed between 3 and 30% of infant cord blood samples with detectable aflatoxins, with an overall rate of 25% (Maxwell, 1998). In Ghana and Kenya, aflatoxin was detected in 28-34% of breast milk samples (Maxwell, 1998). Studies from Uganda, Kenya, Mozambique, Swaziland and South Africa have demonstrated epidemiological evidence of an association between population-level aflatoxin exposure in human or food samples and HCC incidence (Autrup et al., 1987, Peers et al., 1976, Peers et al., 1987, Van Rensburg et al., 1985).

An analysis of population-level exposure samples which incorporated global estimates of cancer potency factors (standardised estimates of incident cancer based on exposure levels) estimated that

between 5 and 28% of cases of HCC are attributable to aflatoxin exposure (Liu and Wu, 2010). The best available population-level data are from a large longitudinal cohort study from China (n=7917) where over 6 years of follow up, a linear relationship between average exposure to aflatoxin and HCC mortality at a regional level was observed (Yeh et al., 1989). Co-exposure to hepatitis B or C has been associated with an enhanced risk of aflatoxin exposure (FAO/World Health Organization, 2017). Chronic HBV may induce cytochrome P450 enzymes responsible for metabolism of aflatoxin metabolites from inactive compounds into carcinogenic metabolites, and nuclear excision repair responsible for removal of aflatoxin-DNA adducts may be inhibited by the HBV X protein (Kew, 2003, Yang et al., 2019). A meta-analysis of case-control studies showed a population attributable risk of 17% from aflatoxin exposure (95% CI 14-19%) overall, with an elevated population attributable risk observed among HBV positive populations (21% vs 8.8%). The summarised odds ratio for combined exposure to aflatoxin and HBV was 73.0 (95% CI (confidence interval) 36.0-148.3), compared to 11.3 (95% CI 6.75-18.9) from HBV exposure and 6.37 (95% CI 3.74-10.86) from aflatoxin exposure alone (Liu et al., 2012). Prevention of aflatoxin formation and mitigation of dietary exposure may be achieved through biological control methods and improving plant resistance through breeding or genetic engineering. Post-harvest control strategies include improved storage methods including control of moisture, temperature and avoidance of fungal inoculum (FAO/World Health Organization, 2017).

1.4. Assessment of HBV Treatment Eligibility

Current guidelines for treatment eligibility are based on evidence of virological activity and hepatic inflammation that represent a risk of hepatic fibrosis, or of evidence of established liver disease manifesting as hepatic fibrosis or cirrhosis (European Association for the Study of the Liver, 2017). HBV activity is assessed by determination of HBeAg status, ALT and HBV DNA quantification. Ascertainment of established liver disease may be achieved through radiological imaging to detect structural impairment, measurement of physical properties of liver stiffness to assess fibrosis, or biochemical markers (biomarkers) that act as surrogate markers of liver fibrosis (Allain and Opare-Sem, 2016).

Several challenges are associated with performing an accurate assessment of treatment eligibility, particularly in low-income country settings. Hepatocyte inflammation and HBV DNA concentration fluctuate and single cross-sectional assessment may lead to misclassification (European Association for the Study of the Liver, 2017). Assessment of hepatic fibrosis based on radiological means alone is relatively insensitive and highly operator dependent, particularly for the diagnosis of early cirrhosis by ultrasound, or may not be cost-effective or available in low income settings as in the case of cross-

sectional imaging (CT or MRI), or transient elastography (TE) (Lemoine and Thursz, 2017). TE has been associated with good correlation with histological assessment of cirrhosis and liver stiffness correlates with the probability of varices (Shi et al., 2013), outcomes such as incident HCC (Kim et al., 2015b) and liver-related mortality (Singh et al., 2013, Wong et al., 2015), but capital and maintenance costs represent barriers to widespread adoption in low resource settings.

Table 1-1 Comparison of assessment of WHO-recommended APRI score for diagnosis of significant fibrosis/ cirrhosis in chronic HBV in sub-Saharan African studies

Study	Country	N	GS	Diagnosis	Cut off	AUROC	Sens (%)	Spec (%)	PPV (%)	NPV (%)
Bonnard 2010	Burkina Faso	59	LB	F2	1.0	0.61	55	55	69	29
Bonnard 2010	Burkina Faso	59	LB	F4	1.2	0.50	50	51	23	75
Lemoine 2016	The Gambia	135	LB	F2	1.2	0.66	50	51	23	75
Lemoine 2016	The Gambia	135	LB	F4	2.0	0.70	25	99	75	87
Lemoine 2016	The Gambia	721	TE	F2 (7.9 kPa)	1.5	0.78	41	99	83	91
Lemoine 2016	The Gambia	721	TE	F4 (9.5 kPa)	2.0	0.87	55	99	82	96
Desalegn 2017	Ethiopia	1015	TE	F2 (7.9 kPa)	1.5	0.79	10	100	88	78
Desalegn 2017	Ethiopia	1015	TE	F4 (11.6 kPa)	2.0	0.86	10	100	90	86
Stockdale 2016[a]	Ghana	100	TE	F2 (7.6 kPa)	0.57	0.62	67	63	29	90
Stockdale 2016[a]	Ghana	100	TE	F4 (9.4 kPa)	0.70	0.59	57	74	14	96

Abbreviations: TE, transient elastography; LB, Liver biopsy; GS, Gold standard used as reference test; F2 Fibrosis score 2 equating to METAVIR histological classification for significant fibrosis; F4 METAVIR Fibrosis score 4 equating to cirrhosis; Sens, sensitivity; Spec, specificity; PPV, positive predictive value; NPV negative predictive value; AUROC area under receiver operating curve [a] This study was conducted among HIV/HBV co-infected patients

Use of biomarkers based on routinely available laboratory tests to diagnosis liver fibrosis represents an attractive alternative, however there are several potential confounding factors unrelated to liver disease that may cause elevations of most biomarker-based tests. Biomarker-based scores including the WHO recommended APRI (AST to platelet ratio index) score have not been derived from sub-Saharan Africa and limited evaluations of the performance of the APRI in sub-Saharan Africa settings to date have shown consistently poor diagnostic performance with low sensitivity for detection of cirrhosis, thus potentially restricting treatment to patients who would benefit (Table 1-1) (Lemoine et al., 2016a, Bonnard et al., 2010, Stockdale et al., 2016, Desalegn et al., 2017, Lemoine et al., 2017).

Table 1-2- Comparison of international guidelines criteria for treatment eligibility

WHO 2015	**EASL 2017**	**AASLD 2018**
Cirrhosis, based on APRI> 2.0, regardless of HBV DNA and ALT	Cirrhosis, regardless of HBV DNA and ALT	Cirrhosis, regardless of HBV DNA and ALT
Age > 30 years and ALT persistently abnormal ALT[a] and HBV DNA>20,000 IU/ml, regardless of HBeAg status	HBV DNA >2000 IU/ml ALT> ULN moderate inflammation or moderate fibrosis[b]	HBeAg positive, HBV DNA >20,000 IU/ml and ALT <2x ULN & fibrosis ≥F2
If HBV DNA testing is not available, treatment can be considered for persistently abnormal ALT alone, regardless of HBeAg	HBV DNA >20,000 IU/ml ALT>2x ULN	HBeAg positive, HBV DNA > 20,000 and ALT >2x ULN
	Age > 30 HBeAg and Normal ALT High HBV DNA	HBeAg negative, HBV DNA >2,000 IU/ml and ALT <2x ULN & fibrosis ≥F2
	Family history of HCC or extrahepatic manifestations	HBeAg negative, HBV DNA > 2,000 and ALT >2x ULN

Abbreviations: ALT alanine transferase; APRI ALT to platelet ratio index, ; ULN upper limit of normal The APRI score is calculated with the following equation: (AST level (IU/L) / upper limit of normal (IU/L)) / platelet count 10^9/L * 100. [a] Persistently abnormal ALT is defined by three ALT measurements above the ULN at unspecified intervals during a 6-12 month period, or pre-determined interval over a 12 month period.[b] Fibrosis can be assessed by liver biopsy or a non-invasive method. In the case of transient elastography, eligibility criteria are a liver stiffness measurement (LSM) >9kPa with normal ALT or LSM >12kPa with elevated ALT below 5x ULN. [c]Fibrosis corresponding to F2 (significant fibrosis) assessed by histological diagnosis or non-invasive method.

1.4.1 Performance of biomarkers for evaluation of hepatic fibrosis

In sub-Saharan African populations, the APRI score does not perform well in chronic HBV or HBV/HIV co-infected populations as a biomarker for cirrhosis (Table 1-1). The APRI was originally derived for use in people with hepatitis C infection in North American populations (Wai et al., 2003). These limited data from Senegal, Ethiopia, Burkina Faso and Ghana show consistently low sensitivity in the diagnosis of cirrhosis which could have the effect of limiting access to treatment for people with established cirrhosis. WHO guidelines for treatment of hepatitis B from 2015 recommended to use an APRI score cut off of >2.0 for the diagnosis of cirrhosis (World Health Organisation, 2015). In the data presented in table 1-1, the optimal cut-off threshold in most populations was significantly lower than 2.0, and even at a range of locally optimised thresholds ranging from 0.7 to 2.0, sensitivity was consistently poor and below acceptable limits, ranging from 10 to 57%. A diagnostic tool should be first evaluated and validated in the specific population of application before clinicians may be confident in the diagnostic performance. Reasons for poor performance in sub-Saharan Africa might be that relative to Asian cohorts, where most validation studies have been conducted, HBeAg prevalence is lower in Africa cohorts and patients have lower average transaminase levels (Desalegn et al., 2017).

Studies that derive and validate diagnostic biomarkers such as the APRI score for the diagnosis of fibrosis or cirrhosis are typically cross-sectional with a paired liver biopsy providing histological assessment of fibrosis and compared to serum biomarkers. These study designs can be subject to a number of important biases. Firstly, spectrum bias, whereby only patients with indeterminate clinical phenotypes that are more difficult to classify undergo biopsy (Ransohoff and Feinstein, 1978). This is due to the risk of iatrogenic injury and side effects including bleeding, pain and death from a biopsy procedure, rendering an ethical imperative not to perform biopsy in patients with clinically well-defined cases such as advanced cirrhosis with features of decompensated liver disease (Friedrich-Rust et al., 2016). The effect of spectrum bias is to reduce the apparent diagnostic performance of the evaluated biomarkers by removing clinical extreme cases (Ransohoff and Feinstein, 1978). The second major limitation is that the diagnostic gold standard reference test usually used in most evaluations of non-invasive biomarkers is histological examination of a liver biopsy. This is subject to a number of substantial limitations which collectively challenge the validity of the gold standard. A liver biopsy samples a very small and variable volume of liver and particularly in early cirrhosis, fibrosis may be inhomogenous and sampling bias may result in histological misclassification. The size of the biopsy specimen has been shown to influence the histological fibrosis score (Colloredo et al., 2003). Substantial inter-observer variability has been noted between histopathologists grading liver biopsy specimens for fibrosis which is only partially mitigated by use of a standardised histological grading

system such as the Ishak or METAVIR classification (Regev et al., 2002, Goldin et al., 1996, Westin et al., 1999). Indeed, in an analysis using latent class analysis (LCA), a probabilistic approach that does not assume a perfect gold standard but combines data from multiple tests, the estimated sensitivity and specificity of non-invasive methods such as TE and APRI were improved, relative to using LB as a solitary gold standard (Fernandes et al., 2017, van Smeden et al., 2014). Ideally, non-invasive scores for fibrosis detection should aim to categorise patients based on predictive risk of liver related events such as hepatic decompensation and incident hepatocellular carcinoma rather than a pair-wise cross sectional evaluation using an imperfect gold standard. A substantial longitudinal cohort study would be required for such an analysis but would provide data on outcomes of patients who do not meet criteria for treatment, and data that could optimise eligibility criteria for HBV treatment.

1.4.2 Treatment eligibility scores

In well-resourced settings, repeated serial measurement 3-6 monthly of HBV DNA, ALT and a non-invasive assessment of hepatic fibrosis are the cornerstone of HBV treatment eligibility assessment (European Association for the Study of the Liver, 2017). International guidelines for HBV management differ on the specific criteria used to determine eligibility, but consider age, ALT as a marker of hepatic inflammation, and HBV DNA as a marker of viral replication to ascertain individuals at risk of developing liver disease. These criteria are based on large long-term cohort studies such as the REVEAL-HBV in Taiwan which demonstrated the correlation between HBV DNA and risk of incident cirrhosis and HCC (Chen et al., 2009). Further data are needed to ascertain the performance of different eligibility classification guidelines in sub-Saharan Africa, requiring longitudinal follow-up of untreated patients who do not meet current treatment criteria to ascertain incident liver disease or liver-related outcomes. Recently, use of an alternative “TREAT-B” score, a simplified treatment algorithm for sub-Saharan Africa has been proposed based on ALT and HBeAg which is associated with improved performance relative to WHO criteria, when considering EASL criteria as a gold standard (Shimakawa et al., 2018). This will require further external validation in other African cohorts. In a recent evaluation in Ethiopia, TREAT-B was associated with moderate sensitivity with a lower sensitivity than that observed in West Africa (Johannessen et al., 2019). The TREAT-B eligibility algorithm was recently externally validated in non-African setting in cross-sectional evaluation in hepatology clinics in Berlin and London. TREAT-B was shown to have good diagnostic performance in both settings using the EASL guidelines as the reference standard. Among African patients in both clinics, the area under the receiver operating curve (AUROC) was 0.90 (95% CI 0.84- 0.96) with

sensitivity of 91.7% (95% CI 73.0- 99.0) and specificity of 73.2% (95% CI 64.0- 81.1) and exceeded the performance of WHO criteria (AUROC 0.69 (95% CI 0.62- 0.77), sensitivity 91.7% (95% CI 73.0 – 99.0) and specificity 47.4% (95% CI 37.9 – 56.9) (Yoshida et al., 2019).

While the TREAT-B score represents a promising and easily applied low-cost score using widely available diagnostic tests (ALT and HBeAg), there are potential issues with HBeAg rapid diagnostic tests (RDTs) which are used in many low resource settings. In a cross-sectional study from West Africa, in Senegal, three commercial HBeAg RDTs were shown to have poor sensitivity with sensitivity of 29.8% (95% CI 17.3- 44.9) for SD Bioline, Alere, South Africa and 42.5% (95% CI 27.0 – 59.1) for OneStep, and 31.1% (95% CI 18.2- 46.6) for Insight. Each test had good specificity. The WHO has recently added HBeAg RDTs to the list of model essential in-vitro diagnostic devices for use at the health clinic level, for its utility in classifying chronic HBV disease (section 1.2.3) and for the potential to use HBeAg as a surrogate marker of high HBV DNA indicating increased risk of transmission, particularly for pregnant women who could benefit from antiviral therapy to prevent mother to child transmission in settings where HBV DNA quantification is not available (World Health Organisation, 2019b, Terrault et al., 2018a). Further assessment of HBeAg rapid diagnostic tests is needed in other sub-Saharan African settings to evaluate diagnostic performance. HBV DNA quantification is becoming increasingly available in low and middle income country settings due to the opportunities to share molecular platforms used for HIV RNA quantification and the development of self-contained HBV DNA quantification platforms such as the GeneXpert HBV DNA viral load cartridge based systems (Peeling et al., 2017, Chevaliez and Pawlotsky, 2018). These opportunities are discussed further in section 1.10.4.

1.5. Antiviral therapy for HBV

1.5.1 Mechanism of action

Currently licenced therapy for HBV can be grouped into two main classes: immunomodulators such as pegylated-interferon alpha (Peg-IFNα) and nucleos(t)ide analogues (NAs) comprising lamivudine, adefovir, telbivudine, entecavir and tenofovir. NAs work by causing chain termination of DNA synthesis, inhibiting reverse transcription of pgRNA into HBV DNA (Trepo et al., 2014). Interferon (IFN) works by stimulating interferon signalling genes (ISGs) which have broad antiviral effects. IFN therapy drives proliferation of innate natural killer cells which exert antiviral activity and induces apolipoprotein B mRNA-editing enzyme catalytic polypeptide-like 3G (Apobec3G) expression which inhibits HBV DNA replication (Woo et al., 2017). IFN alpha degrades cccDNA and interrupts cccDNA

transcription through epigenetic modification (Cornberg et al., 2019). The pegylated alpha-2a form is modified through the addition of a large, branched, 40-kD polyethylene glycol molecule, creating improved pharmacokinetics and facilitating once-weekly dosing (Lau et al., 2005). Peg-IFNα promotes seroconversion among HBeAg positive individuals to an immune control HBeAg negative phase in around 30% of patients and causes HBsAg loss in 3-4% after 48 weeks (European Association for the Study of the Liver, 2017). Barriers to use of IFN include low overall success rates, the significant side effect profile and requirement for parenteral administration.

NA therapy acts by targeting viral polymerase preventing synthesis of viral rcDNA from pgRNA. As a result, recycling via replenishment of rcDNA into the nucleus is suppressed. However, NAs do not inhibit cccDNA translation and cannot target established cccDNA (Trepo et al., 2014). Once NA therapy is discontinued, HBV DNA replication will typically resume (Hu et al., 2019). There is evidence that replenishment of intracellular cccDNA can occur even while established on NAs due to incomplete suppression of intracellular viral DNA synthesis (Boyd et al., 2016). The mainstay of treatment is continuous NA therapy leading to sustained HBV DNA suppression and corresponding reduction of HBV replication and HBV intrahepatic inflammation (Fanning et al., 2019).

From a meta-analysis of 34 studies involving 303,754 person-years of follow up, the pooled annual HBsAg seroclearance rate among chronic HBV patients was 1.02% (95% CI 0.79 – 1.27) with cumulative seroclearance incidence of 4, 8 and 18% at 5, 10 and 15 years (Yeo et al., 2019). On NA therapy, HBsAg declines faster among HBeAg positive relative to HBeAg negative patients (Zoulim et al., 2015). HBeAg negative patients have a higher proportion of HBsAg derived from integrated HBV DNA relative to cccDNA, and since NAs do not affect integrated DNA, HBsAg clearance rates are lower (Cornberg et al., 2017). The decline in HBsAg concentration observed is slower with NA therapy relative to IFN therapy (Reijnders et al., 2011, Yeo et al., 2019). In a meta-analysis, among IFN treated patients, HBsAg loss was 1.8% (95% CI 1.4 – 2.3) compared to 0.8% (95% CI 0.4 – 1.3) with entecavir (Yeo et al., 2019). NA targets only reverse transcription of pgRNA into DNA, whereas IFN inhibits viral replication and augments HBV-specific immune responses at multiple levels including targeting cccDNA (Cornberg et al., 2017). Recent consensus definitions for HBV functional cure are durable HBsAg loss (below 0.05 IU/ml) and undetectable HBV DNA (Cornberg et al., 2019). It is evident from the presented data that functional cure is an uncommon event among patients treated with NA therapy and for the majority of patients, treatment will be of long-term or lifelong duration.

Due to concerns about the rapid emergence of resistance associated with lamivudine (Aoudjane et al., 2014), adefovir and telbivudine, currently entecavir and tenofovir are recommended therapies for

HBV (European Association for the Study of the Liver, 2017). Emergence of resistance usually manifests as an ALT flare together with HBV DNA rebound and may be accompanied by hepatic decompensation among individuals with cirrhosis (Zoulim and Locarnini, 2009). Due to concerns about cross-class resistance that may be acquired while on lamivudine or older NAs, for patients previously exposed to older NA classes or who have failed to achieve HBV suppression on other antiviral agents, tenofovir is recommended (European Association for the Study of the Liver, 2017).

Several novel compounds targeting alternative HBV replicative processes are currently under development including the entry inhibitor targeting the NTCP receptor, bulevirtide, small interfering RNA (siRNA) molecules targeting HBV RNA and HBsAg production, capsid assembly inhibitors and leid acid based polymers targeting HBsAg secretion (Fanning et al., 2019, Hu et al., 2019). Additionally several immunomodulatory compounds are in development including stimulators of the pattern recognition receptors toll-like receptor-7 (TLR-7) and TLR-8, stimulator of interferon genes agonists such as the retinoic acid inducible gene-I (RIG-I) agonists, and therapeutic vaccines (Cornberg et al., 2019). The active drug development pipeline for HBV treatment represents a potential promising future for HBV control, particularly among novel drug classes targeting depletion of cccDNA and functional cure.

In sub-Saharan Africa, the mainstay of HBV treatment is the use of nucleos(t)ide analogues tenofovir, and to a lesser extent, entecavir. These agents have a high genetic barrier to drug resistance and extensive evidence of efficacy. Continuous HBV DNA suppression has been observed in several long-term open-label TDF treatment studies without evidence of emergent resistance (Corsa et al., 2014, Buti et al., 2015). Tenofovir therapy has shown evidence of efficacy at HBV DNA suppression even among patients previously exposed to prior nucleoside therapy with lamivudine or adefovir and with multiple drug resistance mutations (Berg et al., 2014, Baran et al., 2015, Fung et al., 2014, Stockdale et al., 2015). There is also substantial clinical experience with use of tenofovir in sub-Saharan Africa in HIV treatment programmes, facilitating use in HBV programmes.

1.5.2 Evidence for efficacy

Antiviral therapy for HBV with nucleos(t)ide analogues has been associated with regression of fibrosis and reversal of cirrhosis in longitudinal treatment studies, based on histological evidence or use of

repeated transient elastography (Boyd et al., 2010, Marcellin et al., 2013). Regression of fibrosis has also been observed in sub-Saharan Africa among HIV/HBV co-infected patients (Stockdale et al., 2015).

In a five-year open label trial of tenofovir disoproxil fumarate (TDF) for the treatment of chronic HBV in a multicentre study in high-income settings, of 641 patients who were randomised to placebo or TDF therapy, 585 continued in an open label evaluation of ongoing TDF therapy and 348 patients had paired liver biopsies at baseline and a repeated at week 240 (Marcellin et al., 2013). Of 96 patients who had cirrhosis at baseline, 71 (74%) no longer had cirrhosis with a histological improvement of at least 1 on the 6-point Ishak fibrosis scale (ranging from no fibrosis to cirrhosis) and of 252 non-cirrhotic patients, 12 experienced worsening fibrosis (5%) , 135 did not change fibrosis score (54%) and 105 (42%) experienced histological improvement, at 5 years of TDF therapy.

In a study of 106 HIV/HBV co-infected patients in Ghana, among antiretroviral (ART) therapy-experienced patients who had received a median 45 months of lamivudine as the sole HBV-active component of ART, switching to TDF-based ART resulting in a significant reducing in liver stiffness measurement (LSM). From a baseline median liver stiffness by transient elastography of 5.7kPa, LSM reduced by a mean 0.8kPa among patients with pre-treatment HBV DNA <2000 IU/ml and by a mean 1.2kPa among patients with TE values >7.6 kPa) after a median 7.8 months of TDF. Improved HBV DNA suppression was also observed, declining by mean 1.5 $\log_{10}$ IU/ml (Stockdale et al., 2015).

Antiviral therapy has been associated with reduced incidence of HCC. The annual incidence of HCC among 641 patients participating in a multicentre European and North American study was 0.37% (95% CI 0.20- 0.62); 0.65% (95% CI 0.24- 1.40) among patients with cirrhosis and 0.28% (95% CI 0.12-0.56) among people without cirrhosis (Kim et al., 2015d). Using a predictive tool (REACH-B), expected HCC incidence was compared with observed on treatment, and the standardised incidence ratio was 0.40 (0.20 – 0.80), P=0.009 for participants on treatment, providing evidence of markedly lower than expected incidence of HCC on treatment (Kim et al., 2015d). In a Japanese cohort with average follow up of 3.2 years, among 316 patients treated with entecavir, relative to a propensity score-matched untreated control population, the hazard ratio for HCC was 0.37 (0.15-0.91), p=0.003 (Hosaka et al., 2013).

HBV antiviral treatment has also been associated with improved survival. In a large multicentre cohort study of 1951 adult patients with chronic HBV on treatment, 8-year survival was 97.4%, which was not significantly different from the general population (Papatheodoridis et al., 2018). HBV treatment resulting in HBV DNA suppression has been also associated with improved patient-reported outcomes and improved quality of life. In a randomised placebo-controlled clinical trial of a toll-like receptor 7

agonist, among 242 patients in the study on nucleotide analogue therapy with HBV DNA suppression had improved quality of life relative to patients who were unsuppressed. After adjustment for baseline demographics, suppression of HBV viraemia was independently associated with improved quality of life (Younossi et al., 2018b).

Evaluations of effect of HBV treatment on liver-related mortality, incident cirrhosis and HCC, HBV transmission, risk of hepatic decompensation, quality of life and health systems costs are lacking for sub-Saharan Africa to inform economic modelling and to build a case for investment in HBV treatment programmes across the continent.

1.6. Impact of HIV/HBV co-infection

In Southern Africa, a generalised HIV epidemic overlaps with regional intermediate or high HBV prevalence (Dwyer-Lindgren et al., 2019). Around 70% of the total global number of people living with HIV live in Sub-Saharan Africa, and around 10% of people living with HIV in the region are HBV co-infected (Matthews et al., 2014). A recent systematic review estimated that 69% of global cases of HIV/HBV co-infection are located in sub-Saharan Africa and odds of HBV infection are increased among HIV positive, relative to negative individuals (Platt et al., 2019). HIV co-infection affects several aspects of HBV biology and has been associated with increased risk of HBV transmission, increased rates of chronic infection following exposure, increased rates of HBV reactivation, higher HBV DNA concentration, and acceleration of HBV-associated liver fibrosis (Matthews et al., 2014). In a cross-sectional study in Uganda, HIV co-infection was associated with a 50% increased prevalence rate ratio of liver fibrosis (Stabinski et al., 2011). A study of liver biopsy histology from HIV positive patients showed increased rates of hepatic necroinflammation among HIV-HBV co-infected individuals patients who had active HIV replication (Audsley et al., 2012). HIV can infect multiple hepatic cells including hepatic stellate cells and Kupffer cells and can activate hepatic stellate cells giving rise to an increased production of profibrotic and proinflammatory cytokines (Bruno et al., 2010). HIV co-infection may therefore be an important contributor to the overall disease burden and be associated with promotion of HBV transmission in sub-Saharan Africa, by accelerating liver disease progression and increasing infectiousness.

1.7. HBV transmission

Hepatitis B is sub-Saharan Africa is predominantly transmitted in the first five years of life (Spearman et al., 2017). Horizontal transmission occurs between young children who have predominantly hepatitis B e antigen (HBeAg) positive disease and high HBV DNA concentration, although a detailed understanding of mechanisms of horizontal transmission is lacking (Lemoine and Thursz, 2017).

In a study based in the Gambia, phylogenetic analysis was used to examine the relationship between HBsAg positive individuals and family members (Dumpis et al., 2001). From 142 Gambian families who were HBV DNA positive, family members were invited to HBV testing. A total of 75 siblings with infection were identified and 67 HBsAg positive cases were isolated and had no other household cases identified. Following Sanger sequencing of the core/pre-core gene, maximum likelihood phylogenetic tree analysis indicated that cases clustered at the family and village level. Sequences from children from unrelated families were found to cluster at the village level, providing evidence of horizontal transmission (Dumpis et al., 2001).

An epidemiological evaluation in Ashanti region, rural Ghana sampled 1385 individuals using random probability sampling to evaluate risk factors for HBV transmission (Martinson et al., 1998). All individuals living in the same compound as HBsAg positive index cases were tested and behaviours associated with transmission were ascertained relative to negative cases. In the study, serological evidence of HBV exposure (anti-HBc prevalence) increased with age, with anti-HBc prevalence rising from 38.6% (95% CI 30.6 – 46.6) among children aged 1-5 to 79.3% (95% CI 74.8- 83.8) in those aged 11-15. The greatest increase of anti-HBc occurred between 5 and 11 years and the authors inferred that this represented the age group of maximal horizontal transmission. Household-level behaviours including sharing towels, sharing gum or candies, biting fingernails or scratching were most strongly associated with transmission. The number of other carriers in the household and evidence of tribal markings were also associated with transmission (Martinson et al., 1998). Evidence of an association between scarification and HBV transmission was also been observed in a cross-sectional serological survey in Juba, Southern Sudan (McCarthy et al., 1994a). Further biological support for horizontal transmission between children is provided by a study of children in Japan from whom saliva and tear samples were found to have HBV DNA detectable with viral loads in saliva and tears correlating with serum HBV DNA levels. Tear specimens from a child injected into human hepatocyte-transplanted chimeric mouse resulted in HBV transmission indicating replication-competent virus and the potential for HBV transmission (Komatsu et al., 2012).

In several studies from sub-Saharan Africa, male gender has been associated with HBV infection among adults (Bwogi et al., 2009, Martinson et al., 1998). Non-sterile traditional circumcision, shared use of razor blades or use of communal shaving equipment in barbershops without sterilisation may be responsible (Spengane et al., 2018). In a longitudinal cohort study of men who have sex with men in Kenya, being circumcised was associated with reduced risk of HBV acquisition, by contrast, representing that it may be protective against sexual transmission in a similar manner to HIV (Wahome et al., 2017). The lack of population-level association between circumcision and HBV infection may be due to the majority of transmission occurring at an earlier age before adolescents become sexually active (Wahome et al., 2017). In a study of 50 barbers from three townships in South Africa, clippers from barbershops were tested following clean-shave haircuts by rinsing clippers with phosphate buffered saline and submerging into viral transport media prior to testing with HBV DNA PCR. A total of 8% of clippers were positive for HBV, representing a potentially important source of HBV transmission (Spengane et al., 2018).

Detailed evidence on the mechanism and timing of residual HBV transmission in the vaccine era are lacking for sub-Saharan Africa. Longitudinal epidemiological studies among children receiving the infant HBV vaccine to ascertain the timing and risk factors for transmission are needed to improve understanding of HBV transmission and to identify opportunities to implement additional preventative strategies.

1.7.1 Mother to child transmission (MTCT) of HBV

Mother to child transmission (MTCT) represents an epidemiologically important transmission route for HBV and several effective preventative interventions are available (Keane et al., 2016). Risk factors for transmission include high maternal HBV DNA concentration, maternal HBeAg positivity and HIV co-infection (Howell et al., 2014). There are however, conflicting data on the relative importance of MTCT. In a Ugandan cohort study, among 612 mothers, 53 (8.7%) were HBsAg positive and 339 (61.5%) anti-HBc positive, with 10/53 (19%) of HBsAg positive mothers also HBeAg positive. No cases of HBV transmission were observed at the time of the first HBV vaccine dose at 6 weeks and again at 9 months (Seremba et al., 2017). These data highlight that mother to child transmission may not be the major route of transmission uniformly across Africa and highlight the need for additional HBV transmission data to understand and model the need for additional MTCT interventions.

In a systematic review of 15 studies from 11 sub-Saharan Africa countries, among HBeAg positive women, the pooled rate of HBV transmission was 38.3% (95% CI 7.0- 74.4) in the absence of

prophylactic intervention, and 4.8% (95% CI 0.1 -13.3) among HBeAg negative women (Keane et al., 2016). These data suggest that MTCT risk is lower and is less important as a mechanism of transmission relative to Asian cohorts, but also highlights that paediatric HBV transmission remains common in Africa due to lack of implementation of preventative strategies. In the systematic review only three studies were included for southern Africa, of which two were from South Africa and one from Malawi (Keane et al., 2016). In the Malawian study, among HIV positive mothers and their infants, 103/2048 (5%) of women were HBsAg positive and 70 were HBV DNA positive (Chasela et al., 2014). Women and infants in the study received zidovudine and lamivudine from onset of labour for seven days and were randomised to receive placebo, infant nevirapine or maternal antiretroviral therapy with zidovudine, lamivudine and nevirapine for 28 weeks. Among 57 infants tested at 2 weeks, 1 infant experienced HBV transmission (1.8%) and among 51 infants tested at 48 weeks, 5 experienced HBV transmission (9.8%). Two of the mothers whose infants became HBV infected had received lamivudine for 6 months. These data are not applicable to an HIV-negative population and are subject to significant loss to follow up and confounding by receipt of lamivudine in the post-partum period for 28 weeks by a subgroup, but provide evidence of significant HBV transmission among an HIV/HBV co-infected population (Chasela et al., 2014).

Maternal HBeAg positive status represents a risk factor for HBeAg positivity in their children and this group is important in terms of sustaining HBV transmission. In the Gambia, a case-control study comparing 38 HBeAg positive and matched HBeAg-negative mothers showed that maternal HBeAg was a risk factor for HBeAg in their children after adjustment for several confounders (adjusted odds ratio 4.5 (95% CI 1.0 – 19.5), p=0.04) (Shimakawa et al., 2014).

Mother to child transmission may also have implications for the risk for HBV-associated disease. In a longitudinal cohort study in the Gambia, among 405 chronic HBV participants, having a HBsAg positive mother was an independent risk factor for persistent viral replication, and a for significant liver fibrosis and incident HCC (Shimakawa et al., 2016a). The finding of increased risk of liver disease among those with evidence of maternal transmission should highlight the particular importance of interrupting MTCT.

1.7.2 Interventions to prevent mother to child transmission

Interventions with proven efficacy for preventing mother to child transmission include provision of a birth dose vaccine delivered within 24 hours of birth, completion of a 3- or 4-dose vaccination course for infants, provision of hepatitis B immunoglobulin to the neonate, and antiviral therapy with

tenofovir disoproxil fumarate (TDF) for mothers with high HBV DNA concentration (exceeding 200,000 IU/ml), initiated in the third trimester (Andersson et al., 2015).

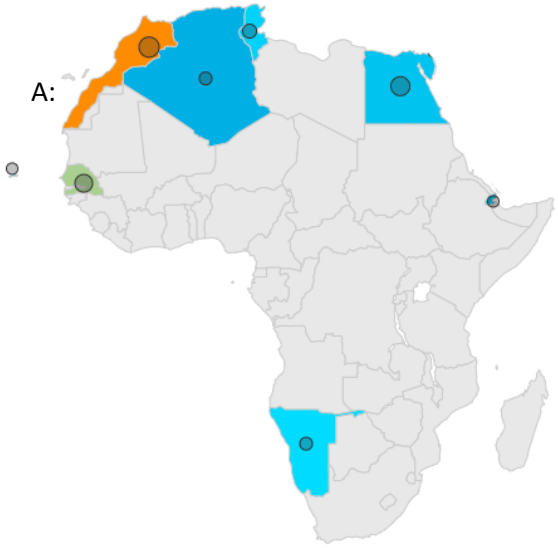
A recent network meta-analysis examined the results of 15 randomised trials including 2706 infants of HBV infected mothers using network techniques to make direct and indirect comparisons (Chen et al., 2017). The addition of an HBV birth dose vaccination reduced HBV risk significantly (relative risk (RR) 0.32 (95% CI 0.21- 0.50)). The addition of hepatitis B immunoglobulin (HBIG) significantly reduced transmission further when used in addition to birth dose vaccination (RR 0.37 (95% CI 0.20-0.67)). Adding prenatal maternal antiviral therapy (with tenofovir) relative to a comparator of HBIG and HBV vaccination was associated with reduced risk of transmission, among mothers with high HBV DNA concentration exceeding $2x10^5$ IU/ml (RR 0.11 (95% CI 0.03 -0.42)). The included studies did not report any adverse profiles among any of the interventions. Data on the applicability of these interventions and efficacy in sub-Saharan Africa are lacking and included studies on MTCT interventions to date have been from Western or Asian settings.

A recent multicentre RCT from Thailand randomised mothers who were HBeAg positive to TDF or placebo starting at 28 weeks of gestation, denoting the start of the third trimester (Jourdain et al., 2018). Infants received HBIG and vaccine at birth and 4 further doses at 1,2, 4 and 6 months. From 322 deliveries, none of the infants in the TDF group experienced HBV transmission, defined by HBsAg confirmed by HBV DNA at 6 months of age, while 3 of 147 (2%) in the placebo group had HBV transmission. Adverse event rate did not different between the two groups. The primary efficacy analysis showed no statistical difference between the two randomised groups (0% vs 2%, p=0.12) but the lack of observed effect was due to a lower than expected rate of HBV transmission in the placebo and HBIG and birth dose HBV vaccine group (Jourdain et al., 2018). That no transmission events occurred in the TDF group is highly encouraging.

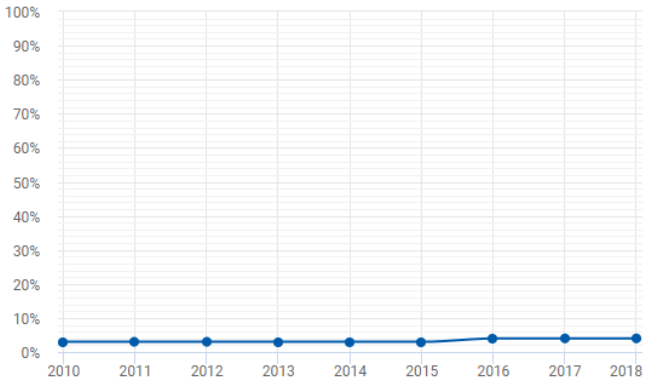
Data are now needed to examine the rate of HBV mother to child transmission in sub-Saharan Africa and to test the efficacy and applicability of MTCT interventions in this setting. The WHO currently recommends a birth dose vaccine but does not provide a recommendation for use of maternal tenofovir on the basis that at the time of the guidleline development (2015) evidence was limited foor efficacy with limited evaluation of potential harms in pregnancy (World Health Organisation, 2015). This recommendation is likely to change in view of more recent evidence of efficacy and extensive evidence of safety of tenofovir in HIV-positive pregnant women.

Figure 1-7: Hepatitis B birth dose vaccine coverage estimates for Africa: A: Map of nations implementing HBV birth dose in 2018, B: Estimated coverage of WHO Africa region by population

A:



B:



Data source: (World Health Organisation and United Nations Children's Fund (UNICEF), 2019)

1.8. Hepatitis B vaccination

The first HBV vaccine was approved in 1981 (Beasley, 2009, Buynak et al., 1976). Originally a purified plasma-derived vaccine, in 1986 it was replaced by the first ever recombinant DNA vaccine, derived from yeast cultures expressing hepatitis B surface antigen (HBsAg) (Huzair and Sturdy, 2017, Ward, 1986). The HBV vaccine was the first vaccine to prevent an infectious disease-related cancer.

HBV vaccination has been recommended for implementation in national immunisation programmes by the WHO since 1991 (van den Ende et al., 2017). Global coverage for the HBV vaccine expanded after the Global Vaccine Alliance (GAVI) started to support HBV vaccination in Mozambique in 2001 (Razavi-Shearer et al., 2018). HBV is now considered a standard component of all national infant immunisation programmes worldwide and approximately 1.1 billion doses have been delivered since 1986 (World Health Organization, 2019, van den Ende et al., 2017). In 2015, 85% of all infants worldwide received a 3-dose HBV vaccination course and 39% of infants received a birth dose (World Health Organisation, 2016b).

Use of a 6, 10 and 14 week HBV vaccine schedule provides protection against horizontal transmission of HBV but does not provide control of mother to child transmission (Nayagam et al., 2019). Since 2004, the WHO has recommended monovalent birth dose vaccination given within 24 hours of birth

for all infants, but there has been limited uptake among African nations (Figure 1-7) with only five nations in the WHO Africa region implementing a birth dose (Algeria, Cabo Verde, Dijibouti, Namibia and Senegal) and 4% of the population receiving it (World Health Organization, 2019, World Health Organisation, 2004).

The importance of the birth dose is greatest among children of women who are HBV infected with HBeAg, for whom the first dose of HBV vaccine would otherwise not be delivered until 6 weeks of age with the opportunity to prevent intrauterine transmission potentially missed. The predominant barriers to implementation of the birth dose recommendation have been the lack of funding support from GAVI for the monovalent birth dose, logistical supply issues, the difficulty in implementation among countries for home birth deliveries outside of health facilities, and the need for cold chain storage which can be difficult in low and middle income countries with inadequate facilities and an unstable power supply (Spearman et al., 2017). A review of UNICEF/WHO data showed a correlation between hepatitis B birth dose administration and skilled birth attendance rates, adult literacy, hospital density and total health expenditure (Allison et al., 2017). Promotion of institutional delivery and training of facility healthcare workers were suggested public health measures to increase uptake.

1.8.1 Out of cold chain and controlled temperature chain (CTC) storage

Recent data have shown that HBV monovalent vaccines are stable outside the standard storage temperatures of 2-8°C, which could facilitate the wider introduction of HBV birth dose implementation in sub-Saharan Africa. Data provided by monovalent HBV vaccine manufacturers show thermostability for 4 weeks at 37°C and 40-45°C (World Health Organisation, 2016b). The WHO recommend use of a controlled temperature chain (CTC) where vaccines are licenced for the purpose, and where additional criteria are met, comprising: i) the vaccine should be heat stable at 40°C, ii) vaccine vial monitors should be used and iii) a peak threshold temperature indicator on each vaccine carrier are used (Immunisation Practices Advisory Committee, 2016).

Field studies have indicated adequate production of anti-HBs after vaccination with vaccines stored outside the cold chain (Scott et al., 2018). A mathematical modelling study estimated that CTC would be cost-saving in sub-Saharan Africa with a lifetime saving of $1920 (95% CI 1540-2140) per 1000 births, representing the greatest cost saving among global regions. This model incorporated assumptions about HBsAg prevalence, disease progression parameters, and costs per disease state, many of which were based on sparse data for the sub-Saharan African region. The most influential parameters associated with cost effectiveness included the HBsAg prevalence and the birth dose

coverage (Scott et al., 2018). In Malawi, rates of health care facilities deliveries are high and as such the CTC method might not be as important as in other regions of Africa where home births predominate. However, interruptions to the electricity supply and lack of infrastructure at the rural district level are significant problems in Southern Africa and an out of cold chain approach might represent an important practical component of birth dose implementation.

1.8.2 Evidence for efficacy of HBV vaccine

Population-level data on HBV vaccine efficacy are available for a number of global populations. In China, sequential serosurveys have observed a significant reduction in HBV prevalence among under 5 year olds from 9.7% in 1992 to 0.3% in 2014, based on use of HBV vaccination incorporating a birth dose (Cui et al., 2017). In addition to the introduction of the universal vaccine in 2002, a catch-up campaign was introduced for children under 15 years during 2009-2011.

The HBV vaccine has been introduced throughout Africa between 1994 and 2012. In the Gambia, the Gambia Hepatitis Intervention Study followed up patients vaccinated originally in 1986 and assessed prevalence in 2007-08. Investigators were able to identify 753/2670 (28%) of participants in the original study and who could be linked to vaccination records. HBV prevalence was 0.8% among the vaccinated cohort relative to a pre-vaccination prevalence of 12.4%. The estimated vaccine efficacy was 94% (95% CI 77 – 99%)(Peto et al., 2014) In a rural population in Nigeria, in Edo state, where vaccine coverage for HBV was 55%, vaccine effectiveness of HBV was estimated at 85% (95% CI: 68, 93%) among a sample of children of mean age 7 years, indicating efficacy even with low overall coverage (Odusanya et al., 2011). In South Africa, a review of four cross-sectional or cohort studies from South Africa found HBV prevalence ranging from 0 to 0.1% among vaccinated children relative to an estimated HBsAg prevalence of 7.8% among children in the pre-vaccination era (Spearman and Sonderup, 2014).

There are a limited number of studies looking at the specific impact of addition of a birth dose in sub-Saharan Africa, relative to use of an infant 6, 10 and 14 week schedule as the comparator. In Abidjan, Cote d'Ivoire, in a non-randomised observational study, infants receiving a 0, 6 and 14 week schedule (including a birth dose) were compared to a 6, 10 and 14 weeks schedule (Ekra et al., 2008). Of a total of 5810 mothers enrolled from four health centres, HBsAg prevalence was 7.7%. No difference in HBsAg prevalence was noted between the two groups born to HBsAg positive mothers (5.8% in among birth dose recipients vs 7.8% among routine vaccine group, p=0.5) (Ekra et al., 2008). The wider applicability of this study is limited by the non-random study design.

The hepatitis B vaccine has been tremendously successful in reducing HBV prevalence in sub-Saharan but there is a paucity of high quality community studies and questions remain regarding the efficacy of the additional birth dose. The lack of data specific to the region may represent a barrier to more widespread implementation. A study is currently ongoing in Burkina Faso to address this question using a stepped wedge design with random selection of phased introduction of the birth dose in 24 vaccination centres (https://clinicaltrials.gov/ct2/show/record/NCT04029454), ClinicalTrials.gov number, NCT04029454) (Nayagam et al., 2019). Evidence from this study should help to provide much needed evidence on birth dose efficacy in this population.

1.9. Issues in the Management of Hepatitis B in Sub-Saharan Africa

In view of the projected increase in deaths from liver disease in sub-Saharan Africa over the next decade, adult treatment programmes are needed, to prevent and reduce mortality from cirrhosis and HCC (Spearman et al., 2017). The treatment cascade in terms of proportion with hepatitis B diagnosed, on treatment and with HBV DNA suppression is very low in sub-Saharan Africa with an estimated 0.3% diagnosed, and globally, an average of 7.9% of those diagnosed on treatment (World Health Organisation, 2017a). The WHO introduced guidelines for management of hepatitis B in 2015 yet several data gaps remain for sub-Saharan Africa. The optimal criteria for assessing treatment eligibility have not been ascertained and there are limited data on cost-effectiveness.

In the Gambia, a large community probability sample population survey was conducted from 5980 community residents from 27 rural and 27 urban communities and 5559 blood donors (Lemoine et al., 2016b). HBsAg prevalence was 8.5% in communities and 13.0% in blood donors. In this study, of 402 HBsAg community participants, 4.4% (95% CI 2.5- 7.7) were eligible for treatment by EASL 2015 guidelines (Lemoine et al., 2016b). In a linked study using a mathematical Markov state transition model, the screen and treat community strategy was found to be cost effective with an incremental cost effectiveness ratio of $540 per disability adjusted life year averted and $511 per quality adjusted life year gained (Nayagam et al., 2016a).

Studies assessing the community level need for treatment have not been conducted to date in sub-Saharan Africa outside West Africa. Rates of HBV treatment eligibility might be different in southern Africa, where genotype A is predominant, since it is associated with an increased risk of liver fibrosis progression in the Gambia, relative to genotype E, which is dominant in West Africa (Shimakawa et al., 2016a). West Africa has higher HBV prevalence relative to other African regions this may also affect the cost-effectiveness of community screen and treat programmes (Razavi-Shearer et al., 2018).

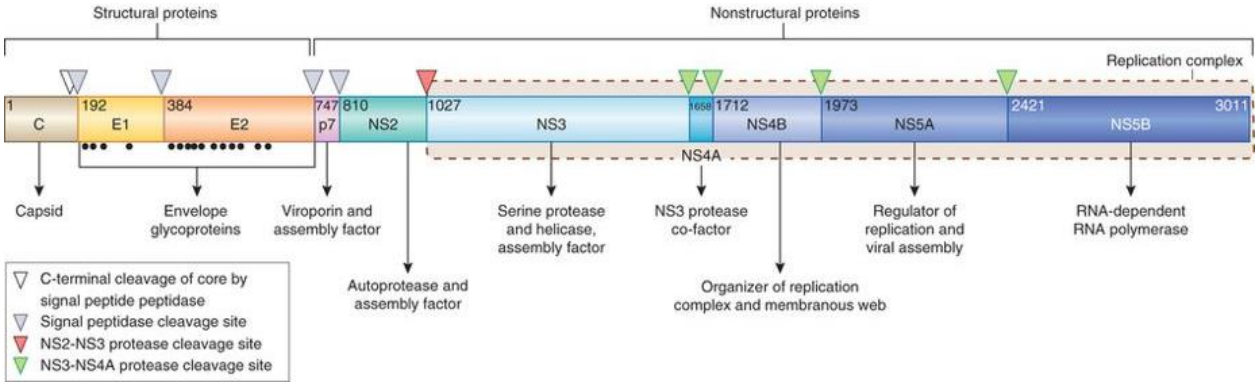
The aims of the present thesis in relation to hepatitis B are to ascertain the prevalence of HBV across different age groups from a community sample in Malawi, to estimate the impact of the HBV vaccine introduced in 2002 and to ascertain rates of treatment eligibility at the community level. The secondary objectives are to recruit an patients with cirrhosis and HCC from a tertiary hospital in Blantyre, Queen Elizabeth Central Hospital, to ascertain the prevalence and population attributable fraction of viral hepatitis B and outcomes. These data will assist in the development of a strategic plan for viral hepatitis control in the country.

1.10. Hepatitis C

1.10.1 Structure and genome

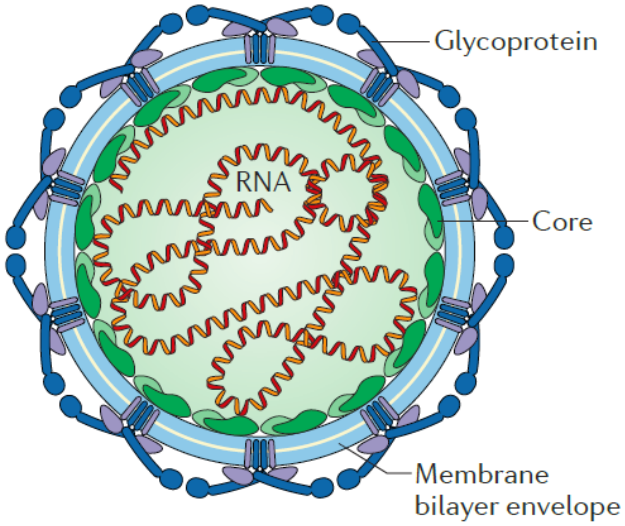
The hepatitis C virus is a positive-sense single stranded RNA virus belonging to the Hepacivirus genus within the *Flaviviridae* family (Scheel and Rice, 2013). Other members of the *Flaviviridae* family include the arthopod borne Flavivirus genus comprising yellow fever, West Nile virus and dengue virus and the Pestivirus and Pegivirus genera (Dubuisson and Cosset, 2014). The HCV genome is approximately 9600 nucleotides and consists of two untranslated regions at the 5' and 3' ends, and a long single open reading frame which is translated to a single polyprotein (Webster et al., 2015). This polyprotein undergoes post-translational processing into structural proteins consisting of core, E1 and E2 proteins which comprise the viral capsid and viral envelope glycoproteins, and non-structural (NS) proteins (Scheel and Rice, 2013). The non-structural proteins consist of p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B proteins and are essential for viral replication and virion assembly (Lindenbach and Rice, 2013) (Figure 1-8).

Figure 1-8: The hepatitis C genome



Reproduced from Scheel and Rice 2013

Figure 1-9: Diagram of HCV virion structure



Reproduced from Lindenbach et al 2013

HCV virions are 50-80nm in size, pleomorphic, and consist structurally of a single-stranded RNA genome, a core protein and with an endoplasmic reticulum-derived lipid bilayer envelope containing envelope glycoproteins E1 and E2 (Catanese et al., 2013) (Figure 1-9). The HCV virion associates with lipoproteins and apolipoproteins, an interaction that may be related evolutionarily to evasion of immune responses through concealment of HCV glycoproteins from antibody neutralisation (Dubuisson and Cosset, 2014). The structure and exact mechanism of the interaction between HCV particles and lipoproteins remains to be fully elucidated, with competing models of a single hybrid particle utilising a shared envelop, or a two-particle model whereby HCV particles transiently interact with lipoproteins (Lindenbach and Rice, 2013).

HCV is associated with markedly high sequence diversity due to the high rate of mutation arising from the error-prone low fidelity viral polymerase NS5B (Adams et al., 2017). To date, eight HCV genotypes and 86 sub-genotypes have been described (Spearman et al., 2019). There is a paucity of available sequence data from southern Africa, with only 15 full-length sequences from sub-Saharan Africa identified in a 2017 report (Niebel et al., 2017). This could have implications for diagnostics and treatment programmes in Africa. Novel genotypes could give rise to diagnostic escape due to primer mismatching in PCR assays, or could be associated with suboptimal treatment responses relative to better-characterised genotypes.

1.10.2 HCV replication life cycle

HCV viral entry is mediated by envelope glycoproteins E1 and E2 and involves a complex and incompletely characterised interaction between viral proteins and associated lipoproteins with several host co-factors. These include the low-density lipoprotein receptor (LDLN), heparan sulphate proteoglycans (HSPG) and hepatocyte surface molecules comprising the CD81 receptor, and several other co-receptors including the scavenger receptor class B member 1 (SRB1), tight junction proteins claudin 1 (CLDN1) and occludin (OCLN) and cholesterol absorption reception Niemann-Pick Ci-like 1 (NPC1L1) (Lindenbach and Rice, 2013).

Following entry to the cytoplasm, the HCV genome is translated into a single polyprotein which undergoes post-translational processing by host cellular, and viral proteases into ten proteins: structural proteins core, E1 and E2 and non-structural proteins p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B (Scheel and Rice, 2013). HCV non-structural proteins are essential for mediation of assembly processes (Adams et al., 2017). NS2 is a viral protease responsible for cleavage from NS3 and p7 and NS2 are essential components of infectious virion production (Jones et al., 2007). NS3 is the major viral protease and cleaves downstream NS proteins at four sites in association with its activator, NS4A (McGivern et al., 2015). NS3 additionally disrupts interferon responses by cleaving two cellular proteins MAVS (mitochondrial antiviral signalling protein) and Toll-interluekin-1 receptor domain containing adaptor inducing IFN-β (TRIF). This leads to disruption of interferon signalling retinoic acid-inducible gene I (RIG-1) and Toll-like receptor 3 pathways (Liang et al., 2008). NS4B is essential for HCV replication, and NS5A is essential for replication and virion assembly (Hoshida et al., 2014). The viral RNA polymerase NS5B acts as the RNA synthesis enzyme and is highly error-prone, leading to high rates of HCV genomic variation. After replication involving a negative-sense RNA intermediate, replicate positive strand RNA genomes are translated into new viral proteins and serve as RNA templates, and are assembled into new infectious virions (Dubuisson and Cosset, 2014). Viral assembly occurs in the endoplasmic reticulum where viral particles transit through the secretory pathway and are exocytosed (Lindenbach and Rice, 2013).

1.10.3 Pathogenesis, natural history and spontaneous clearance

HCV infection can resolve in the absence of treatment, a process termed spontaneous clearance (Grebely et al., 2012). Primary infection in most cases is asymptomatic, with symptoms reported in around 15% of patients with HCV infection (Maheshwari et al., 2008). A systematic review and meta-analysis of 43 studies including 20,110 individuals with HCV, estimated pooled rates of spontaneous

clearance at 12 and 24 months were 36.1% (95% CI 23.5- 50.9) and 37.1% (23.7- 52.8), respectively (Aisyah et al., 2018). People infected with HCV who had not cleared at 12 months were unlikely to do so. HCV clearance was less likely among males and those with HIV co-infection, older age, black individuals, people who inject drugs and those with a history of alcohol excess (Aisyah et al., 2018). Several host genetic factors were associated with HCV spontaneous clearance. These include several interleukin-28B (IL28B) polymorphisms. IL28B encodes interferon lambda 3 and has a well-characterised biological role in clearance of viral infections through signalling type III interferon receptors and stimulating JAK-STAT (Janus Kinase-signal transducers and activators of transcription) pathways (Thomas et al., 2009).

HCV causes chronic hepatitis and this can lead to cirrhosis and HCC (Spearman et al., 2019). Fibrosis progression occurs over a period of decades following infection. In a systematic review which used a Markov maximum likelihood estimation model, stage transition probabilities between METAVIR fibrosis scores were estimated. Of 111 included longitudinal studies of chronic HCV comprising 33,121 people with HCV infection, pooled estimated prevalence of cirrhosis after 20 years of infection was 16% (95% CI 14-19) (Thein et al., 2008). Interestingly, the authors observed that progression through fibrosis stages was non-linear. Transition between F0 to F1 occurred faster than the successive stage from F1 to F2, and progression from F2-F3 occurred at the fastest rate. The authors speculated that histological fibrosis staging may not reflect equal increments of fibrosis progression (Thein et al., 2008). Risk factors for accelerated disease progression in HCV infection include male gender, increasing age, increasing alcohol consumption, and co-infection with HIV (Freeman et al., 2001).

Unlike in HBV infection, with HCV, development of hepatocellular carcinoma occurs in people with established cirrhosis and only rarely among people with lesser degrees of hepatic fibrosis (Hoshida et al., 2014). As a result the treatment options are frequently limited among individuals due to advanced liver disease, such as surgical curative therapy or liver transplantation for HCV-associated HCC. HCV has been associated with a number of specific oncogenic effects including inhibition of tumour suppressor genes by the core protein, NS3, NS5A and NS5B (Kao et al., 2004, Deng et al., 2006). HCV proteins have hepatocarcinogenic effects, such as generation of reactive oxygen species, and induction of cellular proliferative pathways (Hoshida et al., 2014).

1.10.4 Diagnosis of HCV

HCV is diagnosed by the detection of core antigen and/or antibodies directed at several non-structural proteins as an initial screening test, using ELISA or chemiluminescent immunoassay (CLIA), followed

by confirmatory testing with molecular detection of HCV RNA. Third-generation anti-HCV assays detect antibodies directed against multiple viral targets including the core region, and NS3, NS4 and NS5 proteins. Fourth generation assays additionally detect HCV core antigen in a combined antibody/ antigen assay (HCV Ag/Ab) (Irshad et al., 2013, Kamili et al., 2012). The addition of antigen detection improves detection of early infection, shortening the "window" period from infection to detection (Schnuriger et al., 2006, Laperche et al., 2005). In a study of HIV/HCV infected patients who were diagnosed during acute HCV infection, HCV Ag/Ab testing resulted in a mean delay of 27 days after the time of the first HCV RNA positive result, relative to a delay of 77 days by antibody detection alone (Schnuriger et al., 2006). Similarly, in a study of blood donors and haemodialysis patients who experienced HCV infection while under longitudinal follow up, HCV Ag/Ab was detected a mean of 21.6 days prior to the most sensitive anti-HCV assay and a mean of 30.3 days after first HCV RNA detection (Laperche et al., 2005).

An alternative approach to the traditional two-step procedure is to use core antigen (HCVcAg) detection alone. This has been associated with lower sensitivity relative to HCV RNA assays. In a French study, HCV antigen had a clinical sensitivity of 100% (95% CI 97.8 – 100) among 514 patients and HCVcAg levels correlated with HCV RNA concentration. The lower limit of detection of the assay corresponded to an HCV RNA concentration of 1000 IU/ml, assessed among genotypes 1-4 (Chevaliez et al., 2014). Detection of HCVcAg may represent a potential future direction for low-resource HCV diagnosis, but currently commercially available assays are limited to automated immunoassay platforms which are not widely available particularly in decentralised laboratories in rural areas of low and middle income countries, where the majority of the population reside.

In sub-Saharan Africa, anti-HCV detection has been associated with a particularly high rate of false positivity, relative to confirmation by HCV RNA PCR. In a meta-analysis of sub-Saharan African HCV prevalence studies, among 35 cohorts, PCR testing confirmed HCV in 51.3% of anti-HCV positive patients, with substantial heterogeneity in observed rates of PCR confirmation (Rao et al., 2015). Among studies that examined anti-HCV positive, HCV RNA negative patients, in African cohorts, the majority of patients had evidence of a false positive result, rather than a result of spontaneous clearance, by use of line immunoassay (Mullis et al., 2013, King et al., 2015, Twagirumugabe et al., 2017). A study from the Centres for Disease Control and Prevention in the US, identifying an association between sample to cut off ratio (S/CO) of anti-HCV ELISA and CLIA tests, sought to identify optimal S/CO thresholds to prevent unnecessary confirmatory testing (Kamili et al., 2012). Based on a study of 25,000 serum samples from diverse US populations, where prevalence ranged from 0.8 to 25%, cut-offs were derived which predicted true-positive viraemic infections with a probability

exceeding 95% for six Food and Drug Administration (FDA)-approved HCV assays, ranging from a S/CO of >3.8 to >11 (Kamili et al., 2012). Similar cut-offs could be developed for sub-Saharan African populations with potential resource saving implications, by cutting unnecessary confirmatory testing.

Barriers to HCV diagnosis in low and middle income settings are similar to the challenges of HBV diagnosis. Standard diagnostic approaches comprise collection of venous blood by venepuncture, cold storage of serum samples at -20°C or below, batching of samples and testing by ELISA or CLIA to detect anti-HCV followed by confirmatory testing for HCV RNA. In high-income settings this is usually performed using automated molecular diagnostic platforms with robotic nucleic acid extraction and preparation and PCR set up with automated calibration. Automated high throughput systems are expensive, require a reliable electrical supply and frozen or cold shipment of reagents, access to regular maintenance and repair and sufficient laboratory capability (Chevaliez and Pawlotsky, 2018). These cost and logistical issues limit the potential for most people in sub-Saharan Africa to access high quality hepatitis B or C diagnostic tests. Recent innovations in diagnostics include the introduction of point of care rapid diagnostic tests, use of dried blood spots as a sample transportation medium, and use of nucleic acid detection platforms that have been adapted for low-resource settings (Peeling et al., 2017, Easterbrook et al., 2017).

The WHO has defined criteria for ideal diagnostic tests for rapid diagnostic tests designed for use in low and middle income settings: the “ASSURED” criteria. These are affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free and deliverable to end-users (Kosack et al., 2017). Rapid diagnostic tests for HBsAg, HBeAg and anti-HCV are widely available and several have been pre-qualified for use by the WHO. These tests are based on the principle of lateral flow or immunochromographic testing where antigen or antibodies are anchored to a pad along an absorbent test strip and interact with the sample, often using whole blood acquired from a fingerprick sample at the point-of-care (Chevaliez and Pawlotsky, 2018). Limitations of these assays include the potential for error due to subjective interpretation, and reduced sensitivity relative to laboratory ELISA or CLIA. Recently, oral fluid testing for anti-HCV has become available. This has been used to lower barriers to access for HIV testing, allowing individuals to self-test, and facilitating patient autonomy and testing among hard to reach groups (MacPherson et al., 2014). It could represent an opportunity to expand also HCV testing. The reported sensitivity of current WHO pre-qualified anti-HCV RDT assays is very good, exceeding 99.5% in real-world evaluations (Easterbrook et al., 2017).

Commercial point of care HCV RNA detection assays are emerging and may be particularly well-suited to low and middle-income country settings. The GeneXpert HCV RNA Viral Load assay may be used

together with the recently developed GeneXpert Omni system (Cepheid, Kempton Park, South Africa), which is a lightweight (1kg) battery powered system with a small bench footprint, designed for decentralised testing (Chevaliez and Pawlotsky, 2018). This assay has been WHO pre-qualified and validated among genotypes 1-6 with a lower limit of detection of 4IU/ml (Gupta et al., 2017). Other point of care molecular assays such as the Genedrive (Genedrive Diagnostics, Manchester, UK) or the Alere q (Abbott, Chicago, IL, USA) are similar small and portable automated cartridge-based systems currently in development (Chevaliez and Pawlotsky, 2018). These emerging technologies represent a potential for expanded access to diagnosis and treatment in low-income settings.

An important principle of adoption of different diagnostic test strategies is to ensure that any proposed diagnostic tests are evaluated first in the population where they are intended to be used, rather than relying upon validation data from other populations which may not be applicable. Differences in host characteristics, genetics or environment, the prevalence of disease and pre-test probabilities of disease, and prevalence of co-infections or diseases that could interfere with test performance, and perhaps most fundamentally, genetic variation in the viral target may all alter diagnostic test performance (Chevaliez and Pawlotsky, 2018).

1.10.5 Epidemiology of hepatitis C

An estimated 71 million people have active viraemic infection with hepatitis C globally, with 1.75 million new infections in 2015 (World Health Organisation, 2017a). The rate of incident infections exceeded the number of people dying from HCV-associated liver disease and being cured with antiviral therapy, illustrating the need for increased effort to scale up HCV diagnosis and treatment (World Health Organisation, 2017a).

Historically, HCV has been transmitted in low and middle-income countries as a result of unsafe healthcare associated injections, and from injection drug use (Webster et al., 2015). HCV globally is transmitted predominantly through blood transfusion and reuse or sharing of contaminated needles (Spearman et al., 2019). In 2000, the WHO estimated that of 16 billion injections worldwide for healthcare purposes, 40% involved reuse of injection equipment such as the syringe or needle, with informal cleaning of components and re-use or reintroduction of the same injection equipment into multi-dose vials, presenting a risk of nosocomial HCV transmission (World Health Organisation, 2016c, Hauri et al., 2004). WHO now recommends using safety engineered auto-disposable syringes with non-removable needles to prevent re-use, and to introduce sharps injury prevention devices into injection devices (World Health Organisation, 2016c).

People who inject drugs (PWID) represent an important reservoir of HCV infection globally (Spearman et al., 2019). An estimated 17.8% (95% CI 10.8- 24.8) of PWID are infected with HCV, illustrating the central role injecting drug use has in HCV transmission (Degenhardt et al., 2017). A systematic review estimated that 15.6 million people (95% CI 10.2- 23.7) inject drugs globally with a male predominance (3.2 million women and 12.5 million men), based on a pooled random effects meta-analysis of reports of injecting drug use prevalence from 179 countries or territories (Degenhardt et al., 2017). This equates to 8.2 million (95% CI 4.7 – 12.4) PWID with hepatitis C infection. In the review it was noted that injecting drug use is associated with both homelessness and incarceration, highlighting the need for holistic considerations beyond diagnosis and treatment to effect disease control.

A transmission rate from mother to child among HCV infected mothers of 6% has been observed (Benova et al., 2014). Sexual transmission infrequently occurs between heterosexual couples, but transmission between men who have sex with men (MSM) and in particular with high risk sexual practices such as group sex or receptive anal intercourse without condom use have been frequently observed (Spearman et al., 2019). Support for sexual transmission among MSM has been provided by phylogenetic studies demonstrating transmission clusters among international MSM networks (van de Laar et al., 2009).

Natural immunity does not confer protection against re-infection, and re-infection has been observed frequently in MSM cohorts following treatment. Reinfection among high risk groups represents a threat to the success of treatment as prevention elimination strategies (Rossi et al., 2018). A mathematical modelling study estimated the potential impact of different public health interventions, based on existing epidemiological and empirical data on efficacy of different interventions (Heffernan et al., 2019). The proportion of new infections expected in people who inject drugs was 29% (95% credible interval (CrI) 27-32) over the period between 2016 and 2030. The authors projected that implementation of harm reduction services (opiate substitution and needle and syringe programmes for people who inject drugs (PWID)), initiation of directly acting antiviral (DAA) drugs at the time of HCV diagnosis, and outreach screening and diagnosis programmes together could reduce incident HCV by 81% (95% credible interval 78-82) by 2030 and mortality by 61% (95% CrI 60-62), just short of the WHO targets (World Health Organisation, 2016a). The most dramatic decline in mortality was associated with offering directly acting antivirals to all patients at diagnosis and implementation of outreach screening (Heffernan et al., 2019). The limitations of this model relate to the quality of the input data, which was a mixture of assumptions and expert opinions and for many countries involved extrapolating data from neighbours (Wiktor, 2019). The effects of HCV treatment are two-fold; as well

as reducing the risk of liver-related mortality in treated individuals, it also reduces the risk of onward transmission.

Expansion of coverage of HCV screening and treatment to meet WHO targets relies upon adoption of a number of innovative methods for case finding. In high prevalence countries such as Egypt with generalised epidemics, one-time whole population screening has been advocated, or generational specific screening such as in the USA. In countries with focused epidemics concentrated in individuals with specific risk factors, screening activities are targeted at these groups. The two-step anti-HCV followed by HCV RNA testing algorithm has been associated with loss to follow up particularly among hard-to-reach groups such as PWID and reflex testing of all anti-HCV positive cases and innovative approaches such as use of dried blood spots for sample collection or point of care RNA testing are recommended (Chevaliez and Pawlotsky, 2018).

1.10.6 Epidemiology of HCV in sub-Saharan Africa

In sub-Saharan Africa, an estimated 1.0% (95% CI 0.7- 1.6) of the population is infected with viraemic HCV infection, equating to 11 million people (95% CI 7 – 16 million) (World Health Organisation, 2017a). In southern sub-Saharan Africa, the estimated viraemic HCV prevalence is 0.7% (95% CI 0.4-0.9), or 0.5 million people (95% CI 0.3 -0.7) (Polaris Observatory, 2017). In southern Africa, there are no previous community based probability sampling studies of HCV prevalence and estimates are extrapolated from convenience samples (Polaris Observatory, 2017). In key populations at increased risk of HCV such as people who inject drugs (PWID) and men who have sex with men (MSM), there are only limited assessments of prevalence.

The HCV epidemic has been associated with nosocomial infection from healthcare from the reuse of needles. In Egypt, HCV is associated with public health campaigns to treat schistosomiasis using parenteral therapy (Webster et al., 2015). HCV has also been associated with scarification employed for cultural purposes or in traditional medicine (Garve et al., 2017). Currently in sub-Saharan Africa, the progress toward cure has been estimated as net -2.1%, per annum reflecting that incident chronic infections exceed individuals dying of HCV or accessing cure with antiviral therapy (Hill et al., 2017).

A critical step towards implementation of HCV treatment programmes will be improved community level prevalence data from the general population and from specific at-risk groups to identify where to optimally deploy screening and treatment activities.

1.10.7 HCV treatment

Due to the lack of host integration associated with the HCV life cycle, antiviral therapy can result in cure, with clearance of replication-competent virus. HCV cure is assessed by confirmation of no detectable HCV RNA by PCR at 12 weeks after completion of therapy, termed sustained virological response (SVR12) (Spearman et al., 2019).

Treatment of HCV is associated with improved survival, improvements in patient-reported quality of life and a reduction in risk of HCC (Carrat et al., 2019, Younossi et al., 2018a). The WHO recommends that all patients on diagnosis of viraemic HCV infection aged over 12 years should receive treatment as part of an elimination strategy, regardless of stage of liver disease (World Health Organisation, 2018). Genotypic characterisation permits genotypic specific therapy and informs anticipated SVR rates but access to genotyping is limited in low and middle income countries. The WHO recommends use of pan-genotypic directly acting antiviral (DAA) therapy to circumvent the need to perform genotyping (World Health Organisation, 2018).

Targets of directly acting antivirals include the NS3/NS4 protease complex (with compounds suffixed -previr) , the NS5A assembly protein (suffix -asvir) or the NS5B polymerase (suffix -buvir). Since 2014, a large number of directly acting antiviral regimens have been licenced and approved for use as an interferon free regimen and rates of SVR12 now frequently exceed 95% across genotypes, HIV co-infection status, and even among patients with cirrhosis (European Association for the Study of the Liver, 2018b). Combination regimens typically include a NS5B inhibitor such as sofosbuvir, which acts as a chain terminating nucleoside analogue with a high genetic barrier to resistance, together with an NS5A inhibitor such as ledipasvir or velpatasvir (Spearman et al., 2019). Numerous and complex drug-drug interactions have been associated with HCV treatment and a comprehensive evaluation of potential interactions with existing medicines is recommended before treatment (European Association for the Study of the Liver, 2018b). Currently the WHO recommends sofosbuvir/ velpatasvir, sofosbuvir/ daclatasvir or glecaprevir/ pibrentasvir as empirical pan-genotypic regimens with duration based on regimen and increased for patients with cirrhosis (World Health Organisation, 2018).

1.11. Summary

Hepatitis B and C pose serious public health threats in sub-Saharan Africa. To tackle epidemics of these infections and to prevent a projected increase in cirrhosis and HCC-related deaths in the next 20 years will require a concerted effort to screen and treat people for HBV and HCV. These efforts must be informed by accurate epidemiological data in representative samples and in specific at-risk population groups.

In this thesis I aim to:

a. Study the prevalence of hepatitis B and C in a general population cohort in Blantyre, Malawi using random probability community sampling based on a demographic census
b. Use the HBV prevalence survey to estimate the impact of HBV vaccination, which started in 2002 in Malawi, by comparison of prevalence among children born before and after vaccine introduction
c. Assess the proportion of adults with HBV infection who require HBV treatment according to clinical eligibility criteria
d. Determine the aetiology, attributable fraction of HBV and HCV and outcomes of patients with HCC and cirrhosis in Queen Elizabeth Central Hospital, Blantyre
e. Assess the performance of biomarkers commonly used to assess hepatic fibrosis as part of treatment eligibility criteria including the APRI score and the GPR score
f. Ascertain the diagnostic performance of HBeAg rapid tests, used to classify HBV disease

It is hoped that the results of this study will inform the public health response to HBV and HCV within Malawi and regionally, provide much-needed data in a region where there is a paucity of existing epidemiological data and facilitate the introduction of a HBV treatment programme.

Chapter 2. Setting and Methods

2.1. Malawi

Malawi is a landlocked country located in southeast Africa, bordered by Mozambique, Zambia and Tanzania. According to the Malawi Population and Housing Census, the population was 17.5 million in 2018, which has increased by 35% over the last decade from 13.0 million in 2008 (National Statistical Office of Malawi, 2018). The median age of the population is 17 years and 15% are aged under 5 years (National Statistical Office of Malawi, 2018). The country area is 118,000 km^2 with a population density of 192/km^2, which is the highest in the South African Development Community grouping and sixth highest in sub-Saharan Africa, excluding island nations (World Bank, 2019). Overall 83.1% of the population live in rural areas (Food and Agriculture Organisation of the United Nations, 2018). Malawi is widely known as the warm heart of Africa for the kindness of its people. In the Gallup 2016 Global Civic Engagement report, based on a survey of 1000 adults per country, the people of Malawi were the most likely in Africa, and the third most likely in the world, to help a stranger (Gallup, 2016).

Malawi is one of the poorest countries in the world, with per capita gross domestic product (GDP) of US $389 in 2018 (World Bank, 2019). Between 2009 and 2018, annual income growth per capita was an average of 1.6% (World Bank, 2019). Based on data from the Integrated Household Survey 2016-17, an estimated 71.7% of Malawians are living below the international poverty line of USD $1.90, which represents a small decrease from 73.4% in 2004 (National Statistical Office of Malawi, 2017). Very low food security, defined by restriction of food quality, variety, quantity and frequency was reported by 61% of the population, and 66% in urban areas in 2016-17 (National Statistical Office of Malawi, 2017). The major source of export revenue and of employment, comprising 76.9% of the labour force, is the agricultural sector (Central Intelligence Agency, 2019). The main agricultural products are maize, tobacco, tea, and sugar.

Health expenditure per capita for 2016 was USD $30 per year, comprised of donor aid (54%), domestic government expenditure (28%), out of pocket expenditure (11%) and health insurance (3%) (World Health Organisation, 2019a). Life expectancy at birth is 63.7 years, which has been steadily increasing over the past two decades from 46.4 years in 1998 and 54.2 years in 2008 (United Nations Population Division, 2019).

Figure 2-1: Map (A) and satellite image (B) of Malawi

A

B

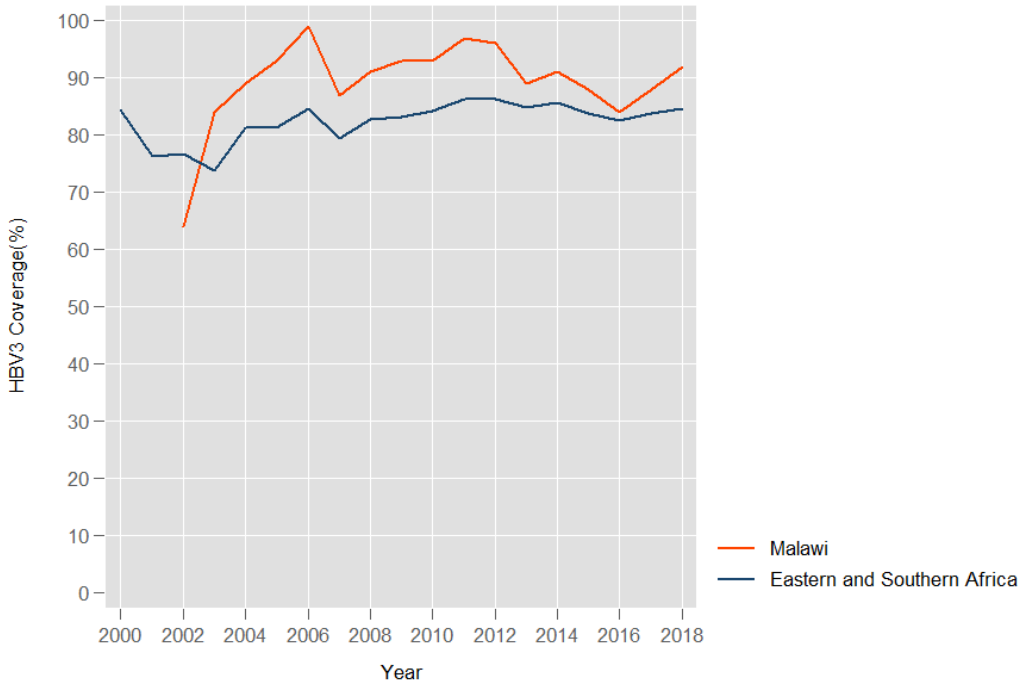
Image source: A: The CIA World Factbook 2019 (public domain) (https://www.cia.gov/library/publications/the-world-factbook/attachments/maps/MI-map.gif) B: NASA Earth Observatory; cropped by Map Library (http://www.maplibrary.org/)

2.2. Epidemiology of HIV in Malawi

A nationally representative household-based survey conducted in 2015-16 estimated HIV prevalence among 15-64 year olds of 10.6% (95% confidence interval (CI) 9.9 – 11.2), with higher prevalence observed among women relative to men (12.5% (95% CI 11.6-13.4) vs 8.5% (95% CI 7.8 -9.2)) (Government of Malawi, 2018). The incidence of HIV among 14-64 year olds was 0.4% (95% CI 0.2 – 0.5) per year or around 28,000 new cases annually. The population-level rate of virological suppression

was 68.3%, with 76.8% of HIV positive people aware of their status, 91.4% of those with HIV aware of their status receiving antiretroviral therapy (ART) and 91.3% of those on ART having virological suppression (2018). In Blantyre city, HIV prevalence was 17.7% (95% CI 16.0 – 19.5) with a virological suppression rate of 59.5% (95% CI 53.9- 65.0).

Figure 2-2: Coverage of 3-dose hepatitis B pentavalent vaccine in Malawi and Eastern and Southern Africa



Abbreviation: HBV3 3 doses of pentavalent infant vaccination including hepatitis B. Data source: United Nations Children's Fund (UNICEF) Immunisation Data 2018 (available at: https://data.unicef.org/wp-content/uploads/2016/07/Immunization-estimates-2018.xlsx)

2.3. Hepatitis B Vaccination Programme in Malawi

The pentavalent infant vaccine comprising hepatitis B together with diphtheria, tetanus, pertussis and *Haemophilis influenza* B, delivered at 6, 10 and 14 weeks of life, was introduced into the routine enhanced programme of immunisation across sub-Saharan Africa between 1994 and 2014, and in Malawi in January 2002. Coverage of the pentavalent vaccine was median 86% (interquartile range (IQR) 73-92) in 2017 in sub-Saharan Africa and 88% in Malawi (Figure 2-2) (WHO-UNICEF, 2018). There are limited existing data on the impact of the hepatitis B pentavalent vaccine in the region. Efficacy was estimated at 94% in the Gambia(Peto et al., 2014) and 85% in rural Nigeria(Odusanya et al., 2011)

and among four cohort studies of vaccinated infants in South Africa, HBV infection rates were 0-1.2% compared to 7.8% in the pre-vaccination era (Spearman and Sonderup, 2014).

Figure 2-3: Aerial photograph of Ndirande township

2.4. Ndirande

Ndirande is an urban township located in the northeast of Blantyre 6km by road from the Queen Elizabeth Central Hospital and 3km from the city centre. Blantyre is the main commercial city in Malawi, located in Southern region. The largest township in Blantyre, Ndirande is an unplanned settlement with high population density and low socioeconomic status relative to Blantyre city (Figures 2-3 and 2-4).

Ndirande is served by a government health centre, located in the centre of the township. This health centre provides general primary medical care, antenatal care and HIV testing and treatment services

for residents without charge, and is staffed by Clinical Officers who have completed a three year diploma in medicine followed by a one year internship.

Figure 2-4: Satellite image of Ndirande township in relation to Blantyre

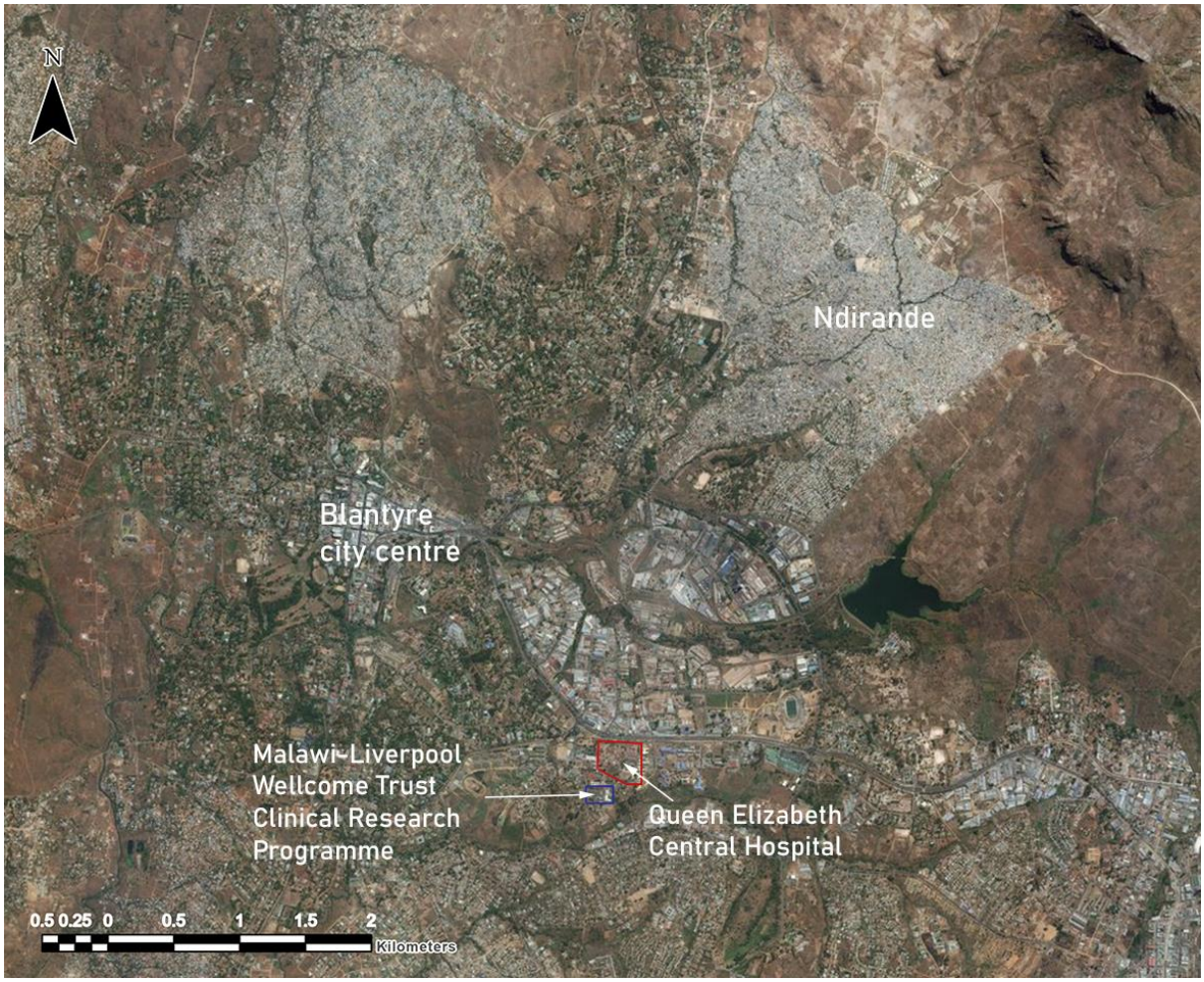


Image credit: ESRI, Digital Globe, GeoEye, Earthstar Geographics, CNS/Airbus DS, USDA, USGS, AeroGrid, IGN and the GIS User Community. Image generated in ArcGIS Pro 2.4 (ESRI). The Malawi-Liverpool-Wellcome Trust Clinical Research Programme is outlined with a blue boundary and the Queen Elizabeth Central Hospital is outlined with a red boundary.

2.5. Queen Elizabeth Central Hospital (QECH)

QECH is the largest government owned and operated hospital in Malawi, serving Blantyre district and acting as a tertiary referral hospital for Southern region, with a total catchment of 7.5 million people.

There were 79,186 admissions, 318,277 inpatient bed days and 379,809 outpatient attendances in Malawi in 2015 (Government of Malawi, 2015).

The Government of Malawi provides medical care, including provision of medicines, free at the point of use. Patients who seek medical care are required to attend a local health clinic to obtain a referral to QECH. Patients who attend the hospital without a referral are requested to attend a local health centre, or are required to pay for access to medical care unless they need emergency medical care.

2.6. Malawi-Liverpool-Wellcome (MLW) Trust Clinical Research Programme

MLW was established in 1995 and is a partnership between College of Medicine, University of Malawi, Liverpool School of Tropical Medicine and the University of Liverpool. It is located next to the Queen Elizabeth Central Hospital and the College of Medicine in Blantyre and is funded by a Wellcome Trust Major Overseas Programme grant. The vision of the organisation is to conduct internationally excellent research to benefit health and to train the next generation of researchers. Laboratory investigations, unless otherwise specified were conducted at the laboratories of MLW. The MLW laboratory is externally quality controlled by participation in the UK National External Quality Assessment Services (UK NEQAS).

2.7. Systematic review of hepatitis B, D and C in Malawi

To evaluate the existing evidence on the epidemiology of viral hepatitis B, D and C in Malawi, a systematic review of prevalence data was conducted.

2.7.1 Literature search

Searches were performed in Pubmed, Scopus and EMBASE using the search terms Malawi and synonymous descriptors of, and diagnostic markers for, hepatitis B, C and D (Table 2-1).

Medical subject headings [MeSH] in Pubmed and EMBASE thesaurus tools were employed. Searches were restricted to publications between 1 Jan 1990 and 1 February 2018 with a search update on 22 June 18, to identify published data from the past 28 years.

Table 2-1: Search strategy for systematic review

Limits: Publication date: 1 January 1990 to 22 June 2018, no language restriction

PUBMED (https://www.ncbi.nlm.nih.gov/pubmed/)
((Malawi AND (hepatitis B[MeSH] or hepatitis C[MeSH] or hepatitis D[MeSH] or Hepatitis, Viral, Human[MeSH] or hepatitis or HBV or HBsAg or or HCV or anti-HCV or HCV antibody or core HCV antigen or HCVcAg or HCV RNA or HDV or anti-HD or anti-HDV or HDV IgG or viral hepatitis)))
SCOPUS (https://www.scopus.com/)
TITLE-ABS-KEY(malawi) AND (TITLE-ABS-KEY (hepatitis B) OR TITLE-ABS-KEY (hepatitis C) OR TITLE-ABS-KEY (hepatitis D) or TITLE-ABS-KEY (Hepatitis) OR TITLE-ABS-KEY (HBV) or TITLE-ABS-KEY (HBsAg) or TITLE-ABS-KEY (HCV) or TITLE-ABS-KEY (anti-HCV) or TITLE-ABS-KEY (HCV antibody) or TITLE-ABS-KEY (core HCV antigen) or TITLE-ABS-KEY (HCVcAg) or TITLE-ABS-KEY (HCV RNA) or TITLE-ABS-KEY (HDV) or TITLE-ABS-KEY (anti-HD) or TITLE-ABS-KEY (anti-HDV) or TITLE-ABS-KEY (HDV IgG))

2.7.2 Inclusion and exclusion criteria

Studies reporting detection of hepatitis B surface antigen (HBsAg), or total or IgG anti-hepatitis delta antibody (anti-HDV) or HDV RNA among HBsAg positive people, or anti-hepatitis C antibody (anti-HCV), hepatitis C core antigen (HCVcAg) or HCV RNA were included, provided they presented details of selection and inclusion criteria and described the laboratory methods used.

2.7.3 Data extraction and quality assessment

The review was conducted in accordance with PRISMA guidelines(Liberati et al., 2009). I extracted details of study design, participant characteristics (age and gender distribution, population group), sampling method, dates, study locations, laboratory test used and prevalence estimates. A quality assessment tool for prevalence estimates was used and study quality was independently evaluated by two authors (AS, CM) with discordance resolved by discussion (Munn et al., 2015).

2.7.4 Statistical analysis for the systematic review

Data were grouped into two categories: “general populations” which provided data from potentially representative community samples, pregnant women, or blood donors; “HIV positive populations” adults or women, or children receiving routine HIV care, and “special groups”, comprising populations likely to be unrepresentative of the general population such as medical inpatients, prisoners or medical students.

Confidence intervals for prevalence were calculated using the Wilson method. Pooled seroprevalence was calculated with the DerSimonian-Laird random-effects model with Freeman-Tukey double arcsine transformation (Freeman and Tukey, 1950). A random effects model was applied due to anticipated heterogeneity. Analyses were performed in Stata release 14.2 (College Station, TX, USA) using the *metaprop* package (Nyaga et al., 2014).

2.8. Inpatient Cohort Study

2.8.1 Screening

Study nurses daily from 8am to 4pm on Monday to Friday screened all patients who were admitted to adult medical or surgical wards or attending the endoscopy unit or general medical clinic of Queen Elizabeth Central Hospital for symptoms or signs of suspected chronic liver disease (CLD) or hepatocellular carcinoma (HCC). These broad, sensitive screening criteria comprised evaluation of patients with any of the following criteria:

1. Clinical features of CLD: Jaundice, ascites, splenomegaly, spider telangiectasia, gynaecomastia, dilated veins of the lower abdominal wall, digital clubbing, asterixis; or
2. Upper gastrointestinal bleeding (UGIB): from oesophageal or gastric varices demonstrated on upper GI endoscopy, or UGIB in patients who had not had an endoscopy and for whom the cause was unknown; or
3. Suspected hepatocellular carcinoma: due to a mass or pain in the right upper quadrant or with a hepatic lesion identified on ultrasound or other imaging

2.8.2 Inclusion criteria

Patients who were screened for potential participation in the study underwent transient elastography and an abdominal ultrasound.

Patients were eligible for inclusion in the study if they met the following criteria:

- Transient elastography (TE) ≥9.5kPa after fasting for at least 3 hours after last meal, subject to exclusion criteria, or
- Hepatic mass >1cm ultrasonographically consistent with HCC identified on ultrasound

Following training in use of TE, study nurses carried out TE under supervision for the first 20 patients and until deemed independently competent, with interval supervised examinations. TE was performed using a Fibroscan Mini 430 (Echosens, Paris, France) in the right anterior axillary line.

Table 2-2 Ultrasound protocol

Structure	**Measurement**
Right lobe of liver	Size measured in sagittal anteroposterior length in mid-clavicular line
Liver parenchyma assessment	i. Normal ii. Heterogenous echotexture, fine scattered hypoechoic and hyperechoic areas iii. Coarse echotexture, irregular pattern
Liver surface outline	i. Normal smooth surface ii. Mild uneven or waveform surface iii. Irregular nodular surface
Left hepatic vein	i. Normal smooth vessel wall ii. Obscured vessel wall with normal diameter iii. Irregular and narrowed vessel
Portal vein	Diameter at porta hepatis in longitudinal plane Peak and minimum flow velocity assessed by pulse wave doppler at caliper angle <60° at porta hepatis
Ascites	Presence of ascites assessed in Morrison's pouch and right paracolic gutter
Spleen	Maximum distance between the most superior margin and inferior margin on a longitudinal section (maximum length). Maximal splenic width: maximal anterior to posterior diameter of spleen on transverse section Splenic vein diameter and presence of splenic varices
Lymphadenopathy	Presence of lymphadenopathy assessed in perihilar, pericolic and para-aortic regions
Inferior vena cava	Assess diameter 1cm inferior to cavo-atrial junction
Overall subjective impression	i. Normal ii. Significant fibrosis (intermediate) iii. Cirrhosis

2.8.3 Ultrasound protocol

In this study, a pragmatic definition of HCC was used, commensurate with the limited available resources and reflecting the lack of availability of contrast enhanced cross-sectional imaging and limited access to histological confirmation in Blantyre. Following training for 6 months and supervised ultrasound examination for one year with >200 patient scans with Dr Karen Chetcuti, Consultant Radiologist, I performed the ultrasound examinations in this study using a standardised protocol with a 4.2MHz curvilinear probe on a CTS 7700 portable ultrasound machine (Shantou Institute of Ultrasonic Instruments, Shantou, China). The ultrasound protocol is shown in Table 2-2. Normal reference ranges for hepatic ultrasound measurements applied in this study are shown in Table 2-3.

Table 2-3 Normal reference values for ultrasound examination

Characteristic	Normal range	Reference
Portal vein diameter	<13mm	(Geleto et al., 2016, Weinreb et al., 1982)
Portal vein peak velocity	16-40cm/s	(McNaughton and Abu-Yousef, 2011)
Pulsatility index (ratio of maximum and minimum PV velocity	>0.5	(McNaughton and Abu-Yousef, 2011)
IVC (measured 1cm inferior to the caval atrial junction)	1.2-1.7cm Dilated >1.7 Markedly dilated >2.5cm	(Lang et al., 2005)
Splenic vein diameter	<0.8cm	(Shen et al., 2006)
Spleen size (maximum spleen length)	8.9- 12.7cm	(Mustapha et al., 2010)
Right lobe sagittal length (craniocaudal diameter) in mid-clavicular line	<16cm	(Sienz et al., 2010)

The ultrasound definition of HCC in this study was a hepatic mass of at least 1cm with a consistent ultrasound appearance including mass effect on surrounding structures and/or macrovascular invasion (typically involving the portal vein). For smaller or indeterminate lesions, repeat ultrasound occurred at intervals of 1-2 months, and enlarging and evolving lesions were considered consistent with HCC.

Where multiple lesions were identified, or where liver metastases were suspected, patients were evaluated to identify a possible primary neoplasm. This comprised: ultrasound of the remaining upper

abdominal viscera, kidneys, bladder, prostate, a chest x-ray, and advice to perform additional examinations where indicated such as breast or prostate examination, bone marrow aspiration, biopsy, magnetic resonance imaging (MRI) or referral for a surgical biopsy.

Guidelines developed for high-income country settings advise use of contrast enhanced multi-phase CT and MRI imaging with supplemental pathological diagnosis for patients with a suspected HCC without cirrhosis (European Association for the Study of the Liver, 2018a). In Malawi, there is one MRI machine available at the University Teaching Hospital where this study was based (0.35 Tesla Signa Ovation Excite, General Electric Healthcare, Chicago, IL, USA) but this provides limited availability for imaging medical patients and contrast is not readily available, a critical component of non-invasive HCC diagnosis. Contrast enhanced computed tomography (CT) imaging is not available in the government health sector in Blantyre and was not available for this study.

2.8.4 Exclusion criteria

The following exclusion criteria were applied:

- Pregnancy (due to manufacturer's recommendation not to use TE)
- History of antiviral treatment for HBV monoinfection, to avoid bias from treatment effects (although participants in receipt of HBV-active agents as part of ART for HIV were eligible)
- Patients with elevated liver stiffness measurements (LSM) for reasons that did not represent cirrhosis (Friedrich-Rust et al., 2016):
    - Ingestion of food <3 hours prior to TE (Lemoine et al., 2014)
    - Acute hepatitis: evidence of alanine transaminase (ALT) elevation > 5 times the upper limit of normal (ULN) and an absence of US features of cirrhosis
    - Cholestasis: elevated alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT)) >2x ULN or evidence of biliary obstruction with dilated biliary tracts on US, and absence of US features of cirrhosis
    - Evidence of right heart failure: peripheral oedema and/or hepatomegaly with US findings of dilated inferior vena cava (IVC) >2cm measured 1cm inferior to the cavoatrial junction with dilated hepatic veins, and absence of US features of cirrhosis

Where US features of cirrhosis were defined as: (Aube et al., 1999, Hung et al., 2003, Lin et al., 1993, Shen et al., 2006)

- Heterogenous or coarse liver parenchymal echotexture

- Waveform or irregular, nodular liver surface
- Narrowed or tortuous hepatic veins, assessed at left hepatic vein
- Small liver with hypertrophy of caudate lobe
- Dilated portal vein and/or splenic vein, presence of splenic varices
- Presence of ascites, assessed in right paracolic gutter and Morrison's pouch
- Reduced portal vein flow velocity
- Presence of splenomegaly (>12.7cm in maximal length)(Mustapha et al., 2010)

2.9. Procedures in the inpatient study

2.9.1 Demographic and risk factor questionnaire

Inpatients with cirrhosis and HCC underwent a questionnaire eliciting risk factors for liver disease and HBV and HCV transmission, and ascertaining details of previous medical problems, the clinical symptoms and signs associated with the current admission and the use of medicines. Alcohol consumption was recorded using the WHO AUDIT tool (World Health Organisation, 2001). The AUDIT tool is an internationally validated screening tool to elicit alcohol consumption in the preceding year. It comprises 10 items with ordinal categorical responses assessing hazardous alcohol use, dependence and harmful use. The sum of the scores provides an overall score which can be interpreted categorically into hazardous, harmful and dependence. It has been used in multiple studies in sub-Saharan Africa to assess alcohol consumption and alcohol dependence (Luchters et al., 2011, Medley et al., 2014, Wandera et al., 2015). This tool was chosen because many people in Malawi drink locally brewed spirits (*kachasu*) which do not have a standardised alcohol content, or maize-based fermented drinks whose alcohol content is variable. As such it is often difficult to quantify alcohol using standardised alcohol content descriptors developed for Western settings.

2.9.2 Clinical examination and investigations

Individuals who consented to participate underwent clinical examination to elicit signs of chronic liver disease and were tested for HIV and schistosomiasis in accordance with the community study procedures as described above. Urinary protein, to look for potential contraindication to tenofovir, was measured by dipstick (Meditest Combi, Germany). Participants reporting symptoms suggestive of malaria (fever, malaise, headache or myalgia) were tested using a rapid diagnostic test (Paracheck Pf-

Device v3, Orchid Biomedical Systems, Goa, India). Female participants had a urinary pregnancy test (OneStep Pregnant Test, SureScreen Diagnostics, UK).

Following enrolment, I undertook a detailed clinical assessment involving review of the medical notes, liver function and haematology test results, fibroscan results, and a study ultrasound as described in section 2.8.3 to determine whether patients met study inclusion and exclusion criteria. Where there was uncertainty about interpretation of ultrasound images, a second opinion was sought from an experienced Consultant Radiologist in Malawi (Dr Karen Chetcuti) or remotely from Liverpool using recorded images (and Dr Liz Joekes).

2.9.3 Inpatient participant follow up

Participants in the inpatient study were followed up monthly by telephone for six months. Details of participants and nominated next of kin contacts were recorded. Field workers conducted home visits for participants who could not be reached by phone, to ascertain outcomes of death and readmission to hospital.

2.10. Demographic Census and Serosurvey

The STRATAA study is a multi-centre observational study aiming to improve understanding of the burden of enteric fever and Salmonella exposure by addressing uncertainties in pathogen exposure, transmission and susceptibility (Darton et al., 2017). One of the three study sites is Ndirande. Ethical permission for the STRATA study was obtained from the National Health Sciences Research Committee (Protocol #15/5/1599).

Between 4th July and 18th October 2016 (107 days), the STRATAA study team conducted a demographic census in Ndirande. The geographic area corresponding to the Government of Malawi Health Surveillance areas for Ndirande were used to demarcate the target population. Census fieldworkers attended each household and a key informant provided written informed consent for participation in the study. At the time of the census, demographic information on all residents within the household and GPS co-ordinates for the household were gathered using a Garmin eTrex 30x (Olathe, KA, USA). Data were recorded in an electronic tablet using Open Data Kit (ODK) (Hartung et al., 2010).

2.11. Serological Survey

Individuals were selected from the census for invitation to participate in the serosurvey conducted between 14th December 2016 and 12th April 2018, using single stage without-replacement probability sampling, with age stratification to oversample younger children.(Table 2-4) (Darton et al., 2017)

Table 2-4: Age stratification used for randomisation of participants in the STRATAA serosurvey[a]

Age group	Sample size (n)
0–4 years	2500
5–9 years	1300
10–14 years	800
>14 years	3900
Total	8500

[a] Reproduced with permission (Darton et al., 2017)

If a randomly selected individual in the serosurvey could not be located or did not consent to participation, in the first instance a household member from the same age-stratification group was requested to participate if available, or secondarily, a further randomisation from the same age stratum from the census population was undertaken to select a replacement. Sample size calculation was based on requirements to ascertain typhoid antibody seroconversion, and targeted recruitment of 8500 (Darton et al., 2017). Vaccination data of children aged ≤10 years were obtained by examining the parent-held child health passport, and if unavailable, parents or guardians were asked to report vaccination status if known.

Venous EDTA samples were collected in participants' households by research nurses, stored in cool boxes and transported to the study laboratory at MLW from the field twice per day. After centrifugation, plasma samples were stored at -80°C.

2.11.1 Testing for hepatitis B surface antigen (HBsAg)

Serosurvey plasma samples were tested for HBsAg using a laboratory ELISA (Monolisa HBsAg ULTRA (Bio-Rad, Marnes-la-Coquette, France) in accordance with manufacturer instructions. Among individuals with intermediate or low-positive results, with a sample to cut-off ratio (S/CO) of between

0.9 and 4.0, samples were retested for confirmation. The assay is a 4th generation one-step sandwich ELISA using three solid phase monoclonal antibodies and peroxidase monoclonal and polyclonal antibodies in conjugate selected for their ability to bind to various subtypes of HBsAg, including HBsAg mutants. The assay is CE (Conformité Européenne)-marked in conformity with European Union Directive 93/42/EEC for use as an in-vitro Medical Device (IVMD).

An evaluation of HBsAg assays conducted by the International Consortium for Blood Safety using a panel of 394 samples and including diverse genotypes A-F showed for this assay an analytical sensitivity of 0.017 to 0.059 IU/ml depending on genotype, a 100% (95% confidence interval (CI) 97.5 – 100) clinical sensitivity based on a clinical panel of 146 geographically diverse HBsAg positive samples, including 32 samples with multiple S gene mutations, and a specificity of 99.5% (95% CI 97.2-100) from 200 HBsAg negative samples (Scheiblauer et al., 2010). A study of commercial HBsAg assay performance showed that, based on a National HBsAg reference panel from the French Society of Blood Transfusion, this assay had an analytical sensitivity of 0.08 IU/ml. The Monolisa HBsAg ULTRA assay was able to detect samples with multiple HBsAg vaccine escape mutations produced by site-directed mutagenesis (n=12) and 16 of 17 natural undiluted samples with multiple HBsAg mutations (Ly et al., 2006). WHO criteria for HBsAg prequalification of ELISAs are a lower limit of detection of HBsAg, sensitivity and specificity of <0.13 IU/ml, 100% and >98% for blood screening and <4.0 IU/ml, 100% and >98% for clinical diagnosis, respectively (World Health Organisation, 2016d).

2.11.2 Testing for hepatitis C

For HCV testing, the sample size required to estimate the prevalence of HCV was calculated using the formula:

$$n = \frac{(Z)^2\, p(1-p)}{e^2} \Big/ \left[1 + \frac{(Z^2 p(1-p)/)}{(e^2 N)}\right]$$

Where Z is the standard normal distribution (z-score) for a given confidence level (where for a 95% confidence interval, Z=1.96), p is the expected true proportion and e is the desired precision (half width of CI). A finite population correction is applied by this formula, where N is the known overall size of the population, in this case, the size of Ndirande from the demographic census.

Based on previous estimates of anti-HCV prevalence from Malawi in the preceding 20 years ranging from 0.5% to 10.0% (Stockdale et al., 2018), we calculated sample size requirements based on a

conservative prior prevalence of 10% with 1.5% precision and 95% confidence, with an estimated sample size of 1537. We randomly selected 1661 individuals aged over 16 years from the total serosurvey population for HCV testing, representing 51% of the total serosurvey population over 16 years, which provided 8% oversampling.

Serosurvey samples were tested for hepatitis C using a 4th generation combined antibody/antigen laboratory ELISA, Monolisa HCV Ag-Ab Ultra, V2 (Bio-Rad). This assay uses a solid phase with two recombinant proteins from the non-structural region (NS3 and NS4), a peptide from the structural region (capsid) and a monoclonal antibody against the hepatitis C capsid. The addition of antigen detection permits earlier diagnosis of HCV among individuals who are recently infected.

Among 1360 serum samples submitted for routine testing in a Spanish hospital, 77 were positive by this assay, while 74 were positive by a comparator assay (Ortho Clinical Diagnostics HCV v3.0, Amersham, UK) (overall agreement 99.5%), while among 183 haemodialysis patients, 17 were positive by this assay and 10 by the comparator. Among the 7 discrepant results, five were positive and two indeterminant by line immunoassay, and three were HCV RNA positive, indicating that these were true positive results and showing improved sensitivity of the HCV Ag/Ab Monolisa assay. (Alados-Arboledas et al., 2007). In an evaluation of the assay in Belgium among high risk groups, the results were completely concordant with two other ELISA assays (Innotest HCV AB IV, Innogenetics, Zwijnaarde, Belgium and Ortho HCV 3.0) among 90 patients of which 27 were HCV RNA positive. The Monolisa HCV Ag/Ab assay detected an additional 4 positive samples that were negative by comparator assays which were also determined as HCV RNA negative, indicating identical sensitivity, with slightly reduced specificity for HCV RNA positive samples in this population (Yagci and Padalko, 2012). In routine clinical practice in a Belgian hospital, the assay had a sensitivity and specificity of 99.4% and 94.9% relative to the AxSYM Immunochemiluminescent automated analyser comparator (Abbott, Chicago, IL, USA) (Yagci and Padalko, 2012). In Yaounde, Cameroon, among 1998 blood donors, relative to confirmatory testing with immunoassay and HCV RNA PCR performed on ELISA or RDT-positive samples, sensitivity of the Monolisa HCV Ag/Ab Ultra was 91.9% and specificity was 98.6%. All 14 HCV RNA positive samples were detected by HCV Ag/Ab ELISA (Tagny et al., 2014). Among a seroconversion panel of 20 French HIV positive patients who were diagnosed with acute hepatitis C virus (HCV) infection, the Monolisa HCV Ag/Ab Ultra detected 13/20 (65%) of patients earlier, an average of 50 days prior to an antibody-only ELISA comparator (Monolisa anti-HCV, Bio-Rad) (Schnuriger et al., 2006). Similar findings have been identified in a haemodialysis and blood donor populations (Laperche et al., 2005). The assay is CE marked as an in-vitro medical device.

Confirmation of active HCV infection was obtained using GeneXpert Xpert HCV viral load (Cepheid, Sunnyvale, CA, USA) run on a GeneXpert IV. The assay has a lower limit of detection of 4.0 IU/ml (95% CI 2.8- 5.2), a lower limit of quantification of 10 IU/ml and uses 1ml of EDTA plasma. The range of linearity is 1 to 8 $\log_{10}$ IU/ml. The assay is fully automated and uses quantitative real time reverse transcriptase PCR with automated RNA extraction, internal on-board low and high quantification standards occurring within an integrated cartridge. The assay is CE marked and WHO pre-qualified. A field evaluation of the GeneXpert in Cambodia showed a sensitivity and specificity of 100% (95% CI 99.2, 100.0) and 98.5% (95% CI 94.8, 99.8) relative to TaqMan HCV Quantitative Test 2.0 (Roche, Risch-Rotkreuz, Switzerland) among 769 patients at an HCV clinic (Iwamoto et al., 2019a). In a tertiary hepatology service in India, relative to the Realtime HCV quantitative assay (Abbott) the GeneXpert HCV showed a good correlation of quantitative concentrations (R^2 = 0.985) with a mean difference of 0.04 $\log_{10}$ IU/ml (95% CI −0.42 to 0.49 $\log_{10}$), sensitivity of 94.4% and specificity of 100% (Gupta et al., 2017).

2.11.3 Statistical analysis of HBV and HCV prevalence

Population HBV and HCV prevalence was estimated using probability sampling weights from the survey age stratification design. Post-stratification iterative proportional fitting using 5-year age groups and sex from the census was used to adjust estimates to reflect the population distribution (Kolenikov, 2014). In a sensitivity analysis the effect of adding geographic area in the post-stratification adjustment to account for variation in response rate across 12 regions in the township was assessed for HBV prevalence. Iterative proportional fitting was conducted using the *ipfraking* package in Stata (Kolenikov, 2019). Where date of birth was unknown, we estimated it as the year mid-point based on reported age. Association between HBsAg and explanatory variables were assessed by binomial logistic regression, applying sampling probability weights, including demographic, educational, marital status, occupational and socioeconomic status (SES) characteristics.

To estimate vaccine impact, HBV prevalence in individuals born in the 5 years prior to vaccine introduction in January 2002 (aged 15- 21 years) was compared with those born in the 5 years after vaccine introduction (aged 10-16 years), and in a sensitivity analysis, compared those born in the 10 years preceding the vaccine (ages 15 to 26 years) with those born in the 10 years after implementation (ages 5 to 16 years). Vaccine impact was calculated as (1-risk ratio (RR)) x 100 where RR was estimated from binomial log-linear regression adjusted for survey weights. For multivariable model selection among participants ≥16 years we considered variables with $p<0.25$ for inclusion and incorporated a

cubic spline variable for age to account for the variation of prevalence with respect to age. To calculate SES, responses to a household economic survey conducted together with the census from 13,717 households were used. This included questions about the number of rooms, type of toilet, water source, ownership of animals, radio, and iron, food adequacy, sleeping material, house and flooring construction materials and use of firewood. A principle component analysis was used to derive relative wealth quintiles for the discrete 44 health-surveillance areas represented in the serosurvey (Fry et al., 2014). In a sensitivity analysis, association between SES and HBV infection was assessed at the household level in the subset of respondents with individual-household SES data. Analyses were conducted in ArcGIS Pro 2.4.1 (Esri, USA) and Stata 16.0 (Statacorp, USA).

2.12. Community Study

2.12.1 Aims of the community evaluation study

Study fieldworkers returned to the houses of participants in the serosurvey who were positive for HBsAg or HCV Ag/Ab and invited them to participate in a community evaluation study. The aims of the community evaluation study were to describe the burden of disease of HBV and HCV and to determine the community-level eligibility for treatment for HBV. Secondary objectives were to evaluate the performance of biomarkers proposed for the determination of treatment eligibility and to assess rapid diagnostic tests for hepatitis B e antigen (HBeAg).

2.12.2 Inclusion criteria

Eligible participants were serosurvey participants who were positive for HBsAg or HCV Ag/Ab, aged over 16 years and with capacity to provide valid informed consent.

2.12.3 Exclusion criteria

Women who were pregnant at the time of the evaluation were excluded, due to recommendations to avoid use of transient elastography during pregnancy, but were invited again to participate from 6 weeks post-partum. Individuals receiving antiviral treatment for HBV monoinfection were excluded. HIV positive participants who were taking ART containing HBV-active components remained eligible for inclusion.

2.12.4 Study procedures

Consenting participants who were positive for HBsAg completed a study questionnaire collecting demographic data and information about risk factors for HBV transmission or acquisition and for liver disease. Responses were recorded using Open Data Kit open-source software on electronic tablets by study field workers (Hartung et al., 2010).

Age- and sex-matched participants were matched ±5 years of HBsAg positive cases, among HBsAg negative individuals from the serosurvey, to facilitate a detailed case-control analysis of factors associated with HBV infection. Similarly 9:1 age-sex matched HCV Ag/Ab negative controls were selected for each HCV Ag/Ab positive individual, with oversampling of controls due to the lower number of HCV Ag/Ab cases identified. If potential controls declined to participate the randomisation procedure was repeated to identify a replacement control.

Trained research nurses performed clinical examination to identify signs of chronic liver disease. HIV testing was offered to participants using rapid diagnostic tests (RDTs) in accordance with national guidelines using Determine HIV (Alere, South Africa) as an initial test with confirmatory testing with a second RDT, Uni-Gold HIV (Trinity Biotech, Ireland). Discordant results were repeated in parallel. Study participants diagnosed with HIV were referred to an HIV treatment clinic for provision of antiretroviral therapy (ART). S*chistosomiasis mansoni* infection was diagnosed using urine circulating cathodic antigen rapid tests (Urine-CCA, Rapid Medical Diagnostics, South Africa). Liver stiffness measurement (LSM) was obtained using transient elastography in the right mid-axillary intercostal space after fasting for >3 hours (Fibroscan 430 Mini, Echosens, Paris, France) (Lemoine et al., 2014). Reliability criteria were IQR/median <0.3 if median LSM >7.1kPa (Boursier et al., 2013). We applied a categorical interpretation of >7.9kPa for significant fibrosis (F2) and >9.5kPa for cirrhosis (F4) for hepatitis B (Lemoine et al., 2016a) and >13.0 kPa for cirrhosis (F4) for participants with HCV (European Association for Study of Liver and Latin American Association for the Study of the Liver, 2015).

2.12.5 Laboratory investigations

Laboratory investigations were performed in Malawi-Liverpool-Wellcome Trust Laboratories in Blantyre (www.mlw.medcol.mw). We tested for HBsAg using Monolisa HBsAg-Ultra (Bio-Rad, France) in accordance with manufacturer instructions as discussed in section 2.11.1. Intermediate samples (ratio 0.9-4.0) were repeated in duplicate. The HBsAg-positive status of community evaluation

participants was confirmed by repeat ELISA. HBeAg, anti-HBe and anti-HDV were tested by ELISA (Monolisa HBeAg-Ab, Bio-Rad and ETI-AB-DELTAK-2, Diasorin, Italy). Liver function tests were tested with an automated analyser (AU480, Beckmann-Coulter, USA) with ALT upper limit of normal (ULN) of 32 IU/L.

2.12.6 Validation of in-house HBV DNA PCR

HBV DNA was quantified using an in-house real-time quantitative PCR calibrated with the 4th WHO standard with a lower limit of quantification of 34 IU/ml. The assay used plasma, collected in BD K2 EDTA tubes, separated by centrifugation at 1500xg for 10 minutes, and frozen at -20°C. Frozen samples were transferred in batches to long term storage at -80°C. The reference standard for absolute quantification was the 4th World Health Organisation (WHO) International Standard for HBV DNA for Nucleic Acid Amplification Techniques (National Institute for Biological Standards and Control, Potters Bar, United Kingdom). A plasma sample from a patient positive for HBsAg by ELISA (Monolisa HBsAg ULTRA, Bio-Rad) was aliquoted and used as a standard, and was quantified and calibrated using the WHO standard. Nucleic acid was extracted using the Qiamp DNA Mini kit (Qiagen, Hilden, Germany). Extraction was performed according to the manufacturer's instructions using 200 µl of plasma. The following modifications were applied: 230µl of 100% ethanol were added following incubation of the lysis buffer and proteinase K and 1 µl of 6 µg/µl poly-A carrier RNA (Qiagen) was added to lysis buffer AL. Extracted nucleic acid was eluted in 60µl of Tris-EDTA buffer, pH 8.0 (Fisher Scientific, Loughborough, United Kingdom) with added poly-A carrier RNA at 10ng/µl (Qiagen). Following extraction, eluted DNA was stored on ice or at -20°C while awaiting PCR. A negative template control consisting of Tris-EDTA buffer from the extraction bench was used to assess for contamination and added to every reaction plate. Primers and probe were adapted from previously published assays (Garson et al., 2005, Ghosh et al., 2016) and targeted a conserved region of the hepatitis B surface gene. Primer and probe locations relative to GenBank reference sequence AB106564.1 are shown in Table 2-5. The amplicon length was 51 nucleotides. The probe used a 5' FAM reporter dye and a ZEN/IowaBlack FQ quencher (Integrated DNA Technologies, Leuven, Belgium).

Table 2-5: Description of Primers and Probe for in-house HBV DNA PCR assay (Garson et al., 2005)

Primer/probe	**Sequence 5' - 3'**	Tm[a]	Location AB106564.1[b]
HBV_Forward	GTG TCT GCG GCG TTT TAT CA	63.1°C	379-398
HBV_Reverse	GAC AAA CGG GCA ACA TAC CTT	63.1°C	456- 476
HBV_Probe	5' FAM- CC TCT KCA T/ZEN/C CTG CTG CTA TGC CTC ATC – 3' IBFQ'		404-430

[a]Primer melting temperature estimated by IDT OligoAnalyser calculator (https://www.idtdna.com/pages/tools/oligoanalyzer). [b]Location relative to GeneBank reference sequence AB106564.1 alignment

PCR plates were prepared using a pipetting robot (Qiagility, Qiagen). Reactions included 25µl TaqMan universal mastermix (ThermoFisher, USA), 400nM of forward and reverse primers and 200nM of the probe. The QuantStudio 7 Flex Real-Time PCR System (Applied Biosystems, Waltham, MA, USA) was used with a 200µl well qPCR plate and thermocycler conditions as shown in Table 2-6. ROX passive reference dye was used.

Table 2-6: Thermocycler Conditions

Step	**Temperature (°C)**	**Time**	**Cycles**
Initial denaturation	95	10 minutes	1
Denature	95	15 secs	42
Anneal/extend	60	1 minute	

The WHO standard was reconstituted to 955,000 IU/ml using molecular biology grade water and diluted 1:10 into five concentrations to 95.5 IU/ml. Sample P004 was diluted in six serial 1: 10 dilutions and run alongside the WHO standard. Both dilutions were run in triplicate (Figure 2-5). The patient sample diluted E^{-4}, was within the measurable linear range of the WHO standard dilutions and was quantified as mean 4.57 $\log_{10}$ IU/ml, and 4.58 $\log_{10}$ IU/ml from six reactions from two separate experiments, with a mean concentration of 4.58 $\log_{10}$ IU/ml. Sample P004 was assigned a value of 8.58 log10 IU/ml. Serial dilutions of the reference sample from E-1 to E-7 were measured in duplicate on each PCR plate alongside patient samples.

The linear range of the assay was assessed with triplicate serial 1:10 dilutions of sample P004 on three separate experiments from 8.58 log_{10} IU/ml to 0.58 log_{10} IU/ml. The assay demonstrated linearity across this range (Figure 2-6). Efficiency was 93.1% and R^2 was 0.996

Repeated serial dilutions of sample P004 were analysed to determine the lower limit of quantification (LLQ) and detection (LLD) of the assay. A probit analysis demonstrated a LLQ of 33.5 IU/ml (95% confidence interval 23.4- 64.2) and LLD of 29.6 IU/ml (95% CI 20.1- 63.4) (Table 2-3)5

Figure 2-5: Serial dilutions of the 4th WHO International standard and reference sample P004

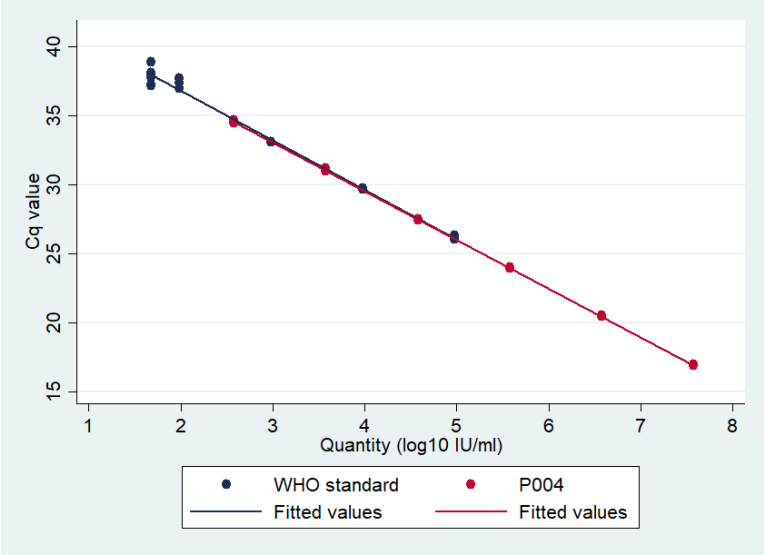


Figure 2-6: Assessment of Linearity of HBV DNA assay

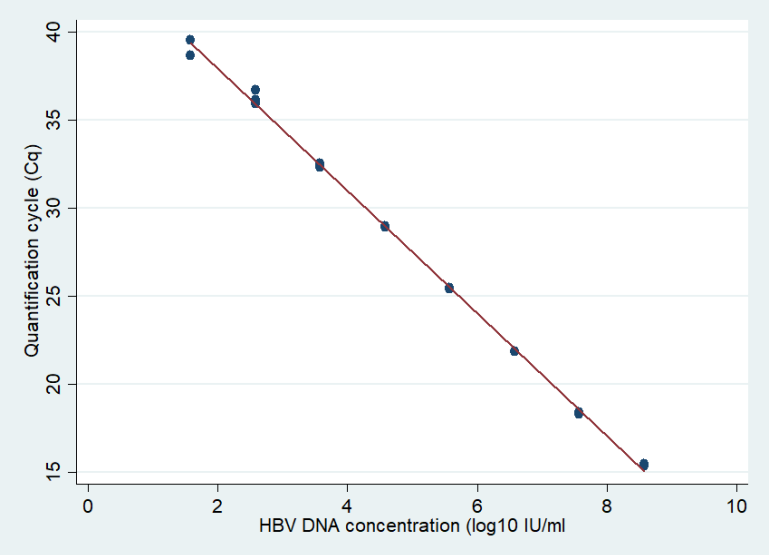


Table 2-5: Analysis of lower limit of quantification and detection of assay using serial dilutions of sample P004

Quantity (log10 IU/ml)	Quantity (IU/ml)	Linear quantification	Detected	Number of replicates
3.58	3783.6	29	29	29
2.58	378.4	31	31	31
2.28	189.2	25	25	25
1.98	94.6	30	30	30
1.58	37.8	35	36	36
1.28	18.9	25	29	30
0.98	9.5	19	19	30
0.68	4.7	11	13	30

2.12.7 HCV sequencing

HCV whole genome sequencing was performed at the Centre for Virus Research, University of Glasgow using a previously described method for target enrichment using probe capture, followed by next generation sequencing using the NextSeq platform (Illumina) among HCV RNA positive participants from the community study and from the hospital study (Chapter 4) (Thomson et al., 2016). RNA was extracted from 200µl of plasma using the Agencourt RNAdvance blood kit (Beckman Coulter, Brea, CA, USA), eluted into 11 µl of water and then reverse transcribed using Superscript III (Invitrogen, Carlsbad, CA, USA) with random hexamers. A NEB Second Strand Synthesis kit (New England BioLabs, Ipswitch, MA, USA) was used for library preparation with the KAPA Library Prep kit (KAPA Biosystems, Wilmington, MA, USA) with index tagging by 16 cycles of PCR using KAPA HiFi HotStart (KAPA Biosystems) and NEBNext Multiplex Oligos (oligonucleotides) for Illumina Index Primer Sets 1 and 2 (New England BioLabs). Libraries were quantified by Qubit (ThermoFisher, Waltham, MA, USA) and TapeStation (Agilent, Santa Clara, CA, USA) and pooled at equimolar concentrations for sequencing. The NimbleGen SeqCap EZ system (Roche, Basel, Switzerland) was used for probe based sequence capture, and then sequenced on the Illumina NextSeq 550 platform using v2.5 chemistry (Illumina) with 150bp paired read length.

2.12.8 Bioinformatic analysis

Trim Galore 0.4.0 (https://www.bioinformatics.babraham.ac.uk/projects/trim_galore/) was used to remove low quality reads with a phred quality score of <20 and adapter sequences were removed

with CutAdapt 1.7.1 (Martin, 2011). Bowtie2 was used to align reads to the hg19 human genome reference and mapped reads were removed (Langmead and Salzberg, 2013). Tanoti (http://bioinformatics.cvr.ac.uk/tanoti.php) was used to align unmapped reads against 223 full length HCV reference sequences from the International Committee on Taxonomy of Viruses (https://talk.ictvonline.org/). The HCV reference sequence with greatest read alignment was used for repeat alignment with a subtype specific reference sequence using tanoti. Samtools was used to convert aligned sequences to a binary bam file and to sort and index the file. Genome coverage was assessed using weeSAM (https://bioinformatics.cvr.ac.uk/weesam-version-1-5/). HCV Glue was used to assign a genotype and subtype and to assess resistance associated mutations (Singer et al., 2018). For sequences with sub-optimal coverage, repeat alignment with tanoti was performed using a subtype-specific reference sequence. A consensus reference sequence was obtained using UGene (Okonechnikov et al., 2012). Multiple sequence alignment of reference sequences and consensus sequence data was performed using MAFFT with local alignment (Katoh et al., 2002). JModeltest2 was used to identify the optimal phylogenetic tree model with selection based on the lowest negative log likelihood and Akaike Information Criteria (Darriba et al., 2012). MEGA compute core v6 (MEGA software, was used to generate a phylogenetic tree using genotype 4 reference sequences using a maximum likelihood model with 500 bootstrap replications using the JModelTest2 recommended model, a general time reversible model with gamma distribution with invariant sites with nearest-neighbour-interchange heuristics (Kumar et al., 2012).

2.13. Ethical review

Ethical permission to conduct this study was obtained from the National Health Sciences Research Committee of Malawi (16/11/1698 and 15/5/1599) and the University of Liverpool (reference 1954) (see Appendix) Census informants gave witnessed, recorded verbal consent, and participants in the serological survey and community evaluation provided written informed consent. The study was conducted in accordance with the Declaration of Helsinki (2013).

Chapter 3. Epidemiology of hepatitis B, C and D in Malawi: Systematic Review and Meta-Analysis

3.1. Introduction

Data on the epidemiology of viral hepatitis are required to inform an effective public health response. In the Global Health Sector Strategy on Viral Hepatitis 2016–2021, the World Health Organisation (WHO) identified the need to define the national disease burden and to strategically target limited resources to counter the local epidemic. There is a call for data on transmission and risk factors, to identify specific populations at risk and to quantify the health burden in terms of cirrhosis and hepatocellular carcinoma (World Health Organisation, 2016a).

The Malawi Ministry of Health (MoH) has resolved to respond to viral hepatitis in a concerted and strategic manner. As part of the response in 2017, a National Viral Hepatitis Unit was created in the MoH to guide the direction of policy and practice. In order to consolidate the current available evidence on epidemiology of viral hepatitis, identify the gaps in knowledge, practice and policy, together with colleagues from Malawi Ministry of Health, a systematic review of all published epidemiological data on the prevalence of chronic hepatitis B, C and D in Malawi was conducted to ascertain existing knowledge and identify data gaps and further research needs.

3.2. Methods

The methodology for the conduct of this systematic review are set out in Chapter 2. The searches were conducted on 22nd June 2018 and included data published from 1990-2018.

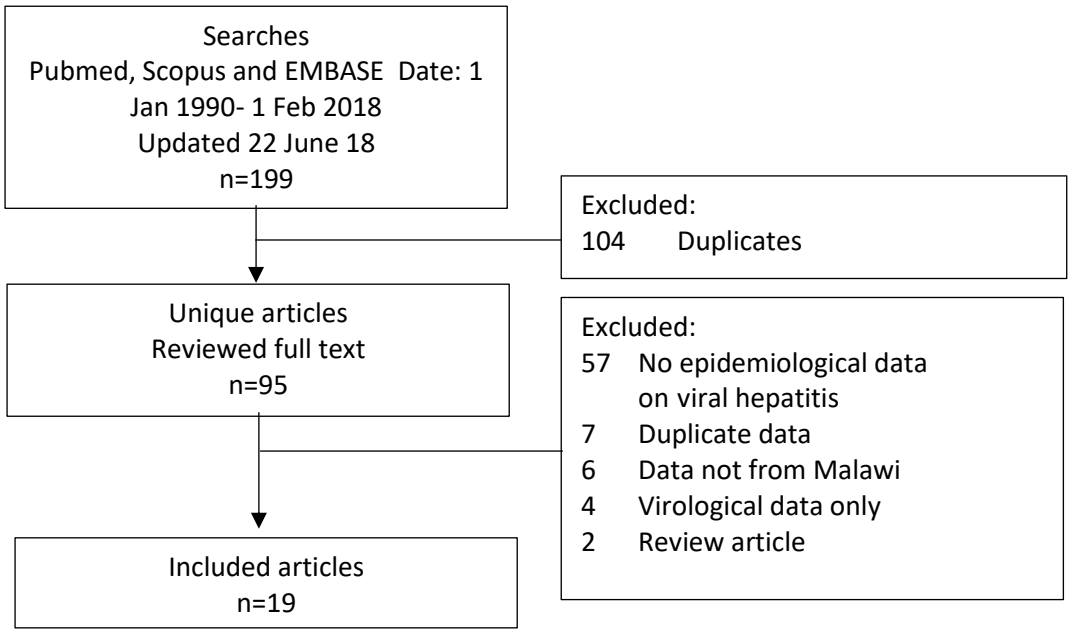
3.3. Results

The literature search identified 199 studies. Following removal of duplicates, 95 full-text articles were reviewed and 19 studies that reported epidemiological data on hepatitis B, C and D in diverse populations in Malawi were included (Figure 3-1).

3.3.1 Description of included studies

The 19 included studies described a total of HBsAg seroprevalence data from 16 different cohorts that were from general or HIV-positive populations (Table 3-1; Figure 3-2) and three cohorts from specific unrepresentative subgroups (Table 3-2). Hepatitis D antibody (anti-HDV) data were available from a single study (Table 3-3) and hepatitis C antibody (anti-HCV) data were available from 15 general or HIV-positive cohorts (Table 3-4; Figure 3-4) and from four cohorts describing specific subgroups (Table 3-5). Fourteen studies were from urban centres.

Figure 3-1: Flowchart of literature searches



3.3.2 Hepatitis B prevalence

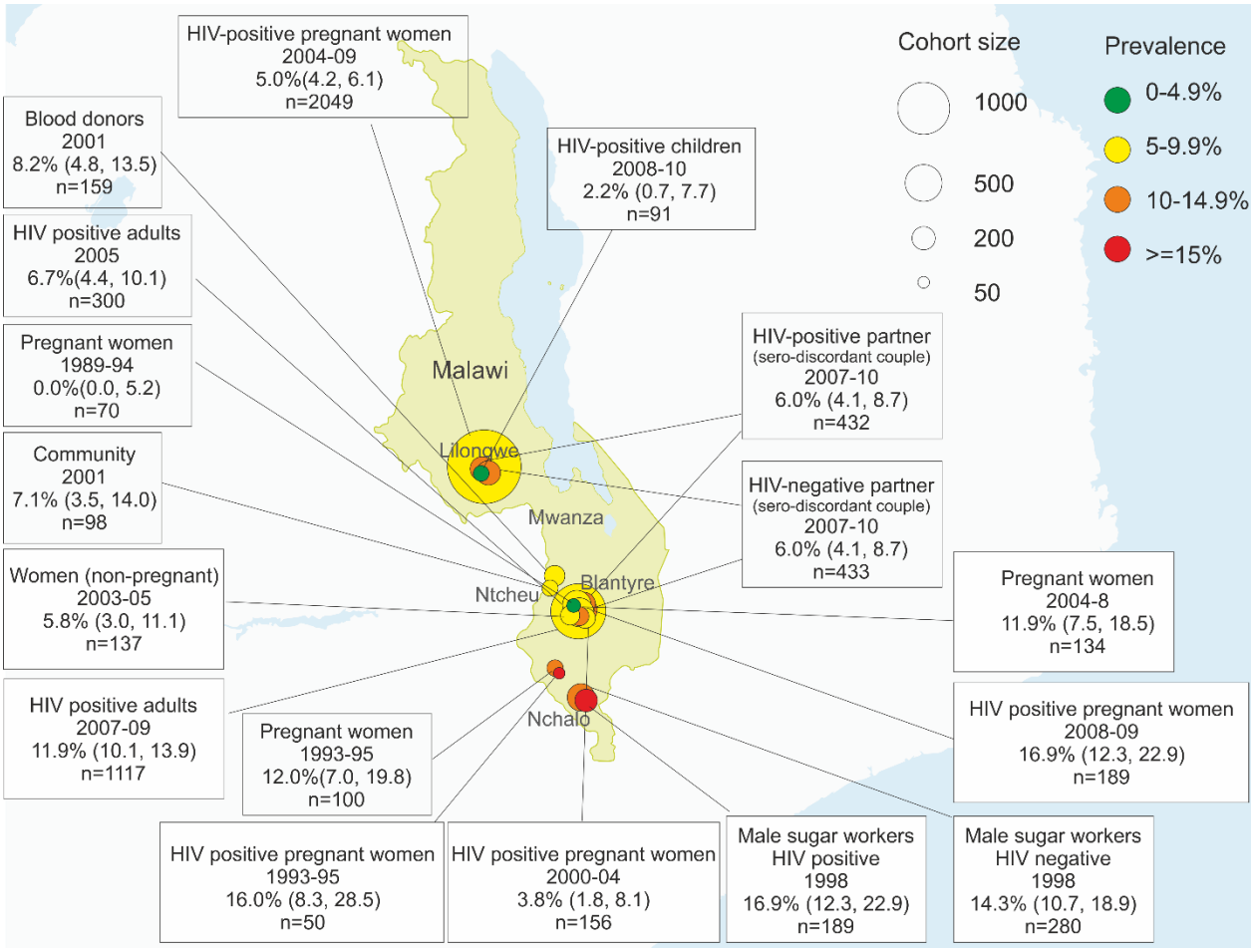
HBsAg seroprevalence estimates ranged from 0.0 to 14.3% in general populations and 3.8 to 16.0% in HIV positive populations (Table 3-1). One small study reporting from HIV positive children aged 3 months - 15 years (median 36 months) reported seroprevalence of 2.2% [95% confidence interval (CI) 0.6, 7.7]. This study did not estimate HBV vaccine efficacy as the vaccine was introduced in Malawi in 2002 and both vaccinated and non-vaccinated cohorts were combined. Pooled estimates of HBsAg seroprevalence among adult general populations was 7.6% (95% CI 4.6, 11.2) and 8.5 (95% CI 5.7, 11.7) in HIV positive populations (Figure 3-3). The overall pooled estimate of HBsAg seroprevalence in adults was 8.1% (95% CI 6.1, 10.3).

Table 3-1: Hepatitis B surface antigen (HBsAg) seroprevalence in Malawi

General Populations						
Population	**Ref**	**Year**	**Location**	**Laboratory method**	**Prevalence (n/total)**	**Prevalence (%), (95% CI)**
Pregnant women	(Taha et al., 2015)	1989-1994	QECH, Blantyre	MONOLISA HBsAg ULTRA (Biorad)	0/70	0.0 (0.0, 5.2)
Pregnant women, at delivery	(Ahmed et al., 1998)	1993-1995	Shire Valley	Bioelisa HBsAg (Biokit, S.A.)	12/100	12.0 (7.0, 19.8)
Male workers at sugar estate	(Sutcliffe et al., 2002)	1998	Nchalo	Auszyme monoclonal EIA (Abbott)	40/280	14.3 (10.7, 18.9)
Community, rural adults	(Taha et al., 2015)	2001	Mwanza District	MONOLISA HBsAg ULTRA (Biorad)	7/98	7.1 (3.5, 14.0)
Blood donors	(Candotti et al., 2001)	2001	Ntechu	HBsAg ELISA NS	13/159	8.2 (4.8, 13.5)
Non-pregnant women (intravaginal MTZ gel RCT)	(Taha et al., 2015)	2003-2005	QECH, Blantyre	MONOLISA HBsAg ULTRA (Biorad)	8/137	5.8 (3.0, 11.1)
Pregnant women	(Taha et al., 2015)	2004-2008	QECH, Health Centres Blantyre	MONOLISA HBsAg ULTRA (Biorad)	16/134	11.9 (7.5, 18.5)
HIV-negative partners in a serodiscordant couple	(Greer et al., 2017)	2007-2010	Blantyre Lilongwe	HBsAg ELISA NS	26/433	6.0 (4.1, 8.7)
HIV-positive populations						
Population	**Ref**	**Year**	**Location**	**Laboratory method**	**Prevalence (n/total)**	**Prevalence (%), (95% CI)**
HIV-positive pregnant women, at delivery	(Ahmed et al., 1998)	1993-1995	Shire Valley	Bioelisa HBsAg (Biokit, S.A.)	8/50	16.0 (8.3, 28.5)
HIV positive: male workers at sugar estate	(Sutcliffe et al., 2002)	1998	Nchalo	Auszyme monoclonal EIA (Abbott)	32/189	16.9 (12.3, 22.9)
HIV-positive pregnant women	(Taha et al., 2015)	2000-2004	QECH, Blantyre	MONOLISA HBsAg ULTRA (Biorad)	6/156	3.8 (1.8, 8.1)
HIV-positive pregnant women	(Chasela et al., 2014)	2004-2009	Lilongwe	Vitros Chemiluminescence Immunoassay (Ortho Clinical Diagnostics)	103/2049	5.0 (4.2, 6.1)
HIV-positive adults	(Moore et al., 2010)	2005	QECH, Blantyre	Bioelisa HBsAg (Biokit, S.A.)	20/300	6.7 (4.4, 10.1)
HIV positive adults, ART starters	(Aoudjane et al., 2014)	2007-2009	QECH, Blantyre	Bioelisa HBsAg (Biokit, S.A.)	133/1117	11.9 (10.1, 13.9)
HIV-positive adults in sero-discordant couple	(Greer et al., 2017)	2007-2010	Blantyre Lilongwe	HBsAg ELISA NS	26/432	6.0 (4.1, 8.7)
HIV-positive pregnant women	(Andreotti et al., 2014)	2008-2009	Blantyre	Murex HBsAg Version 3 with Confirmatory Assay (Murex Biotech)	27/309	8.7 (6.1, 12.4)
HIV-infected children	(Varo et al., 2016)	2008-2010	Lilongwe	Genetic Systems HBsAg 3.0 (Bio-Rad)	2/91	2.2 (0.6, 7.7)

Abbreviations: QECH Queen Elizabeth Central Hospital, Blantyre; MTZ metronidazole; RCT randomised controlled trial. Biorad: HBsAg ELISA, Biorad, Hercules, CA, USA; Bioelisa: HBsAg 3.0 Biokit SA Barcelona, Spain; Ortho: Siemens: ADVIA Centaur, Siemens, Munich, Germany; Abbott: Murex HBsAg, Abbott, Illinois, USA; NS- manufacturer not specified

Figure 3-2: HBsAg seroprevalence in Malawi: published data 1990-2018



Boxes refer to individual studies and list clinical setting, year of study, HBsAg prevalence estimate and sample size. The colour of the circles indicates prevalence and area indicates the sample size for each sample.

No significant difference in HBsAg prevalence was noted between HIV-positive and -negative populations (p=0.74). The effect of HIV status on HBV seroprevalence was assessed directly in three studies, with a total of 1484 participants, that tested HBsAg prevalence, stratified by HIV status within the same population. These populations comprised male workers at a sugar factory (n=469) (Sutcliffe et al., 2002), pregnant women recruited at delivery (n=150) (Ahmed et al., 1998) and HIV positive and negative serodiscordant couples recruited for a randomised control trial of antiretroviral therapy for prevention of transmission (n=865) (Greer et al., 2017). Among the three groups, the odds ratio of HBsAg positivity among HIV positive compared to HIV negative individuals from within the same population was 1.2 (95% CI 0.8, 1.6, p=0.80), indicating no evidence of association between HBV infection and HIV infection status (Figure 3-4).

Studies among three unrepresentative groups deemed at altered risk of HBV infection: (adult medical inpatients, prisoners and medical students) found HBsAg prevalence rates of 17.5%, 3.0% and 0% respectively (Table 3-2).

Table 3-2: HBsAg seroprevalence among special unrepresentative populations in Malawi

Population	Ref	Year	Location	Laboratory method	Prevalence (n/total)	Prevalence (%, (95% CI))
Adult medical inpatients	(Nyirenda et al., 2008)	2004	Medical ward, QECH, Blantyre	Determine HBsAg Rapid Test (Alere)	34/194	17.5 (12.8, 23.5)
Prisoners	(Chimphambano et al., 2007)	2007	Chichiri Prison, Blantyre	HBsAg kit (Abbott)	5/164	3.0 (1.3, 6.9)
Medical students	(Chipetah et al., 2017)	2013	College of Medicine, Blantyre	SD Bioline Rapid Test (Alere)	0/89	0.0 (0.0, 4.9)

Abbreviations: QECH Queen Elizabeth Central Hospital; ART antiretroviral therapy

3.3.3 Hepatitis D prevalence

A single study was available reporting HDV prevalence among HIV/HBV co-infected individuals commencing ART in Blantyre(Stockdale et al., 2017) (Table 3-3). This demonstrated anti-HDV prevalence of 2/133 (1.5%) but none of the participants were HDV RNA PCR positive.

Figure 3-3: Forest Plot of HBsAg prevalence in general and HIV-positive populations, Malawi 1990-2018

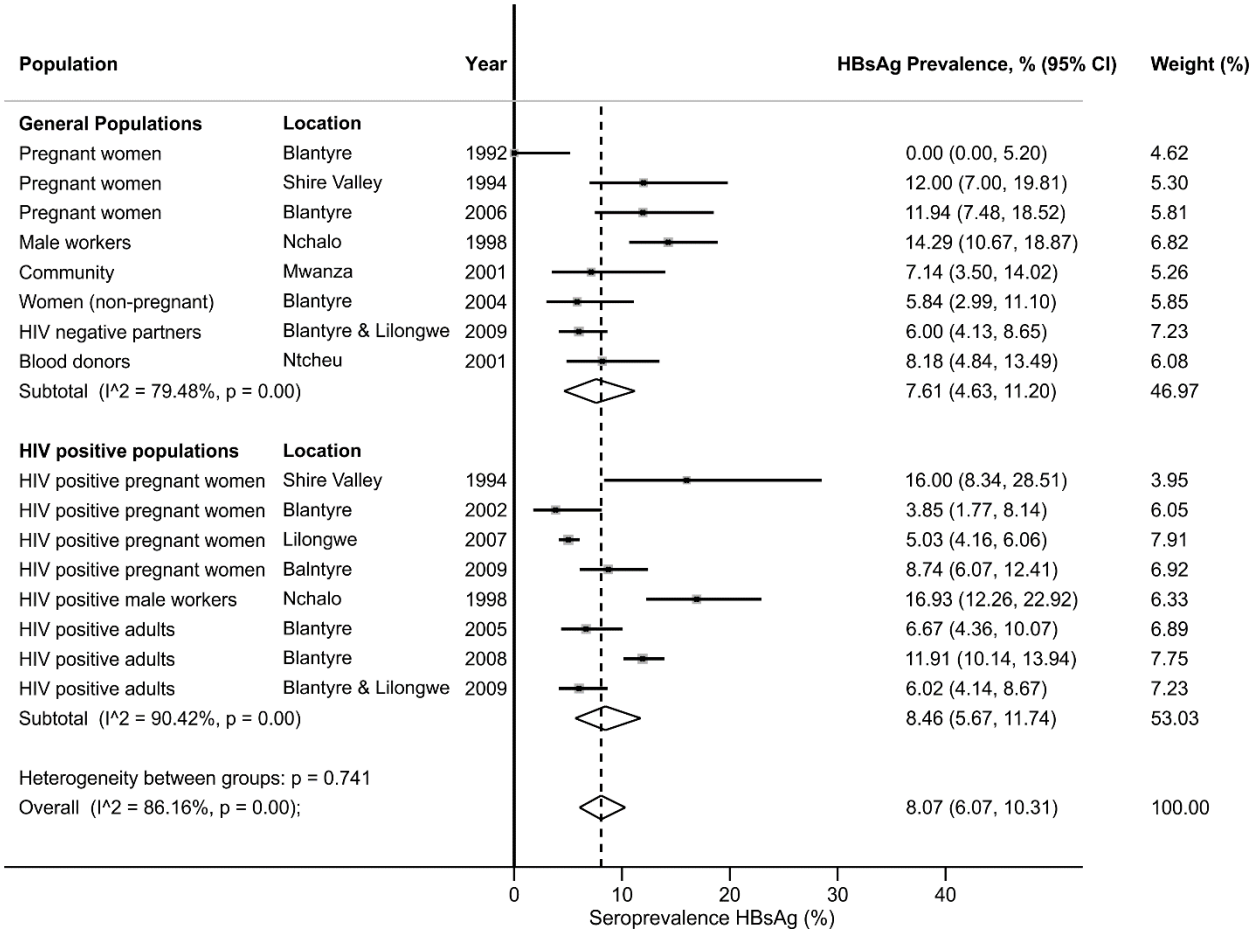


Figure 3-4: Odds ratio of HBsAg seropositivity according to HIV status

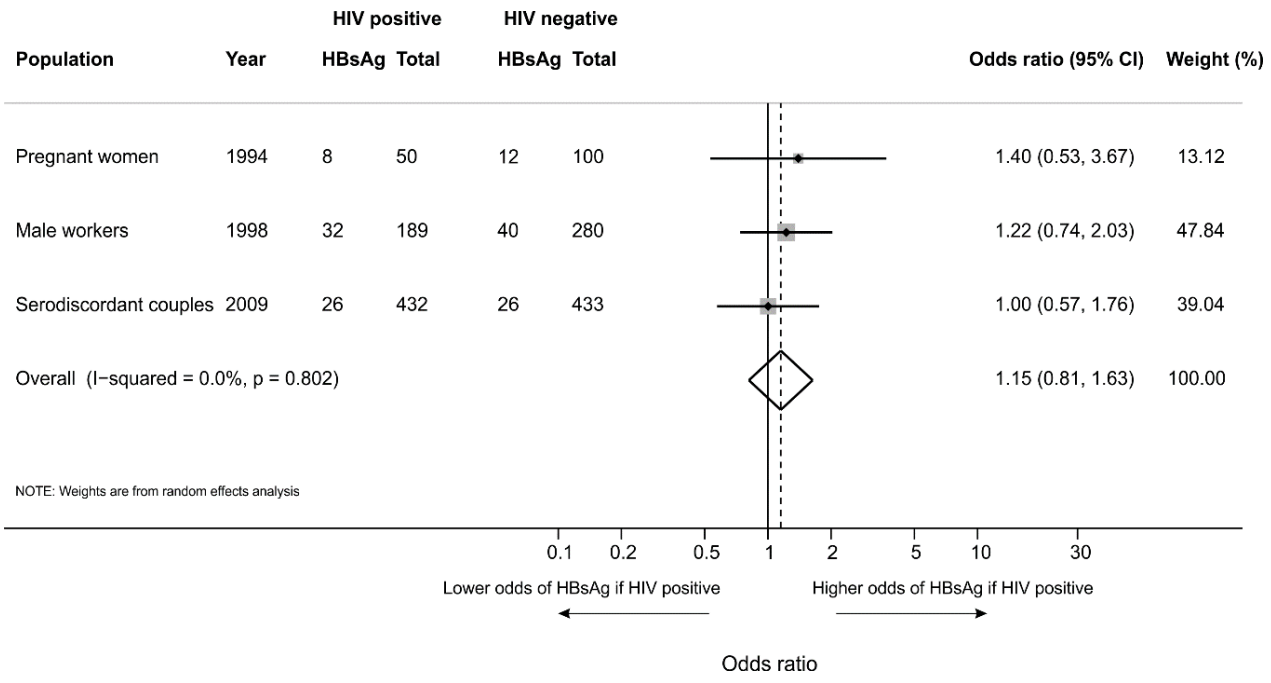


Table 3-3: Hepatitis D seroprevalence in Malawi among HBsAg positive individuals

Population	Ref	Year	Location	Method	Prevalence (n/total)	Prevalence (%, (95% CI))
HIV-HBV infected adults	(Stockdale et al., 2017)	2007-2009	HIV clinic, QECH Blantyre	1. ETI-AB-DELTAK (Diasorin) 2. HDV RNA PCR (in-house)	2/133 0/133	1.5 (0.4, 5.3) 0.0 (0.0, 2.8)

3.3.4 Hepatitis C prevalence

Among general populations, anti-HCV prevalence ranged from 0.7 to 18.0% and among HIV-positive populations from 0.0 to 12.0% (Table 3-4). Three studies confirmed active HCV infection using RNA PCR. These comprised a study of HIV-positive adults commencing ART in Lilongwe(Demir et al., 2016), a study of blood donors in Ntcheu(Candotti et al., 2001) and a study of HIV-positive pregnant women in Blantyre(Andreotti et al., 2014). In these studies, anti-HCV prevalence was 2.2, 6.8 and 2.6% respectively but HCV RNA PCR demonstrated active HCV prevalence of 0, 0.7 and 0.3% respectively, with a pooled rate of HCV RNA confirmation among anti-HCV positive participants of 7.3% (95% CI 0.0-24.3).

Among four studies assessing HCV prevalence in unrepresentative special subgroups comprising: prisoners; medical inpatients in Blantyre and Lilongwe; and children with malignancies and their mothers, the prevalence of anti-HCV was 0; 3.9 and 4.5; 0.2 and 0.5% respectively (Table 3-5)

Table 3-4: Hepatitis C seroprevalence in Malawi

General Populations						
Population	**Ref**	**Year**	**Location**	**Method**	**Prevalence (n/total)**	**Prevalence (%, (95% CI))**
Pregnant women	(Taha et al., 2015)	1989-1994	QECH, Blantyre	Anti-HCV (Biorad)	2/70	2.9 (0.8, 9.8)
Pregnant women, at delivery	(Ahmed et al., 1998)	1993-1995	Shire Valley	Ortho anti-HCV (Ortho Diagnostics)	18/100	18.0 (11.7, 26.7)
Blood donors	(Maida et al., 2000)	1996	KCH, Lilongwe	Anti-HCV EIA (Roche) Confirmed with Anti-HCV (Abbott)	4/100	4.0 (1.6, 9.8)
Male workers at sugar estate	(Sutcliffe et al., 2002)	1998	Nchalo	Ortho anti-HCV (Ortho Diagnostics)	35/279	10.0 (7.0, 14.1)
Community, rural adults	(Taha et al., 2015)	2001	Mwanza District	Anti-HCV (Biorad)	9/99	9.0 (4.9, 16.4)
Blood donors	(Candotti et al., 2001)	2001	Ntechu	1. Murex anti-HCV 2. HCV RNA by in-house PCR	10/148 1/140	6.8 (3.7, 12.0) 0.7 (0.1, 3.9)
Non-pregnant women (intravaginal MTZ gel RCT)	(Taha et al., 2015)	2003-2005	QECH, Blantyre	Anti-HCV (Biorad)	9/146	6.1 (3.3, 11.3)
Pregnant women	(Taha et al., 2015)	2004-2008	QECH, Health Centres Blantyre	Anti-HCV (Biorad)	8/138	5.8 (3.0, 11.0)
HIV positive populations						
Population	**Ref**	**Year**	**Location**	**Method**	**Prevalence (n/total)**	**Prevalence (%, (95% CI))**
HIV-positive pregnant women, at delivery	(Ahmed et al., 1998)	1993-1995	Shire Valley	Ortho anti-HCV (Ortho Diagnostics)	6/50	12.0 (5.6, 23.8)
HIV-positive male workers at sugar estate	(Sutcliffe et al., 2002)	1998	Nchalo	Ortho anti-HCV Ab (Ortho Clinical Diagnostics)	28/280	10.0 (7.0, 14.1)
HIV-positive pregnant women	(Taha et al., 2015)	2000-2004	QECH, Blantyre	Anti-HCV (Biorad)	8/148	5.4 (2.8, 10.3)
HIV positive patients	(Moore et al., 2010)	2005	QECH, Blantyre	Monolisa HCV Ag-Ab (Biorad) confirmed with ADVIA Centaur anti-HCV) and InnoLIA HCV immunoassay (Innogenetics)	17/300	5.7 (3.6, 8.9)
HIV-positive pregnant women	(Andreotti et al., 2014)	2008-2009	Blantyre	1. Innotest HCV Ab IV (Immunogenetics), 2. Versant HCV RNA 1.0 assay (Siemens)	8/309 1/309	2.6 (1.3, 5.0) 0.3 (0.1, 1.8)
HIV positive adults starting ART	(Demir et al., 2016)	2014-15	Lilongwe	1. HCV IgG Architect (Abbott) 2. RealTime HCV RNA (Abbott)	5/227 0/227	2.2 (0.9, 5.1) 0.0 (0.0, 1.7)
HIV positive patients on ART for >10 years	(Loarec et al., 2017)	2014-16	Chiradzulu	OraQuick HCV Rapid antibody test (Orasure)	2/385	0.5 (0.1, 1.9)

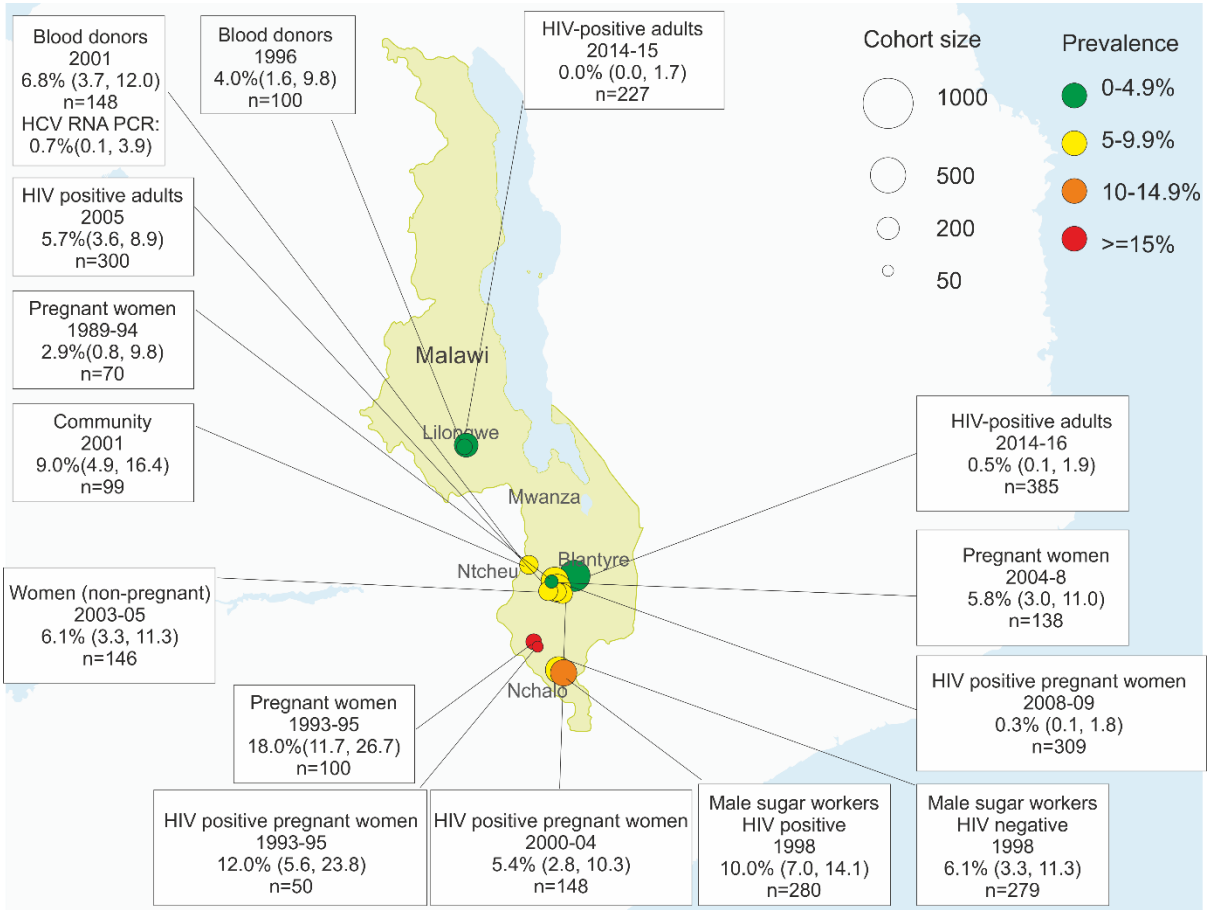
Abbreviations: QECH Queen Elizabeth Central Hospital; HCV hepatitis C virus

Table 3-5: Hepatitis C seroprevalence among special unrepresentative populations in Malawi

Population	Ref	Year	Location	Method	Prevalence (n/total)	Prevalence (%, (95% CI))
Adult inpatients (Dermatology, Urology)	(Maida et al., 2000)	1996	KCH, Lilongwe	Anti-HCV EIA (Roche) Confirmed with Anti-HCV (Abbott)	13/333	3.9 (2.3, 6.6)
Adult medical inpatients	(Nyirenda et al., 2008)	2004	Medical ward, QECH, Blantyre	HCV Ag/Ab (Monolisa, Biorad) confirmed with Immunoassay (innogenetics)	9/202	4.5 (2.4, 8.2)
Prisoners	(Chimphambano et al., 2007)	2007	Chichiri Prison, Blantyre	Anti-HCV (Biotec)	0/164	0.0 (0.0, 2.3)
Malawian women and children with childhood malignancies	(Fox et al., 2015)	2006-10	QECH, Blantyre	HBV ELISA (MP Biomedicals) Confirmed by HCV BLOT (MP Biomedicals)	Mothers: 2/418 Children: 1/418	0.5 (0.1, 1.7) 0.2 (0.0, 1.3)

Abbreviations: KCH Kamuzu Central Hospital; QECH Queen Elizabeth Central Hospital; HCV hepatitis C virus

Figure 3-5: Prevalence of hepatitis C antibody: published data 1990-2018



Boxes refer to individual studies and list clinical setting, year of study, HBsAg prevalence estimate and sample size.

Table 3-6: Assessment of quality of included studies

	Was the sample representative of the target population	Were study participants recruited in an appropriate way?	Was the sample size adequate?	Were the study subjects and setting described in detail?	Was the data analysis conducted with sufficient coverage of the identified sample?	Were valid methods used for the identification of the condition?	Was the condition measured in a standard, reliable way for all participants?	Was the response rate adequate, and if not, was the low response rate managed appropriately?
Ahmed, 1998	Y	Y	Y	Y	Y	Y	Y	Y
Andreotti, 2014	Y	Y	Y	Y	Y	Y	Y	U
Aoudjane, 2014	Y	Y	Y	Y	Y	Y	Y	U
Candotti, 2001	N	U	N	N	U	Y	Y	U
Chasela, 2014	Y	Y	Y	Y	Y	Y	Y	Y
Chimphambano, 2007	N	Y	Y	Y	U	Y	Y	U
Chipetah, 2017	N	Y	Y	Y	Y	Y	Y	Y
Demir, 2016	Y	Y	Y	Y	Y	Y	Y	Y
Fox, 2015	N	Y	Y	Y	U	Y	Y	U
Greer, 2017	Y	Y	Y	Y	Y	Y	Y	Y
Loarec 2017	Y	U	Y	Y	U	Y	Y	U
Maida, 2000	N	Y	N	Y	U	Y	Y	U
Moore, 2010	U	Y	Y	Y	Y	Y	Y	Y
Nyirenda, 2008	N	Y	N	Y	U	Y	Y	U
Stockdale, 2017	Y	Y	Y	N	Y	Y	Y	U
Sutcliffe, 2002	N	U	Y	Y	U	Y	Y	U
Taha, 2015	Y	Y	N	N	U	Y	Y	U
Varo, 2016	Y	Y	U	Y	U	Y	Y	U

Abbreviations: Y Yes; N No; U Unclear

3.4. Discussion

In this systematic review, the existing epidemiological evidence on HBV, HCV and HDV prevalence in Malawi was compiled, highlighting a number of key findings and important knowledge gaps. Data from studies reporting from general and HIV-infected populations showed a pooled HBsAg seroprevalence estimate of 8.1% (95% CI 6.1, 10.3). This finding is in keeping with regional estimates from Mozambique (8.3%), Tanzania (7.2%) and Zambia (6.1%) (Schweitzer et al., 2015). The review has benefitted from the inclusion of significantly more data than previous pooled estimates for Malawi (Schweitzer et al., 2015, Rao et al., 2015). It is notable that available data were biased toward the two main urban centres of Lilongwe and Blantyre, that the Northern region was under-represented and that there were no nationally representative community survey data.

Hepatitis C antibody seroprevalence estimates ranged from 2.9 to 18% from general or HIV-infected populations. Among the three available studies that reported HCV RNA confirmation, only 7.3% of 676 participants with anti-HCV antibody were confirmed to have HCV RNA replication. This finding has been consistent with other cohorts across the region and highlights issues with using anti-HCV as the basis for obtaining epidemiological estimates in the absence of confirmatory testing (Sonderup et al., 2017). Confirmation of anti-HCV results with PCR or core HCV antigen testing are required to obtain reliable prevalence estimates (World Health Organisation, 2017b). Accordingly, due to the paucity of studies reporting PCR data, a pooled HCV prevalence estimate was not provided in this review. Furthermore, an assessment of possible association between HCV, HBV and HIV infection was not possible based on the limited data. Based on the available evidence, it is likely that HCV prevalence is low in Malawi, and was below 1% in all studies using RNA confirmation (Andreotti et al., 2014, Candotti et al., 2001, Demir et al., 2016), but larger representative samples employing confirmatory PCR testing are required to confirm these findings. Further work to establish whether false positive anti-HCV antibody tests or failure of HCV RNA assays to detect local HCV strains is required. Existing data from other sub-Saharan Africa cohorts using line immunoassay among anti-HCV positive, HCV RNA negative patients has indicated that most such results represent false positive anti-HCV IgG detection rather than evidence of past infection (King et al., 2015, Twagirumugabe et al., 2017, Chasela et al., 2012).

Only a single study reporting HDV prevalence was available, demonstrating a low rate of anti-HDV among HIV/HBV co-infected patients in Blantyre (1.5%), with none of the participants showing replication of HDV RNA by PCR. This finding is in keeping with available limited data demonstrating a low rate of HDV seroprevalence from Southern Africa relative to Central or West Africa, though the paucity of available data from the Southern Africa region should be noted (Stockdale et al., 2017). Due

to the rapid progression to fatal liver disease associated with HBV/HDV superinfection or co-infection, cross-sectional community estimates of HDV seroprevalence are unlikely to reliably estimate the true burden of disease caused by HDV. Studies of hospitalised patients with well-characterised liver disease are required and will facilitate the ascertainment of the attributable fraction of viral hepatitis to liver disease (Lempp et al., 2016).

There are several limitations in this analysis, highlighted by the assessment of study quality (Table 3-6). The epidemiological evidence presented in this study is drawn from predominantly small cohorts studies in diverse populations employing convenience sampling. A striking bias toward urban centres was observed with only four of 18 included studies drawn from rural areas, despite an estimated 85% of the Malawian population residing in rural areas (Government of Malawi, 2008). There were no available data from the Northern region of Malawi, where 13% of the population live (Government of Malawi, 2008). To overcome these issues of lack of nationally representative unbiased community data, the use of the demographic health survey using dried blood spot sampling represents a promising solution. Dried blood spots have excellent diagnostic performance relative to venous blood sampling for HBsAg and anti-HCV screening and this method has been recently recommended for large surveys by the WHO (World Health Organisation, 2017b). Use of dried blood spots for hepatitis D screening of the demographic health survey has recently been used in Burkina Faso (Tuaillon et al.), and represent an efficient method to obtain samples without requiring a cold chain or venepuncture.

The finding of lack of an association between hepatitis B seroprevalence and HIV status is in keeping with previous studies from sub-Saharan Africa (Matthews et al., 2014). This is likely due to distinct transmission epidemiology, with hepatitis B predominantly acquired perinatally or horizontally in early childhood, and HIV acquired predominantly during adolescence or adulthood by sexual transmission in sub-Saharan Africa. By contrast, recent evidence of incident transmission of HBV in HIV-infected adults has highlighted the risk of HBV acquisition in adulthood (Seremba et al., 2017). Hepatitis B vaccination is provided as a component of the pentavalent vaccine (also containing polio, diphtheria, tetanus and *Haemophilis influenzae* type B) in the expanded programme of immunisation schedule for Malawian infants, provided at 6, 10 and 14 weeks since 2002. The Demographic Health Survey 2015-16 estimated 3-dose coverage of the vaccine of 93.0%, with consistently high coverage exceeding 90%, regardless of socioeconomic status or geographic location (Government of Malawi, 2017). The WHO has recently proposed that gathering data on hepatitis B seroprevalence among a vaccinated cohort at 5 years of age is a priority in order to generate evidence on the efficacy of HBV vaccination programmes and this is a priority area for research highlighted by this review (World Health Organisation, 2017c).

3.5. Conclusions

Hepatitis B is highly prevalent in Malawi with an estimated seroprevalence among the general population of 8.1% based on convenience samples from diverse populations from 1990-2018. HCV prevalence was below 1% in three general population cohorts that used nucleic amplification confirmatory testing. There is a need for representative unbiased community seroprevalence estimates of HBV, HDV and HCV prevalence. These should include confirmatory PCR testing to establish reliable HCV prevalence estimates indicating active viraemic infections. Future studies examining seroprevalence among community samples, with a particular focus on rural areas and the Northern region, are required. Assessment of the effectiveness of the hepatitis B vaccination programme introduced in 2002 and data on HDV prevalence among HBsAg positive individuals represent further research priorities. Prevalence estimates of viral hepatitis among people with well-characterised liver disease with cirrhosis and HCC are required to ascertain the attributable fraction and burden of disease. These data will help to support a viral hepatitis strategy for Malawi and facilitate the introduction of screening and treatment programmes for HBV and HCV.

Chapter 4. Aetiology and outcomes of cirrhosis and hepatocellular carcinoma (HCC) In Blantyre, Malawi

4.1. Introduction

Hepatitis B and C are the leading causes of liver cirrhosis and HCC in sub-Saharan Africa. There is a paucity of data on the aetiology and outcomes of well-characterised patients receiving routine care in the region. These data are important to inform clinicians and policymakers, to estimate the impact of public health interventions and to facilitate estimation of the disability and mortality associated with liver disease in the region.

A systematic review of liver-cirrhosis mortality conducted as part of the Global Burden of Disease study estimated that liver cirrhosis deaths doubled in sub-Saharan Africa between 1980 and 2010 (Mokdad et al., 2014). Malawi was estimated to be in the highest global decile of cirrhosis mortality rates, although data for Malawi were modelled and extrapolated from neighbouring countries. The population attributable fraction (PAF) for cirrhosis mortality was estimated as 37% for hepatitis B and 18% for hepatitis C in southern Africa (Mokdad et al., 2014). For HCC mortality, the estimated PAF in sub-Saharan Africa was 40% for alcohol, 29 % for hepatitis B and 20% for hepatitis C for 2015 (Global Burden of Disease Liver Cancer et al., 2017). These estimates for sub-Saharan Africa are modelling estimates based on sparse epidemiological data, with many countries providing no empirical data.

Improving data on the relative contribution of viral hepatitis B, C and D is an important step to inform an effective public health response and improve understanding of the burden of disease associated with viral hepatitis in sub-Saharan Africa. This study aimed to recruit patients with cirrhosis and HCC in a tertiary hospital in Blantyre, Malawi, to provide descriptive data on clinical features, aetiology, outcomes and the attributable fraction of viral hepatitis to the disease burden.

4.2. Methods

Study recruitment procedures and investigative methods are detailed in chapter 2. The population attributable fraction for cirrhosis and HCC was calculated among patients, using the community serological sample data to provide an estimate of background population prevalence for hepatitis B and C. To calculate PAF, first a logistic regression model was fitted with a binary dependent variable denoting cases (cirrhosis or HCC) or community control data and explanatory independent variables indicating exposure (HBV, HCV or alcohol). Community control data were obtained from community

serosurvey prevalence data, as described in chapter 2 and chapters 5 and 6 for community HBV and HCV prevalence. For alcohol consumption data and for schistosomiasis exposure data, community control data were obtained from HBsAg community evaluation participants and randomly selected age- and sex-matched HBsAg negative control patients from the serosurvey as detailed in chapters 2 and 5. Since alcohol and schistosomiasis exposure prevalence estimations were obtained from a sub-population of the serosurvey, alcohol and schistosomiasis prevalence was compared between HBsAg positive participants and matched controls to ensure that HBsAg status did not correlate with these exposures. Following logistic regression, a post-estimation predictive margin of response was estimated for a modelled scenario with zero exposure (for example estimating the logit for a case with an HBsAg prevalence of zero). The ratio between the logit of the baseline likelihood (at the real prevalence) and the zero exposure scenario was calculated, representing the population unattributable fraction (PUF). PAF was calculated using (1- PUF). PAF calculations were implemented in the *punafcc* package in Stata version 16.0 (Statacorp, College Station, TX, USA) (Newson, 2013). Survival estimates were produced using Kaplan Meier survival function, censored for outcomes at 6 months to account for some patients having their final outcome assessed beyond the 6-month period. To estimate survival at intervals of 1, 3 and 6 months following hospital admission, and to ascertain explanatory variables associated with mortality risk, a Cox proportional hazards model was used to obtain regression estimates for patients with cirrhosis and HCC using the exact marginal likelihood method for tied failure time. Confidence intervals of proportions were calculated using the Wilson method. Comparisons of median age by aetiology of cirrhosis and HCC were made using the Wilcoxon rank sum test.

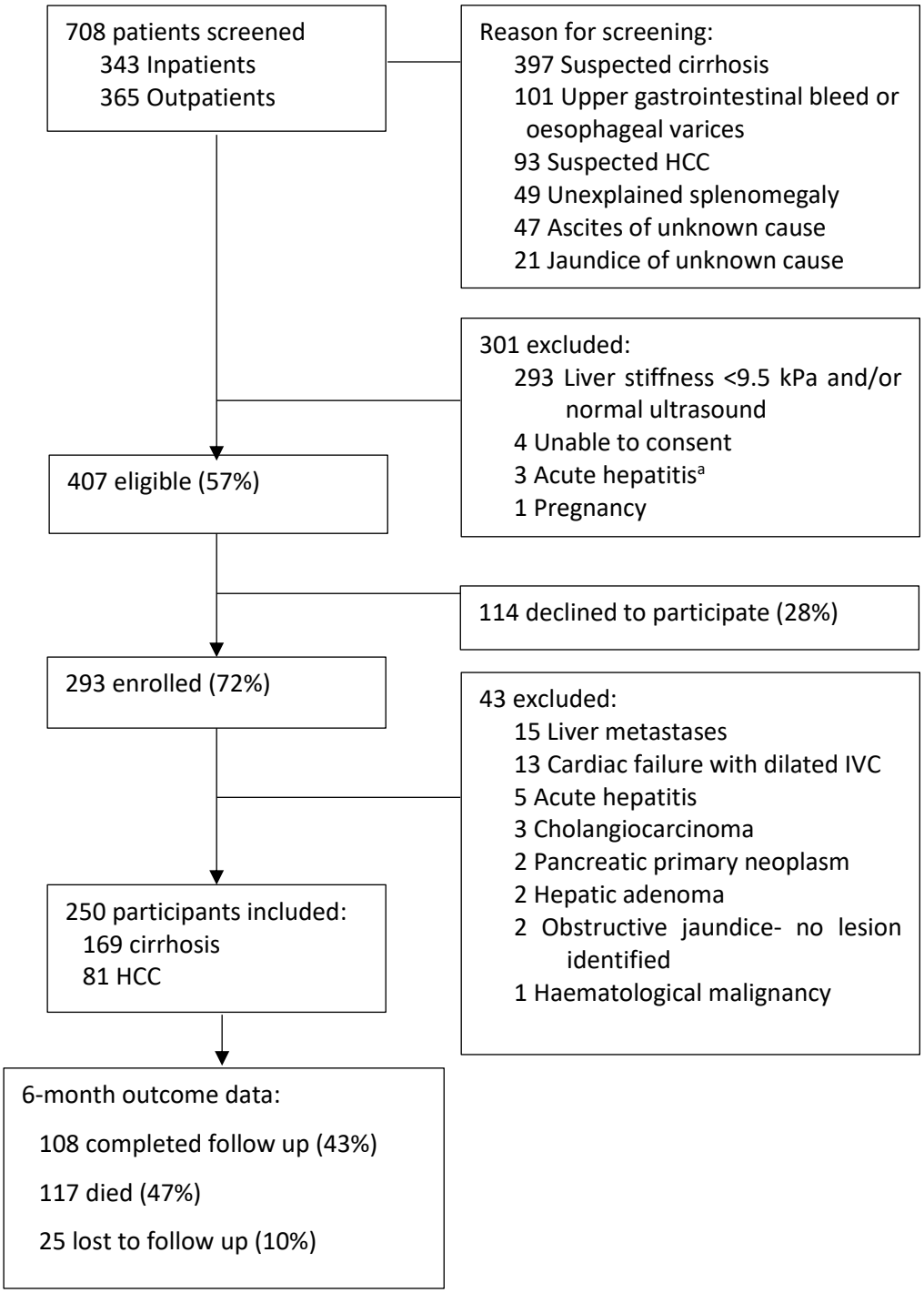
4.3. Results

4.3.1 Cohort description

During the 18 month recruitment period from November 2017 to April 2019, 708 patients were screened for suspected cirrhosis or HCC, in the medical and surgical wards, medical clinics and endoscopy unit of Queen Elizabeth Central Hospital (QECH) in Blantyre. A total of 407/708 (57.5%) patients were potentially eligible based on screening criteria and 293 agreed to participate in the study (72%). The majority of patients were reviewed due to suspected cirrhosis (397/708, 56%), followed by an upper GI bleed (101/708, 14%) or due to suspected HCC (93/708, 13%) (Figure 4-1). Following a study ultrasound and clinical evaluation, 250 patients met criteria for cirrhosis or HCC and were included in the study (Figure 4-1), comprising 169 patients with cirrhosis and 81 patients with HCC.

Reasons for exclusion following clinical evaluation included a diagnosis of liver metastases (15), cardiac failure (13), an alternative neoplastic process (10) or acute hepatitis (5) (Figure 4-1).

Figure 4-1: Flowchart of study recruitment



[a] Acute hepatitis was defined by ALN >5x upper limit of normal with no features of cirrhosis on ultrasound.

Table 4-1: Characteristics of included participants

Characteristic	n(%) or median (IQR)	
	Cirrhosis (n=169)	**HCC (n=81)**
Sex, female, n(%)	73 (43)	25 (31)
Age (years), median (IQR)	41 (33, 52)	40 (36, 50)
Body mass index (kg/m^2), median (IQR)	21.3 (19.6, 22.9)	21.4 (18.8, 22.8)
Obese (body mass index >30 kg/m2), n(%)	0 (0)	0 (0)
Hypertension (BP >140/90 mmHg)	23 (14)	10 (13)
Grade 1 ≥140/90	18 (11)	5 (6)
Grade 2 ≥160/100	4 (2)	3 (4)
Grade 3 ≥180/110	1 (1)	2 (3)
HIV positive[a], n(%)	50 (30)	23 (29)
CD4 count (cells/mm^3), median (IQR)	198 (112, 346)	307 (159, 552)
On ART, n(%)	20/50 (40)	8/23 (35)
Reason for screening, n(%) [b]		
Suspected cirrhosis	150 (89)	75 (94)
GI bleeding	40 (24)	4 (5)
Suspected HCC	13 (8)	61 (76)
Presenting symptoms, n(%)		
Abdominal swelling	93 (57)	46 (58)
Abdominal pain	47 (29)	57 (71)
Leg swelling	51 (31)	21 (26)
Weight loss	20 (12)	15 (19)
Shortness of breath	21 (13)	14 (18)
Jaundice	13 (8)	14 (17)
Vomiting	22 (13)	5 (6)
Cough	14 (9)	7 (9)
Weakness	7 (4)	5 (6)
Fever	2 (1)	3 (4)
Duration of symptoms (days), median (IQR)	30 (8, 90)	45 (14, 90)
Past medical history, n(%)		
Previous admission for liver disease	19 (11)	1 (1)
History of tuberculosis	22 (13)	3 (4)
History of diabetes	4 (2)	2 (2)
History of jaundice	15 (9)	1 (1)
Signs of chronic liver disease, n(%)	150 (89)	76 (94)
Cachexia	126 (75)	69 (86)
Ascites	108 (64)	49 (60)
Peripheral oedema	77 (46)	37 (46)
Icterus	47 (28)	31 (39)
Dilated abdominal veins	29 (17)	24 (30)

Bruising	1 (1)	3 (4)
Finger clubbing	5 (3)	2 (3)
Gynaecomastia (among men)	1 (1)	2 (4)
Full blood count		
Haemoglobin (g/dL), median (IQR)	10.4 (7.7, 12.5)	11.5 (9.8, 12.8)
Platelets (10^9/L), median (IQR)	81 (53, 173)	231 (161, 385)
White cell count (10^6/L), median (IQR)	3.9 (2.2, 5.5)	6.7 (5.1, 10.3)
Urine protein dipstick		
Negative	155 (92)	68 (85)
0.3g/L	8 (5)	9 (11)
1.0g/L	6 (4)	3 (4)
Schistosomiasis urine CCA positive, n(%)	50 (30)	27 (33)
Weak positive	31 (18)	13 (16)
Strong positive	19 (11)	14 (17)
Liver stiffness measurement (kPa), median (IQR)	23.6 (14.9, 50.6)	>75 (45, >75)

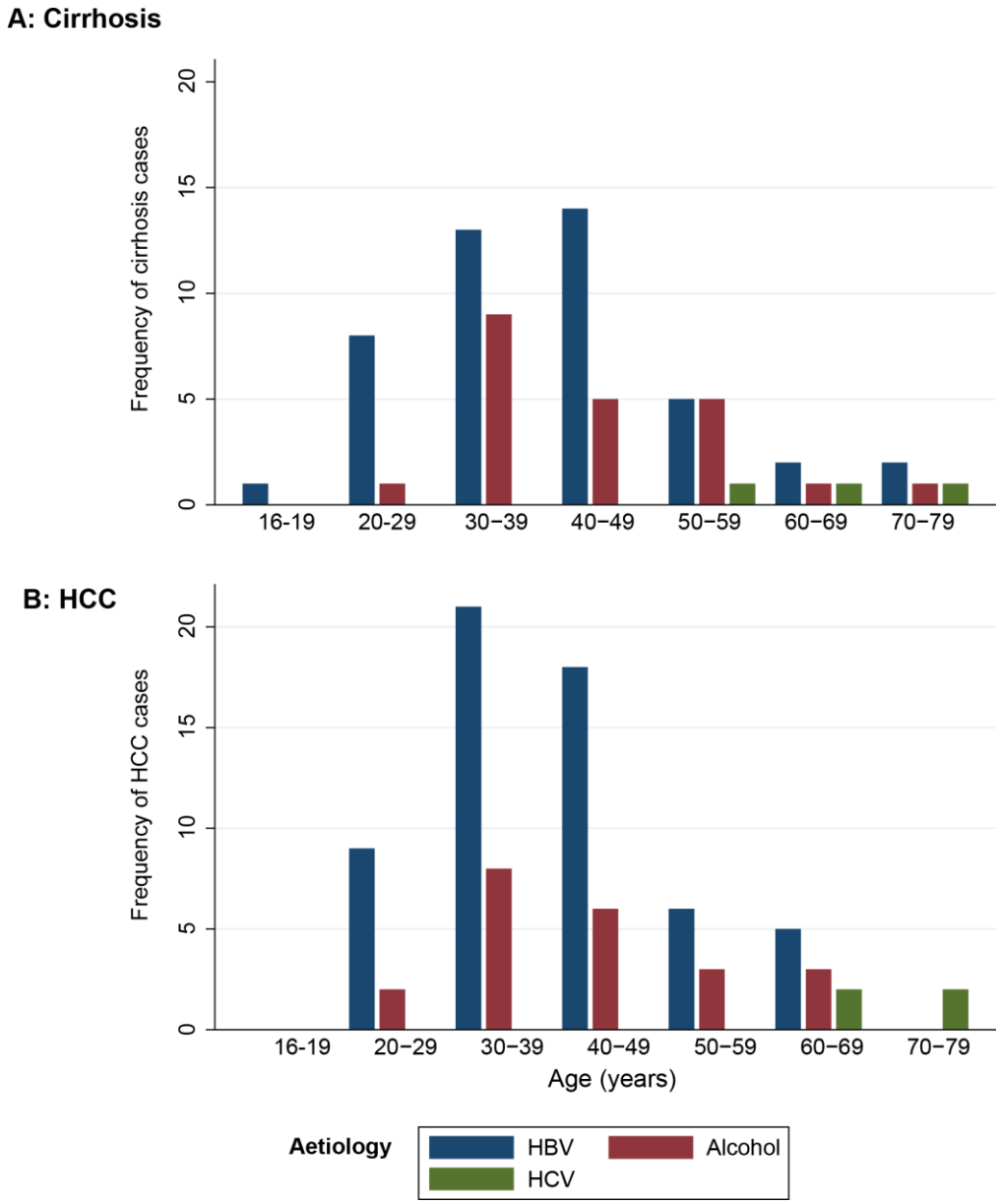
[a] HIV status was available for 246/250 (98.4%) who agreed to HIV testing or were previously known to be HIV positive. [b]There could be more than one reason for screening.

Patients were median 40 years and male gender was predominant (56 and 70% males for cirrhosis and HCC respectively) (Table 4-1). Participant age at diagnosis was associated with the aetiology of disease (Figure 4-2). Median age of diagnosis of cirrhosis for hepatitis B (HBsAg positive) related cases was 40 (IQR 32, 45), and hepatitis C related cirrhosis, was 69 (IQR 59, 73), p=0.005. For HCC patients, age of diagnosis for HBV associated cases was 39 (IQR 34, 46) while for HCV associated cases it was 68 (IQR 34, 75), p= 0.0001. Among participants reporting harmful alcohol consumption, age of onset of cirrhosis (median 40 years (IQR 35, 50)) and HCC (median 41 years (IQR 36, 50)) did not differ from HBV associated cases, p=0.59 and 0.22 respectively (Figure 4-2).

HIV prevalence was 30% in both groups and HIV positive patients with cirrhosis had more advanced immunosuppression relative to those with HCC (median CD4 count 198 vs 307), p=0.04. This compares to an estimated population HIV prevalence of 17.7% (16.0 – 19.5) for Blantyre city and 16.0 (95% CI 14.1- 18.0) for the South West region (Government of Malawi, 2018). A total of 40% of people with cirrhosis and 33% of those with HCC who were HIV positive, were already on ART, relative to a population level ART coverage of 70.2% based on the community Malawi population-based HIV impact assessment for 2015-16 (Government of Malawi, 2018, Justman et al., 2018).

Patients had experienced symptoms for a median of 30 and 45 days for cirrhosis and HCC respectively. Predominant complaints included abdominal swelling, pain, leg swelling or weight loss (Table 4-1).

Figure 4-2: Age of diagnosis for cirrhosis and HCC, stratified by aetiology [a]

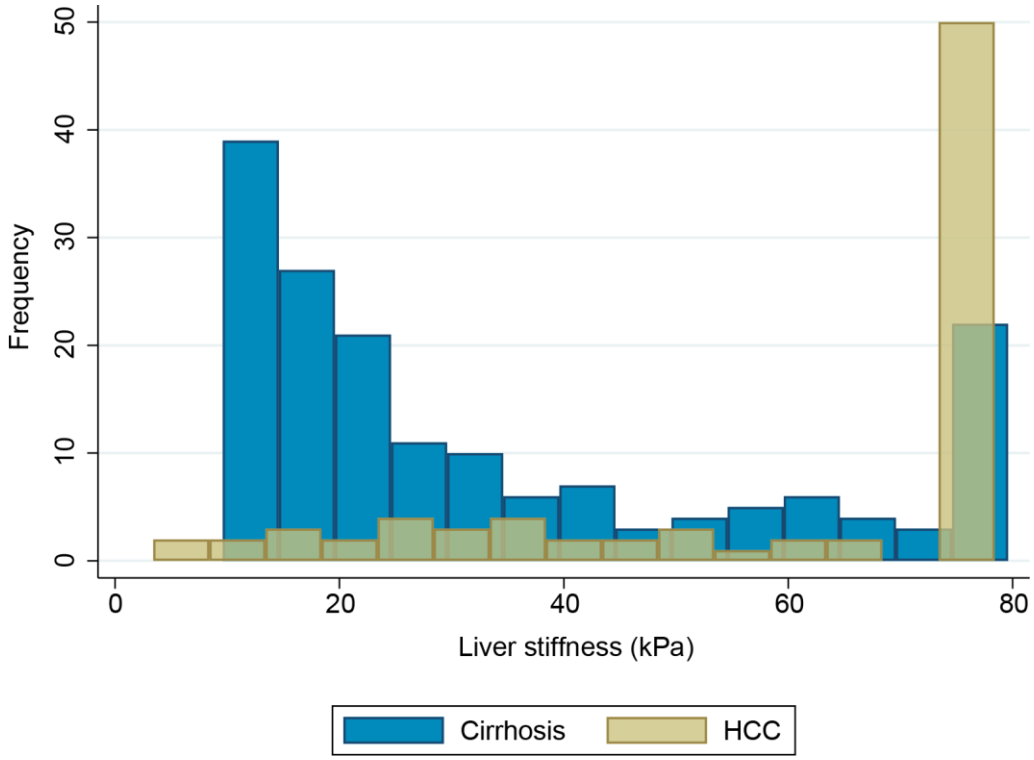


[a] Definitions: HBV, HBsAg positive; HCV, HCV RNA positive; Alcohol, harmful alcohol consumption (WHO AUDIT score ≥8/40)

Previous admissions to hospital with liver disease were reported in 11% of cirrhosis and 1% of HCC patients. A high proportion had clinical signs of chronic liver disease (89% among patients with cirrhosis and 94% among people with HCC) with cachexia, ascites and peripheral oedema the most common signs (Table 4-1). Schistosomiasis urinary CCA, indicating infection with *Schistosomiasis mansoni,* was positive in 30 and 34% of people with cirrhosis and HCC respectively. Liver stiffness measurement among cirrhosis patients was median 23.6 (IQR 14.9, 50.6) and exceeded the upper limit of quantification for most HCC patients (median >75.0 kPa (IQR 45, >75.0) in keeping with malignant invasion of the liver (Figure 4-3).

Among the included patients 21 (8%) had heard of hepatitis B prior to the study: 14% among HBV positive and 5% among HBV negative participants.

Figure 4-3: Histogram of liver stiffness measurement distribution for patients with cirrhosis and HCC



4.3.2 Utility of APRI, GPR and TREAT-B scores for diagnosis of cirrhosis

Among cirrhosis patients with hepatitis B (HBsAg positive), median AST to platelet ratio index (APRI) values were median 3.58 (IQR 1.78 – 6.39). Applying the WHO recommended cut off threshold for the diagnosis of cirrhosis (APRI >2.0) (World Health Organisation, 2015), a total of 30/44 HBsAg positive

patients with cirrhosis had an APRI exceeding 2.0, which represents a sensitivity of 68.2 (95% CI 52.4 – 81.4). Employing a cut-off threshold for APRI of 1.5, in keeping with data from West Africa suggesting improved performance with a lower threshold (Lemoine et al., 2016a), the sensitivity for the diagnosis of cirrhosis was 79.6 (95% CI 64.7 – 90.2). The gamma glutmyl transferase (GGT) to platelet ratio score (GPR) has been proposed as an alternative biomarker of cirrhosis (Lemoine et al., 2016a). Using the recommended cut-off of 0.56 derived from a study in the Gambia, the sensitivity of GPR was 81.8% (95% CI 67.3 – 91.8). The TREAT-B score, a points-based system based on HBeAg status and ALT level was also evaluated. Among the 44 HBsAg positive individuals with cirrhosis, 26 (59%) had a TREAT-B score of 2 or above, giving a sensitivity of 59.1% (95% CI 43.3 – 73.6).

4.3.3 Findings from evaluation by ultrasound

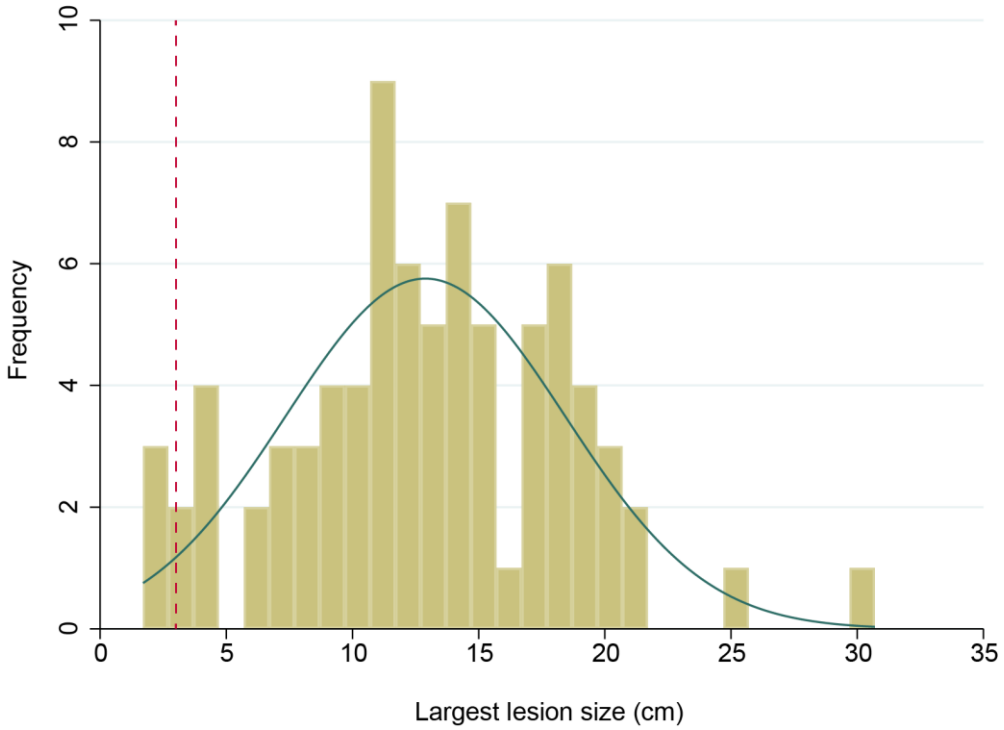
Ultrasound findings in the cohort are shown in Table 4-2. Among patients with cirrhosis, a coarse (61%) or heterogenous (26%) liver parenchyma was frequently observed, with similar proportion having a nodular (58%) or uneven waveform (29%) liver surface. In the majority of patients (56%), the hepatic vein appearance was deemed to be normal and was deemed to be tortuous in 28%. Ascites was a common finding, visualised ultrasonographically in 60% and 74% of cirrhosis and HCC patients, respectively and exceeding the prevalence detected by clinical examination (Table 4-1). Portal vein size was within normal reference values for the majority of patients, with a median size of 1.2cm, while splenomegaly was observed in 71% of cases of cirrhosis and 54% with HCC (Table 4-3). Among patients diagnosed with HCC in this study, a solitary lesion was seen in 60% and three or more in 32%. The largest lesion size was median 12.6cm (IQR 9.5, 17.3) (Figure 4-4) and compression or invasion of the portal vein was observed in 88% of cases. Most HCCs involved multiple segments and the right lobe was more commonly involved, with segment 5 in the medial right lobe involved in 95% of cases (Figure 4-5). Relative to community HBsAg positive individuals, patients with cirrhosis had smaller livers (median size of right lobe 11.1cm (IQR 9.2, 12.7) vs 13.9cm (IQR 12.4, 15.9)), and a higher proportion with a heterogenous or coarse liver parenchyma, a liver surface that was uneven or irregular, and significantly higher rates of ascites (61% vs 0%) and splenomegaly (71% vs 4%) (Table 4-2). Example ultrasound images are shown in Images 4-1 to 4-8.

Table 4-2: Ultrasound findings among patients with cirrhosis and HCC and community HBV patients without cirrhosis

	Cirrhosis n=149[a]	**HCC n=81**	**Community HBV patients n=76**
Size of right lobe (cm), median (IQR)[b]	11.2 (9.2, 12.7)	14.5 (12.8, 16.5)	13.9 (12.4, 15.9)
Parenchymal appearance[c], n(%)			
Normal	20 (13)	8 (11)	71 (93)
Heterogenous	39 (26)	11 (15)	4 (5)
Coarse	90 (61)	56 (75)	1 (1)
Liver surface[c], n(%)			
Normal smooth	19 (13)	13 (17)	72 (95)
Uneven waveform	43 (29)	14 (18)	4 (5)
Nodular irregular	87 (58)	51 (65)	0 (0)
Left hepatic vein appearance[c], n(%)			
Normal smooth	83 (56)	16 (22)	72 (95)
Obscured vessel wall with normal diameter	24 (16)	12 (16)	4 (5)
Irregular tortuous and narrowed vessel	41 (28)	46 (62)	0 (0)
Ascites visualised, n(%)	89 (60)	58 (74)	0 (0)
Portal vein size (cm), median (IQR)	1.2 (0.9, 1.4)	1.2 (0.9, 1.4)	1.1 (0.9, 1.3)
Spleen size (maximum length)	16.6 (12.2, 19.4)	13.0 (11.2, 15.1)	11.6 (9.9, 13.9)
Spleen size (maximal width)	6.0 (4.8, 7.9)	4.8 (4.0, 5.5)	
Splenomegaly (>12.7cm), n(%)	106 (71)	43 (54)	3 (4)
Splenic varices, n(%)	22 (15)	3 (4)	0 (0)
HCC appearance			
Solitary lesion	-	48 (60)	-
Two lesions	-	6 (7)	-
Three or more lesions	-	26 (32)	-
Largest lesion size (cm), median (IQR)	-	12.6 (9.6, 17.3)	-
Compression or invasion of portal vein, n (%)	-	71 (88)	-

[a]Study ultrasounds were performed for 149/169 (88%) patients with cirrhosis. [b]Right lobe size was measured in sagittal length at the midclavicular line in longitudinal section. [c]Could not be assessed in all HCC patients due to tumour obstructing residual liver appearance. Categorical scoring system adopted from (Hung et al., 2003)

Figure 4-4: Histogram and normal distribution plot of largest lesion size among patients with HCC



Red dashed line indicates 3cm, denoting early stage lesions (BLCL stage A) for which curative treatment may be considered such as resection or transplantation, according to EASL 2018 HCC guidelines.

Figure 4-5 : Location of hepatocellular carcinoma visualised on ultrasound, by hepatic segment involvement, as a percentage of total number of cases

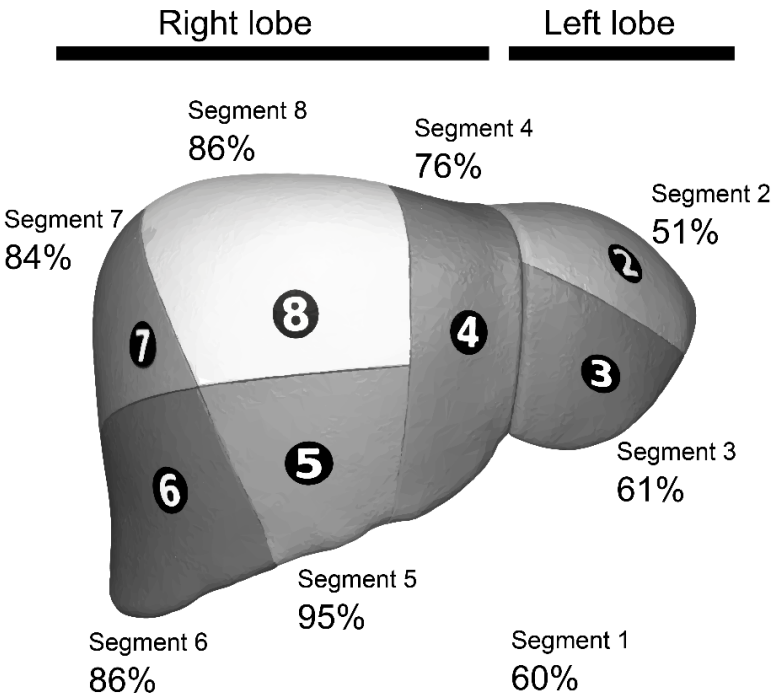
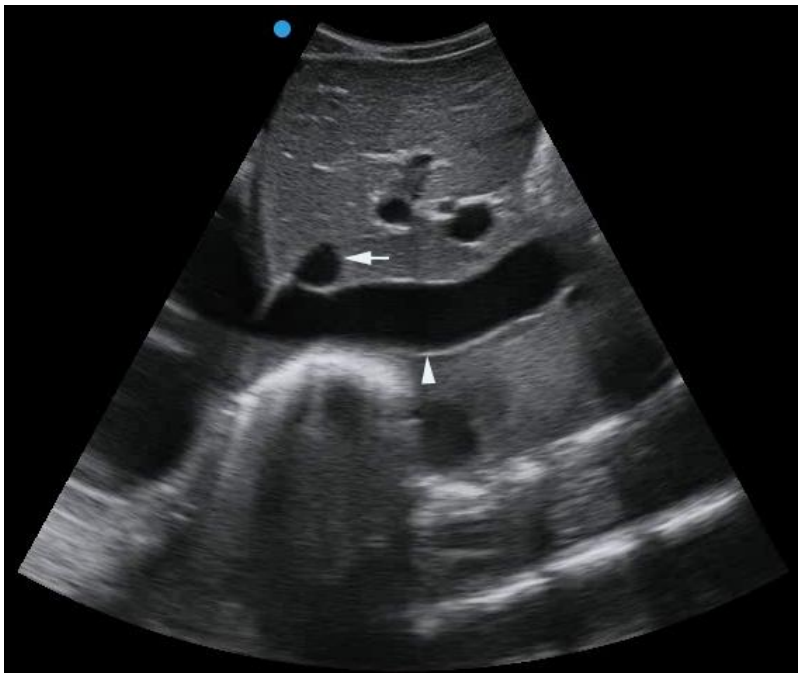


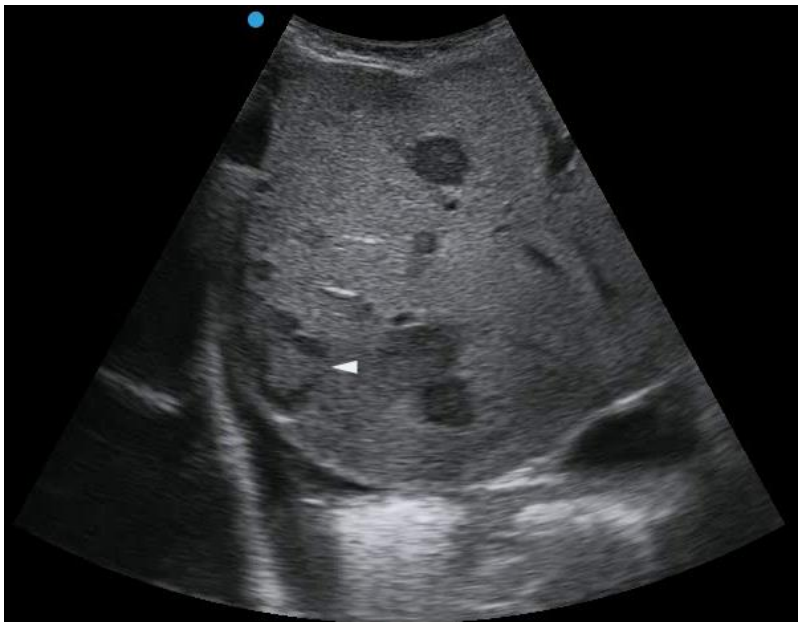
Image 4-1: Exclusion by ultrasound: Dilated inferior vena cava and hepatic veins due to cardiac failure. Hepatic parenchyma was normal



The IVC measured 2.3cm in AP diameter at 1cm inferior to the cavoatrial junction (posterior wall of IVC denoted by arrowhead) and hepatic veins (dilated left hepatic vein branching from IVC seen by white arrow).

Severe hypokinesis of the left ventricle was observed. The patient had ascites. The hepatic parenchyma was otherwise normal, consistent with elevated liver stiffness due to cardiac failure

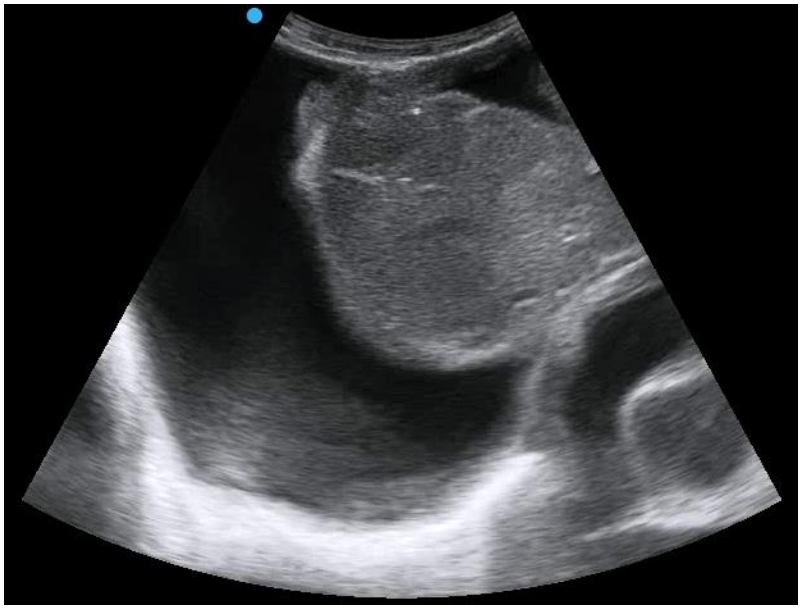
Image 4-2: Exclusion by ultrasound: Hepatic metastases



Multiple lesions of mixed echogenicity are seen in the right lobe.

The lesion highlighted with a white arrowhead indicates a hyperechoic lesion with a surrounding hypoechoic rim indicating surrounding oedema or proliferation. Appearances were consistent with diffuse hepatic metastases and a pancreatic lesion was suspected to represent the primary.

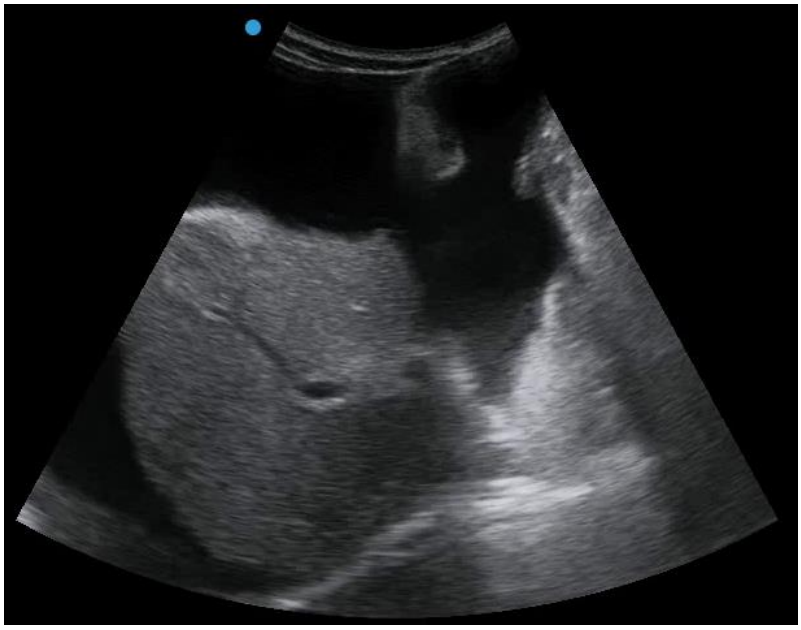
Image 4-3: Ascites and coarse hepatic parenchyma in patient with cirrhosis



This sagittal view of the right lobe of the liver shows a small, atrophic right lobe, with coarse hepatic parenchyma and nodular surface with gross ascites, consistent with cirrhosis.

The patient was negative for HBsAg and HCV Ag/Ab and reported abstinence from alcohol.

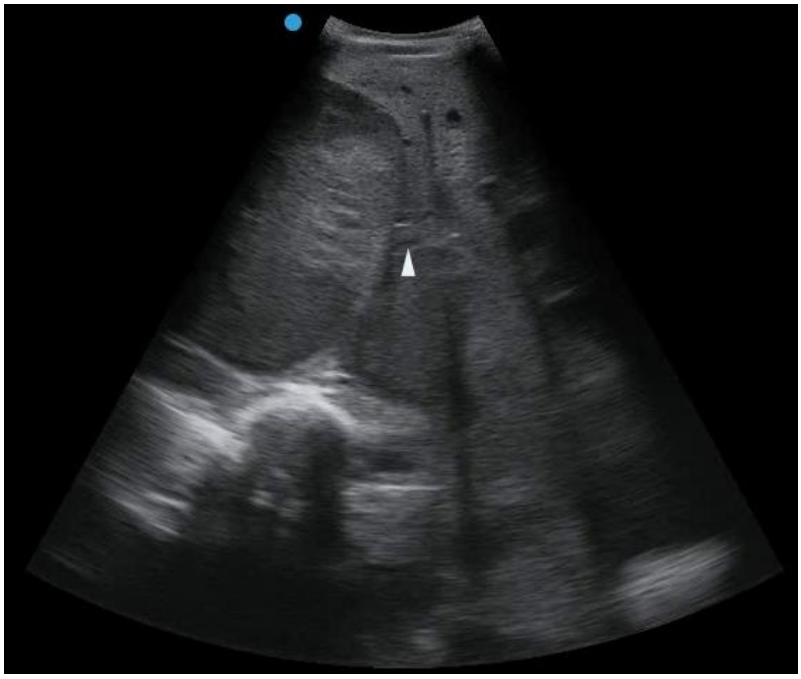
Image 4-4: Nodular liver surface, coarse echotexture and ascites in a patient with cirrhosis



An atrophic right lobe is visible with a heterogenous parenchymal echotexture, a nodular, irregular liver surface and gross ascites consistent with cirrhosis. The patient had splenomegaly (maximal length 14.6cm).

HBsAg and HBeAg were positive with a HBV DNA concentration of 6.5 $\log_{10}$ IU/ml.

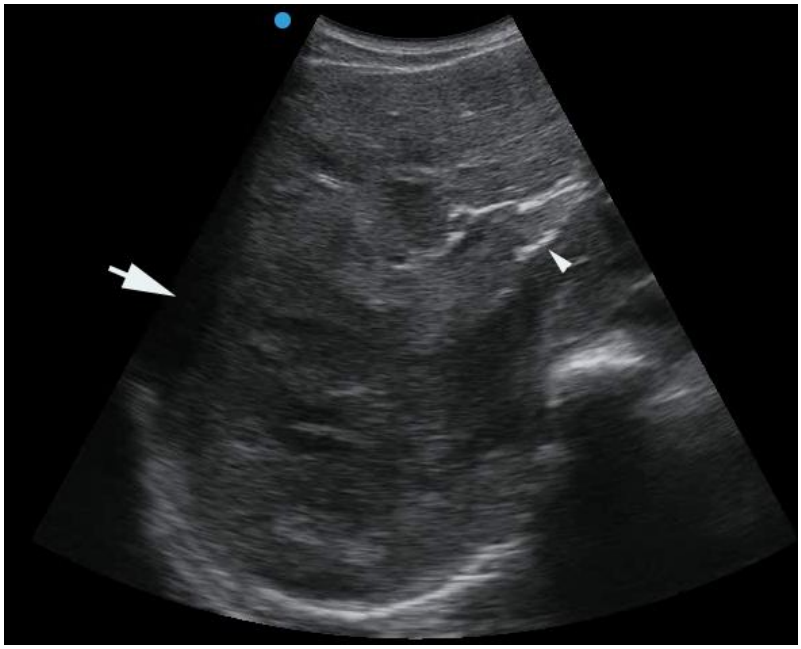
Image 4-5: Large HCC with mass effect arising from right lobe of liver



A large well-defined mass in the right lobe measuring 17cm in maximal length is visible. The lesion is of mixed echogenicity and causes compression of the portal vein (white arrowhead) and mass effect on surrounding vasculature. Ascites is visible. The mass is consistent with HCC on background cirrhosis.

The patient is HBsAg negative, anti-HBc positive with negative HBV DNA. He has low risk alcohol consumption.

Image 4-6: Large HCC with invasion of portal vein and tumour thrombosis

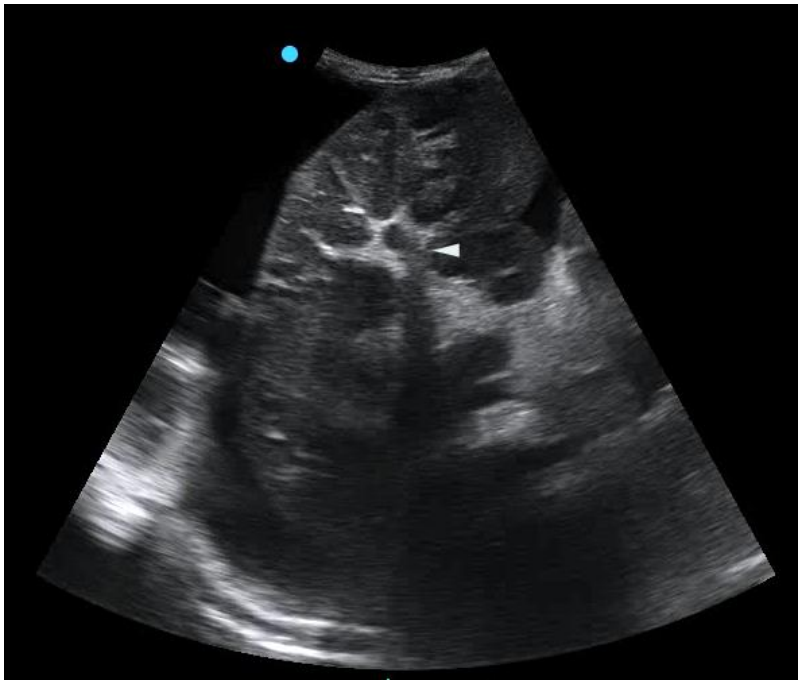


A 14cm heterogenous mass in segments 5-7 of the right lobe is visible (white arrow), with invasion and tumour thrombosis of the proximal right portal vein (arrowhead). The lesion is consistent with HCC.

The background liver echotexture is coarse and splenomegaly was evident suggesting background cirrhosis.

The patient was HBsAg positive with a HBV DNA concentration of 2.4 $\log_{10}$ IU/ml.

Image 4-7: Features of periportal fibrosis consistent with schistosomiasis

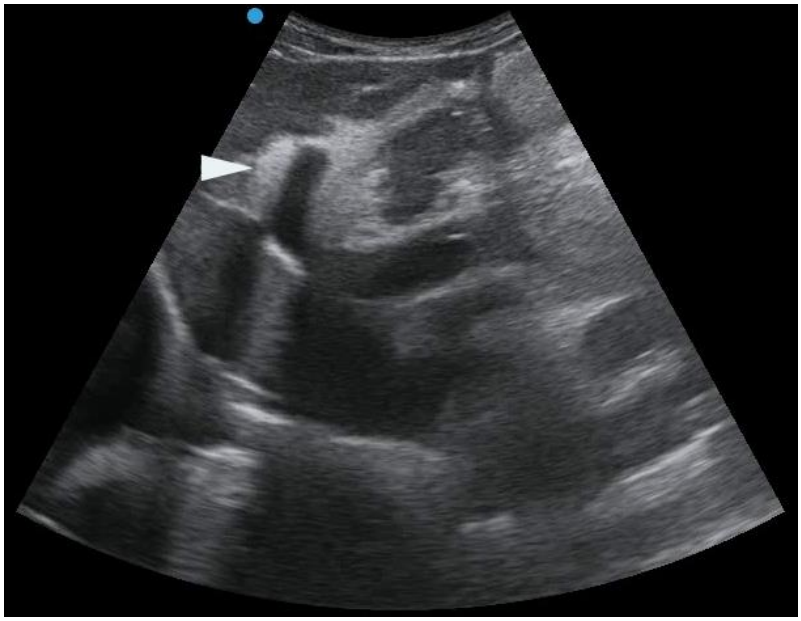


This sagittal view of the right lobe of the liver shows the proximal right portal vein (white arrowhead) with surrounding periportal fibrosis.

The patient had ascites (visible at the upper left region of the image).

Investigations for HBV and HCV were negative and the patient reported abstinence from alcohol.

Image 4-8: Extensive periportal fibrosis of the right portal vein consistent with schistosomiasis



The right portal vein (arrowhead) has an extensive surrounding hyperechoic region of fibrosis which extends along the length of the portal venous system.

The portal vein is dilated (1.4cm) and the patient has splenomegaly (19cm).

Urine schistosomiasis CCA is negative, but the patient has previously been treated several times with praziquantel.

Table 4-3: Aetiology of cirrhosis and HCC

Aetiology	n(%) or median (IQR)		
n(%) or median (IQR)	Cirrhosis (n=169)	HCC (n=81)	Community controls
Hepatitis B			
HBsAg positive	45 (27)	59 (73)	150/3253 (5)
Among HBsAg positive			
HBeAg positive	19/45 (42)	22/59 (37)	10/94 (11)
HBV DNA log10 IU/ml	4.0 (2.3, 6.3)	4.3 (2.7, 6.1)	2.3 (1.5, 3.4)
HBV DNA>2000 IU/ml	26/45 (58)	39/59 (66)	19/94 (20)
HBV DNA>20,000 IU/ml	20/45 (44)	31/59 (53)	11/94 (12)
Anti-HDV positive	0/45 (0)	2/59 (3)	2/94 (2)
Among HBsAg negative			
Anti-HBc positive[a]	65/124 (52)	14/22 (64)	219/634 (35)
Occult HBV	1/169 (1)	2/81 (2)	-
Hepatitis C			
HCV Ag/Ab positive	10 (6)	5 (6)	13/1661 (0.8)
HCV RNA positive[b]	3 (2)	4 (5)	3/1661 (0.2)
HCV RNA (log_{10} IU/ml)	6.0 (4.9, 6.1)	6.2 (5.7, 6.5)	6.6 (5.9, 7.1)
Alcohol			
WHO AUDIT score	0 (0, 2)	2 (0, 8)	0 (0, 3.5)
Abstinent (0)	123 (73)	40 (49)	152/224 (68)
Low risk (1-7)	24 (14)	18 (22)	46/224 (21)
Hazardous (8-15)	14 (8)	12 (15)	19/224 (8)
Harmful (16-19)	4 (2)	4 (5)	3/224 (1)
Alcohol dependence (20-40)	4 (2)	6 (7)	4/224 (2)
Schistosomiasis			
Urine CCA positive	50 (30)	27 (34)	4/222 (2)
Ultrasound evidence of schistosomiasis	18 (11)	0 (0)	-
HIV status			
HIV positive	50/166 (30)	23/80 (29)	11/118 (9)
Previous tuberculosis	22/169 (13)	3/81 (4)	3/119 (3)
Previous TB, among HIV negative participants	9/116 (8)	3/57 (5)	2/107 (2)
Multiple aetiology[c]			
HBV and HCV co-infection	0 (0)	0 (0)	0 (0)
HBV and alcohol	5 (3)	17 (21)	13 (0.4)
HCV and alcohol	1 (0.6)	0 (0)	0 (0)

[a] Anti-HBc was tested among HBsAg negative participants [b]Occult HBV was defined as HBsAg negative, anti-HBc positive with detectable HBV DNA. [b]HCV RNA was tested among HCV Ag/Ab positive individuals. [c]HBV is defined

here as HBsAg positive, HCV by HCV RNA positive and alcohol by ≥hazardous alcohol consumption by the WHO AUDIT score)

Table 4-4 Population attributable fraction to cirrhosis and HCC of hepatitis B, D and C, alcohol and HIV

Aetiology	**Population attributable fraction (%) (95% CI)**	
	Cirrhosis	**HCC**
Hepatitis B virus		
HBV infection (HBsAg positive)	23.1 (15.7 – 29.8)	71.5 (59.3 – 80.1)
Hepatitis D virus		
Anti-HDV positive	[a]	0.6 (-5.7 – 6.6)
Hepatitis C virus		
HCV Ag/Ab positive	4.7 (1.0 – 8.3)	5.5 (0.0 – 10.7)
HCV RNA positive	1.6 (-0.4 – 3.6)	4.8 (-0.1, 9.5)
Alcohol		
≥Hazardous consumption	1.6 (-6.1 – 8.7)	18.0 (5.3, 28.9)
≥Harmful consumption	1.7 (-2.5 – 5.6)	9.7 (1.5, 17.1)
HIV		
HIV positive, all participants	22.9 (13.5 –31.4)	21.4 (8.6 – 32.4)
HIV positive, among HBsAg negative people	23.4 (12.6 -33.0)	19.1 (-5.2 – 37.8)
HIV positive, among HBsAg and HCV Ag/Ab negative people	25.3 (12.7 – 36.0)	25.3 (-4.9, 46.8)
Not receiving ART, among HIV positive individuals[b]	39.1 (5.8- 60.7)	46.7 (15.3 – 71.2)

[a]No patients with cirrhosis were positive for anti-HDV. [b]The "zero" comparison scenario for the PAF calculation is where all HIV positive individuals are receiving ART.

4.3.4 Aetiology of cirrhosis and HCC

Among patients with cirrhosis and HCC 27% (44/169) and 73% (59/81) were HBsAg positive, relative to a community prevalence of 5% (150/3253) from the census-based serological survey of Ndirande among individuals aged >16 years (Table 4-3). Of the HBsAg negative population, 65/125 (52.0%) of cirrhosis patients and 15/22 (68.2%) of HCC patients were anti-HBc positive, relative to a prevalence of 219/634 among HBsAg-negative community controls (34.5%). A further 0.6% (1/169) and 2.4% (2/82) of cirrhosis and HCC patients had evidence of occult HBV, with HBsAg negative, anti-HBc positive and detectable HBV DNA. Among the three patients with occult HBV, HBV DNA was 613 IU/ml

among the patient with cirrhosis and 426 IU/ml and 288 IU/ml among the HCC patients respectively. Anti-HDV was not detected among any of the cirrhosis patients and among 2/82 (2.4%) of the HCC patients, relative to a community prevalence of 2/94 from the community evaluation study (2.1%) (Table 4-3).

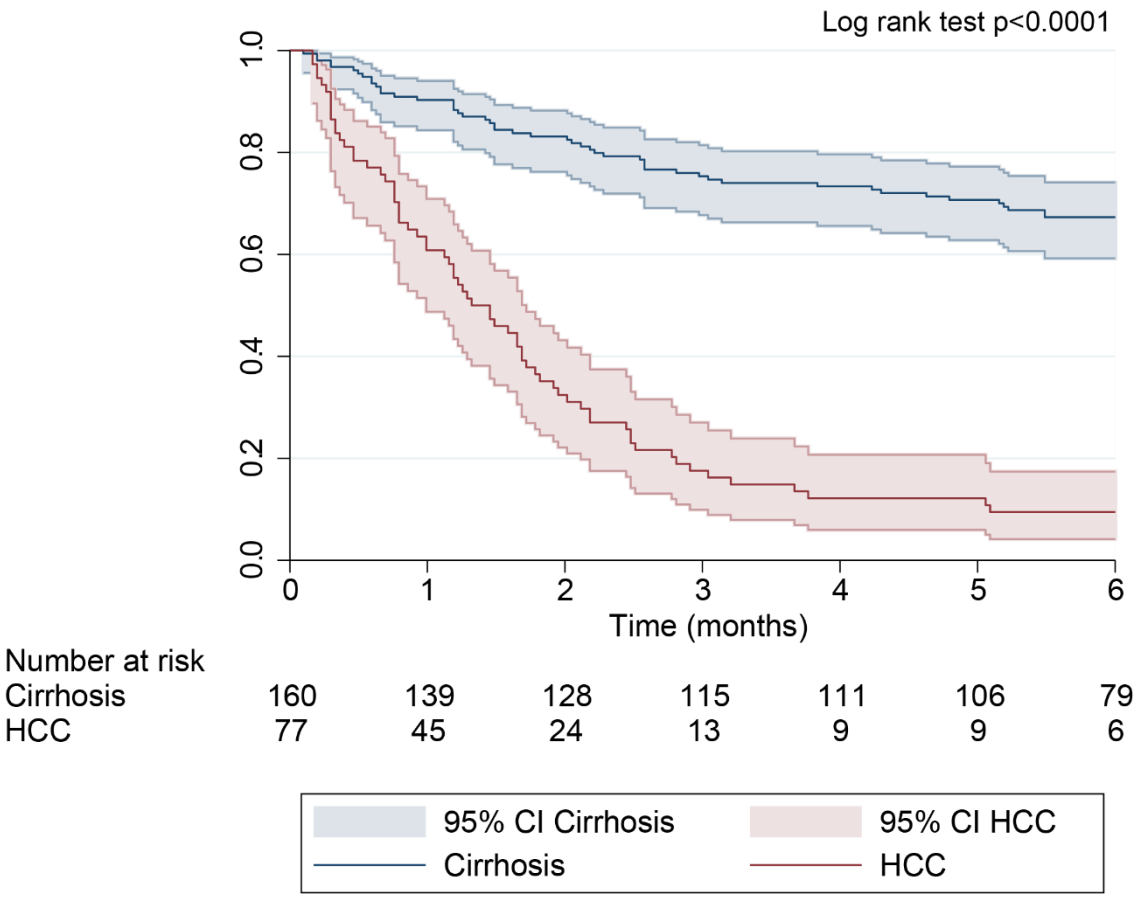
Hepatitis C Ag/Ab was positive in 11/169 (6.5%) of cirrhosis patients and 4/82 (4.9%) of HCC patients, and HCV RNA was confirmed in 4/169 (2.4%) and 3/82 (3.7%) of cirrhosis and HCC patients respectively. This compares to community HCV Ag/Ab and HCV RNA prevalence of 0.8% and 0.2% respectively (Table 4-4).

The majority of patients assessed by the WHO audit tool reported abstinence from alcohol (Table 4-3). Among cirrhosis patients, hazardous or greater drinking (WHO audit score ≥8/40) was reported in 22/169 (13.0%) which compared to a community control prevalence of 11.6% (26/224). Among HCC patients, 22/81 (27.2%) reported ≥ hazardous drinking. Schistosomiasis urinary CCA was positive in 50/169 (29.6%) of cirrhosis patients, 27/81 (33.3%) of HCC patients and 4/222 (1.8%) of community patients.

The PAF of HBV infection to cirrhosis was 23.1% (95% CI 15.7- 29.8). HCV was a less important cause of cirrhosis with an attributable fraction for HCV RNA of 1.6% (95% CI -0.4 – 3.6). Harmful alcohol consumption was not a major contributor to cirrhosis in this cohort with similar rates to community controls (PAF 1.6% (95% CI -6.1 to 8.7) for alcohol (Table 4-4). By contrast, HIV infection was strongly associated with cirrhosis with a PAF of 22.9% (13.5- 31.4) among all participants and 23.4% (95% CI 12.6-33.0) among HBsAg negative participants. Among patients who were HIV positive, not receiving antiretroviral therapy was associated with a PAF of 39.1% (95% CI 5.8- 60.7) for cirrhosis. Similar PAF rates were observed for HIV status in relation to HCC (Table 4-5). Previous TB therapy was also associated with cirrhosis (prevalence of previous TB (2.5% (95% CI 0.9 -7.2) among community participants vs 13.0% (95% CI 8.8 – 18.9) among people with cirrhosis). The association between previous TB and cirrhosis persisted after adjustment for HIV status (adjusted odds ratio, for odds of previous TB in cirrhosis patients vs community 4.0 (95% CI 1.1 – 14.2), p=0.03).

HBV infection was strongly associated with HCC. The PAF for HBsAg to HCC was 71.5% (95% CI 59.3 – 80.1). In contrast to cirrhosis, harmful alcohol consumption was a significant contributor to HCC, with a PAF of 18.0% (95% CI 5.3, 28.9)

Figure 4-6: Kaplan-Meier survival estimates: six month outcomes for patients with cirrhosis and HCC



Abbreviations: CI = confidence interval, HCC= hepatocellular carcinoma. Shaded areas represent pointwise 95% confidence interval around the survivor function.

4.3.5 Outcomes and prognostic factors for mortality from cirrhosis and HCC

Outcome data at 6 months were available for 225/250 patients (90%). A total of 25/250 (10%) patients were lost to follow up at 6 months and outcomes could not be ascertained, despite extensive efforts to trace participants at their homes. Among the remaining 225 patients, 108 (43%) completed 6 months of follow up and 117 (47%) had died (Figure 4-6). Median survival for patients with HCC was 1.26 months (38 days). One, three and six month survival for HCC was 59.2% (95% CI 48.9 – 68.3), 18.9% (11.6 – 27.6) and 9.8% (95% CI 4.8 – 16.9) respectively. For cirrhosis, one, three and six month survival was 91.4% (95% CI 87.5 – 94.1), 75.0% (95% CI 67.8 – 80.9) and 67.0% (95% CI 59.0 – 73.8). Among people with cirrhosis, predictors of survival by 6 months included the liver stiffness measurement (LSM) by transient elastography with a 2.5% (95% CI 1.3 – 3.6) increased hazard of

mortality at 6 months per 1kPa increase in LSM (Table 4-5, Figure 4-7 and Figure 4-8). By univariate analysis, age and the presence of ascites on ultrasound were also significant predictors of mortality among patients with cirrhosis by 6 months (Table 4-5). HIV status, gender, and disease aetiology were not associated with differences in mortality hazard. Splenomegaly was found to be associated with a reduced risk of mortality. This finding persisted whether considering a dichotomous threshold of 12.7cm in maximal length(Mustapha et al., 2010), or continuous variables of maximal length, or length x maximal splenic width (maximal anterior to posterior diameter of spleen on transverse section). It was observed that splenomegaly was negatively associated with age (Spearman's rho for association between age and maximal splenic length= -0.167, p=0.006). After adjustment for age, splenomegaly was not associated with mortality (adjusted HR = 0.88 (95% CI 0.74 – 1.04), p=0.23.

Table 4-5 Univariable predictors of mortality from cirrhosis: Cox proportional hazards model

Explanatory variable	**Hazard ratio (95% CI)**		**P value**
Age, per year increase	1.03	(1.01 – 1.05)	0.003
Sex, female vs male	0.62	(0.34 – 1.11)	0.11
HIV positive	1.13	(0.62 – 2.04)	0.69
Hepatitis B (HBsAg positive)	1.23	(0.66 – 2.28)	0.52
Hepatitis B positive, referred for HBV antiviral treatment	0.20	(0.03 – 1.47)	0.12
Hepatitis C (HCV RNA positive)	0.96	(0.13 – 6.92)	0.96
Alcohol (hazardous consumption)	0.98	(0.44 – 2.18)	0.96
Schistosomiasis (urine schistosomiasis CCA positive)	0.78	(0.42 – 1.47)	0.45
Signs of schistosomiasis on ultrasound	0.89	(0.32 – 2.51)	0.83
Presence of ascites on ultrasound	10.38	(3.19 – 33.8)	<0.0001
Splenomegaly on ultrasound >12.7cm	0.47	(0.25 – 0.89)	0.02
Liver stiffness measurement, per 1 kPa increase	1.03	(1.01 – 1.04)	<0.0001
AST to platelet ratio index (APRI), per 1 unit increase	1.10	(1.00 – 1.22)	0.06

Figure 4-7: Kaplan Meier survival function among patients with cirrhosis, stratified by liver stiffness measurement

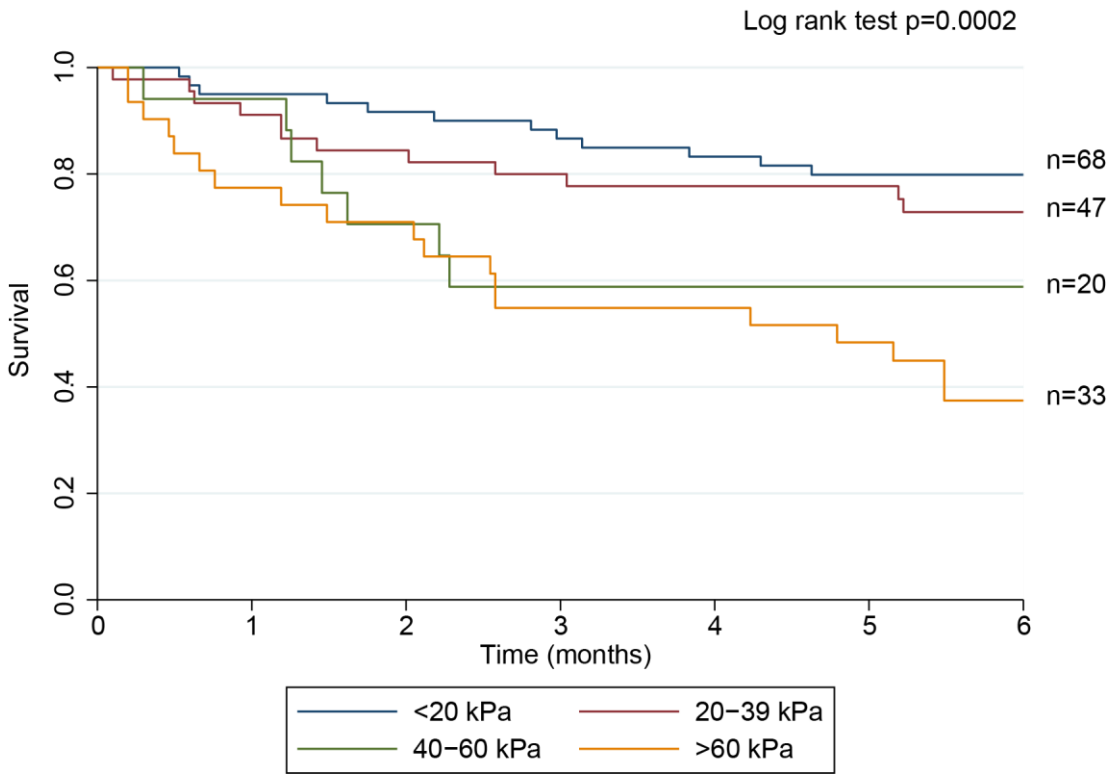


Figure 4-8: Mortality at six months among patients with cirrhosis, stratified by liver stiffness measurement by transient elastography

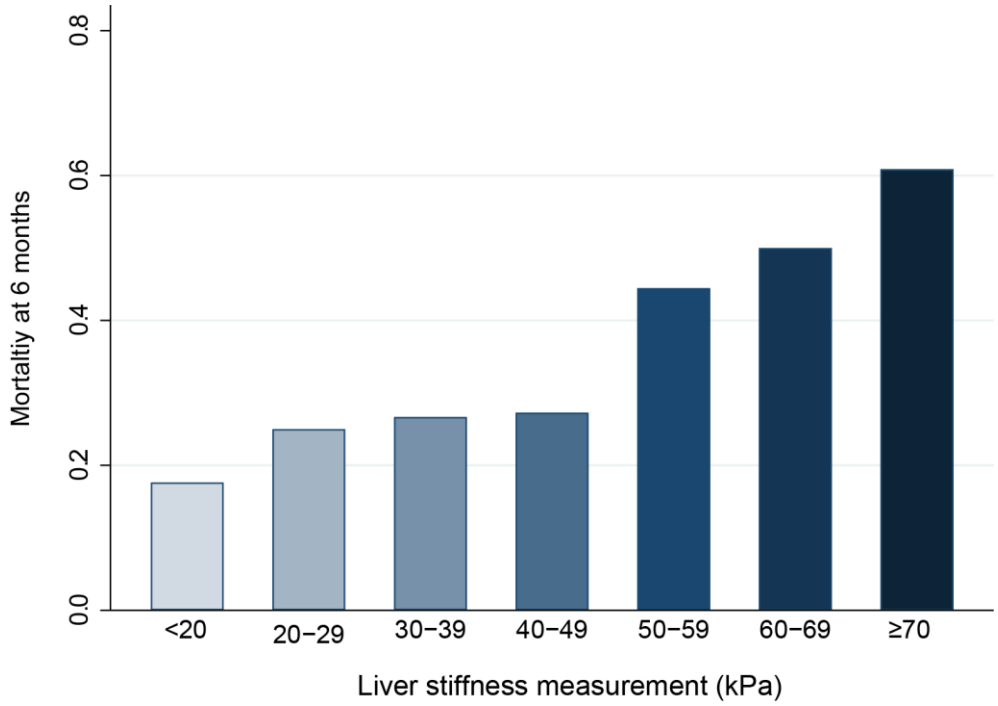


Table 4-6: Predictors of mortality among patients with cirrhosis: multivariable Cox proportional hazards models

Explanatory variable	Model 1			Model 2		
	Hazard ratio (95% CI)		P value	Hazard ratio (95% CI)		P value
Age, per year increase	1.03	(1.01 – 1.05)	0.006	1.04	(1.01- 1.07)	0.004
Liver stiffness measurement, per 1 kPa increase	1.03	(1.01 – 1.04)	<0.0001	1.01	(1.00 – 1.03)	0.17
Presence of ascites on ultrasound				8.46	(1.86 – 38.59)	0.006

Among the subset of HBsAg positive patient with cirrhosis, liver stiffness measurement (LSM) was associated with mortality (hazard ratio (HR) per 1kPa increase in LSM = 1.04 (95% CI 1.01 – 1.08), p=0.005) but the APRI score was less well correlated with mortality, either considered as a continuous variable (HR per 1.0 increase in APRI = 1.10 (95% 1.00 – 1.22), p=0.06) or as a dichotomous variable at the cut-off of 2.0 (HR 1.33 (95% CI 0.42 – 4.25), p=0.63). Patients with cirrhosis who were HBsAg positive were referred to the HIV clinic to receive lifelong tenofovir/lamivudine (300mg/300mg). Among the subset of HBsAg positive patients referred for treatment, relative to the remaining patients with cirrhosis, the hazard ratio for mortality was 0.20 (95% CI 0.03- 1.47), p=0.12 showing a trend toward reduced mortality.

In a multivariable model, age, liver stiffness and presence of ascites were strong predictors of mortality at 6 months among patients with cirrhosis (Table 4-6).

4.4. Discussion

In this study of patients diagnosed with cirrhosis or HCC at a tertiary referral hospital in Blantyre, Malawi, we observed several important findings with implications for clinical practice and public health, relating to the aetiology, diagnosis and clinical outcomes of liver disease. Patients diagnosed with a pragmatic definition of HCC in this study presented to hospital at an advanced stage, with a median tumour size of 12.4cm, multiple lesions visualised in 40% and with compression or invasion of the portal vein observed in 88%. Only 4/82 (5%) HCC lesions were less than 3cm, and only 2/82 (2%) were <3cm size and did not have invasion of the portal venous system, representing lesions that could be amenable to surgical resectable according to EASL guidelines (European Association for the Study

of the Liver, 2018a). These findings represent particularly poor outcomes by global comparison and in relation to other countries from the region. In a multicentre cohort study of HCC from 19 tertiary referral centres from sub-Saharan Africa, including Nigeria, Ghana, Cameroon, Cote d'Ivoire, Sudan, Ethiopia, Tanzania and Uganda, the median age at diagnosis was 46 years (IQR 36, 58), HBV was the cause in 55% of cases (597/1082) and the size of the largest tumour at diagnosis was a mean of 8cm (standard deviation (SD) 4) (Yang et al., 2017). By comparison, our cohort had a significantly more advanced presentation with larger tumour size, our patients were diagnosed at a younger age (median 40 years), and HBV was responsible for a higher proportion of disease (73% HBsAg positive). The median survival in the multicentre cohort from sub-Saharan Africa was 2.5 months (IQR 2.0 -3.1), relative to 1.3 months in Malawi. In Malawi, HCC diagnosis occurs late and with a poorer prognosis relative to other tertiary centres in sub-Saharan Africa. At the point of diagnosis, it is too late to offer potentially curative therapy and to prevent HCC- associated mortality.

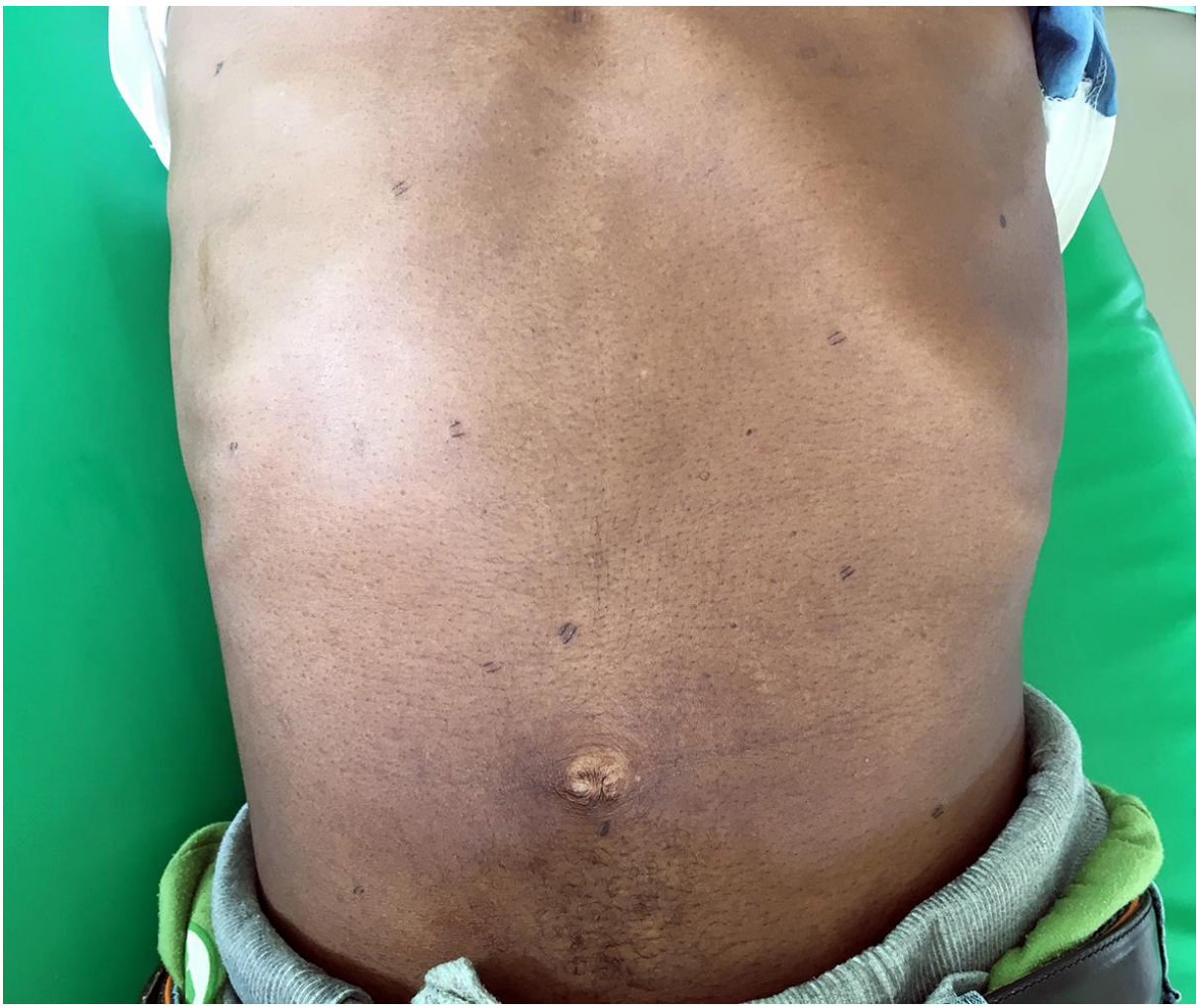
4.4.1 Delays in diagnosis

These findings should act as a stimulus to identify additional preventative public health interventions and to introduce strategies to diagnose HCC before presentation to hospital. The latter objective may be problematic in Malawi, where presentation to a healthcare facility is often delayed. This may be due to the need for referral from lower levels of the healthcare system where clinical staff often make attempts to try empirical therapy before arranging referral, and where patients often seek care outside the health service from a traditional healer prior to or alongside seeking medical care. An example clinical vignette is provided by Figure 4-9, as an example of a typical presentation on a ward round in the Department of Medicine in Queen Elizabeth Central Hospital in Blantyre.

The 34-year old patient pictured in Figure 4-9 presented with five months of right upper quadrant pain and had received repeated empirical therapy with proton pump inhibitors for peptic ulcer disease and attempts to eradicate *Helicobater pylori* with combination antibiotic therapy. A large and firm mass was palpable in the right upper quadrant. The photograph shows a visible exophytic mass in the right upper quadrant and multiple scarification scars from having sought previous assistance from a traditional healer which he described seeking several months prior to attending the hospital. Scarification has been described in many societies across sub-Saharan Africa both as a marker of cultural identity and in the practice of traditional medicine (Garve et al., 2017). Due to the frequent lack of instrument sterilisation among traditional healers, scarification has also been epidemiologically linked to HBV and HCV transmission.(McCarthy et al., 1994b, Adewole et al., 2009) This patient had

an 11.8cm mass in the right lobe of the liver on ultrasound causing compression of the portal vein consistent with HCC and died six weeks after the diagnosis.

Figure 4-9: Photograph of a 34 year old male patient with visible exophytic mass in right upper quadrant and scarification marks



The patient provided written informed consent for publication of this photograph and case history

Medical pluralism, defined as the use of more than one health system for diagnosis and treatment is common in sub-Saharan Africa, frequently involving switching between traditional, faith-based and biomedical healthcare (Moshabela et al., 2017). In a series of in-depth interviews conducted among people living with HIV in Eastern and Southern Africa, including Malawi, patients reported often trying traditional medicine as a first option after experiencing symptoms, then waiting to ascertain if any

response, before seeking an alternative healthcare source (Moshabela et al., 2017). Concurrent utilisation of both traditional/faith-based medicine and biomedical healthcare was also frequently reported and could in some cases cause conflict between the two systems or represent bottlenecks to accessing care (Legare and Gelman, 2008). Significant delays were evidenced by the extensive size of HCC lesions (median 12.4cm) and suggests that patients might have minimised their report of duration of symptoms (median reported duration for HCC 45 days) to avoid stigmatisation or challenge for their delay in seeking medical care. It is unclear to what extent an earlier diagnosis could be facilitated through community engagement to promote symptom awareness. It is likely that earlier diagnosis of HBV through community screen and treat programmes, using existing voluntary counselling and testing infrastructure for HIV, and introduction of additional measures to prevent HBV transmission, to be explored in Chapter 5, would be more feasible avenues to reduce HCC-associated mortality.

4.4.2 Opportunities for HBV disease control

While our six-month follow up data of HCC and cirrhosis patients demonstrate a bleak prognosis from the time of diagnosis in Blantyre, the finding of a very high attributable fraction to hepatitis B also offers hope since there are a number of public health tools available to treat and to prevent infection. We observed that among patients with HCC, a very high proportion had active or occult HBV infection with a population attributable fraction from HBV infection of 71.5%. Furthermore, even among HBsAg and HBV DNA negative patients, a high proportion had evidence of past infection with HBV with isolated anti-HBc. The very high proportion of HCC cases attributable to HBV represents a considerable opportunity for prevention. A modelling study illustrated that while in the long-term infant vaccine will begin to reduce HBV-associated mortality through prevention of infant infection, rising life expectancy in sub-Saharan Africa is projected to lead to rising adult mortality, and this can only be tackled among HBsAg positive individuals by introduction of adult screening and treatment programmes (Nayagam et al., 2016b). In keeping with other cohorts from sub-Saharan Africa, the age of diagnosis was substantially younger than in Western or Asian cohorts, although this may reflect the different demographic structure of the population with equivalent age-specific incidence, in view of the substantially younger population structure relative to high-income country settings (Shimakawa and Lemoine, 2017).

4.4.3 Role of HIV in cirrhosis and HCC

A striking association between HIV infection and both cirrhosis and HCC was noted. This association was observed in HBV positive patients and in patients negative for both HBV and HCV. HIV prevalence among people with cirrhosis and HCC, at 30%, exceeded the estimated population prevalence for Blantyre city at 17.8%, and a greater proportion were not receiving ART relative to the population rates of ART coverage (Government of Malawi, 2018). Not being on ART was attributable to a third of cirrhosis cases and almost half of HCC cases among patients with HIV. HIV has been associated with multiple alterations in hepatocyte biology as discussed in Chapter 1, including interactions with hepatic stellate cells and promotion of a pro-inflammatory and pro-fibrotic environment, thus accelerating fibrosis progression associated with HBV and HCV (Bruno et al., 2010, Audsley et al., 2012). The main mechanism of action of HIV in the oncogenesis of HCC have been described in terms of modulation of the immune response to HBV or HCV co-infection (Degos and Tural, 2007). In this cohort, the finding of a direct association with HIV even in the absence of HBV or HCV co-infection suggests HIV might be causally responsible for the pathogenesis of these diseases. Opportunistic infections affecting the liver remain potential alternative causes. In the context of HIV infection, hepatitis E virus infection, particularly genotypes 3 and 4, can develop into chronic infection with persistent viraemia and progression to cirrhosis but it is uncommonly described with a prevalence ranging from 0 to 0.5% in high income country settings among HIV positive populations (Rivero-Juarez et al., 2019). Alternative explanations include that older antiretroviral therapy regimens associated with hepatic fibrosis such as stavudine or didanosine could be responsible. We did not ascertain ART history and composition of past regimens of people living with HIV to facilitate an assessment of this hypothesis, since our patients were mostly unable to accurately recall previous ART regimens. A further possibility is that other opportunistic infections such as tuberculosis and TB treatment could be responsible, since TB is strongly associated with HIV infection, and that multiple antituberculous agents have been associated with drug-induced liver injury including rifampicin, isoniazid and pyrazinamide (Saukkonen et al., 2006). Indeed, an association between cirrhosis and previous TB was observed, a finding that persisted after adjustment for HIV status, suggesting that TB or its treatment may have played a contributory role in the pathogenesis of cirrhosis in this cohort. A further explanation for increased HIV prevalence among cirrhosis and HCC patients, relative to community controls, is that HIV patients are more likely to be in receipt of medical care and thus have improved ascertainment of, and more rapid referral for diagnosis of incident cirrhosis and HCC. Among patients with HIV, not receiving ART was significantly associated with cirrhosis and HCC and this lends support to these hypotheses since those not receiving ART are less likely to be under HIV clinic attendance and

medical supervision. The substantial attributable fraction of HIV to cirrhosis and HCC observed offers further reason for optimism since, in keeping with other countries in southern Africa, Malawi is expanding provision of ART and has achieved considerable progress towards UNAIDS targets in the coverage and community virological suppression of HIV (Government of Malawi, 2018, Dwyer-Lindgren et al., 2019).

4.4.4 Hepatitis C and D

Hepatitis C was a less frequent cause, and did not contribute to the same degree as HBV or alcohol to the development of cirrhosis or HCC. HCV-associated cases were observed to occur at a significantly older age (median 69 years) relative to HBV or alcohol. The association between HCV and older age suggests that HCV transmission is related to risk factors that have changed and decreased over time, such as historical nosocomial transmission through needle reuse in healthcare settings (Spearman et al., 2019). The finding of an absence of cases of co-infection between HBV and HCV in either cirrhosis, HCC or community patients supports epidemiologically distinct risk factors for infection. In keeping with a meta-analysis of HDV epidemiology in sub-Saharan Africa, hepatitis D was not found to be a significant contributor to liver disease with no cases identified among HBsAg positive cirrhosis patients and anti-HDV positive among 3% of HBsAg positive HCC patients (Stockdale et al., 2017). This study provides additional evidence that HDV is of low endemicity in southern Africa, relative to West and Central Africa, which have among the highest rates of HDV in the world.

4.4.5 Evaluation of non-invasive biomarkers and ultrasound for the diagnosis of cirrhosis

In this study, several proposed non-invasive and routinely available biomarkers for the diagnosis of cirrhosis were assessed. These include the APRI score, which is recommended by the WHO in the hepatitis B treatment guidelines of 2015 (World Health Organisation, 2015) the GPR (Lemoine et al., 2016a) and the TREAT-B score (Shimakawa et al., 2018). This cohort consisted of patients with cirrhosis without normal comparators, so full diagnostic performance evaluation was not possible and only sensitivity of these scores could be assessed. Evaluation in this setting provides insight into how scores perform among the subgroup of patients with advanced cirrhosis who clearly need treatment. In this cohort, APRI and TREAT-B performed poorly with sensitivities of 68 and 59% respectively, while GPR had improved sensitivity at 82%. In Ethiopia, the sensitivity of APRI for the diagnosis of cirrhosis in a hospital-based hepatitis B clinic was 10%, relative to a fibroscan reference test with a threshold of

11.7 kPa. At a lower APRI threshold of 1.5, the sensitivity was 29% (Desalegn et al., 2017). In the Gambia, the sensitivity of APRI at the threshold of 2.0 was 25%, and was 40% at the cut-off of 1.0 (Lemoine et al., 2016a). The relatively better performance of APRI in this study is likely to be due to the more advanced spectrum of cases. Despite advanced cirrhosis, APRI and TREAT-B did not perform well and this may be due to the reliance of the scores on transaminases, which can provide evidence of active hepatic inflammation but are less able to identify established hepatic fibrosis. The findings suggest that alternative measures should be used in an inpatient setting to diagnose cirrhosis. GPR performed well in this setting, similar to the sensitivity observed in the Gambia and this merits further study in similar populations (Lemoine et al., 2016a).

Our finding of distinct ultrasound features among patients with cirrhosis relative to community controls shows that ultrasound might have potential particularly among patients with advanced cirrhosis. The frequent rate of exclusions based on ultrasound for cardiac failure, liver metastases and biliary disease shows the important role of ultrasound as a complement to transient elastography and liver function tests in the accurate classification of patients with liver disease. The most discriminatory ultrasound features for cirrhosis were the presence of a coarse hepatic parenchyma, nodular liver surface and splenomegaly and ascites in keeping with previous evaluations of ultrasound for use in diagnosis of cirrhosis in high-income settings (Aube et al., 1999, Chen et al., 2008, Hung et al., 2003). Notably, ascites was a strong predictor of mortality among patients with cirrhosis, portending an 8-fold increased odds of 6 month mortality. Although ultrasound had a highly discriminant value in this study, the comparison represents spectrum bias, with advanced cirrhosis compared to community controls (Ransohoff and Feinstein, 1978). It should also be noted that as the ultrasonographer in this study, I was unblinded to the referral origin of patients, such that scans were conducted for inpatients on the wards and community patients attended separately so this could have introduced observer bias (Delgado-Rodriguez and Llorca, 2004). Before ultrasound could be recommended more widely as a tool to assess HBV treatment eligibility, a mix of cases should be assessed that is representative of the spectrum that will be seen in setting in which it would be adopted, for example in a viral hepatitis clinic, and the sonographer should be blinded to the results of the reference test.

4.4.6 Schistosomiasis

Schistosomiasis was frequently identified among people with cirrhosis and HCC. In particular among cirrhosis patients, 30% were positive for urine CCA relative to 2% among community control patients. Schistosomiasis is a common infection in Malawi and transmission is dependent upon a life cycle

involving fresh water bodies and intermediate snail hosts. Following cutaneous invasion, schistosomes migrate to the portal venous system and deposit eggs giving rise to periportal chronic inflammation and fibrosis (McManus et al., 2018). It is possible that schistosomiasis was a significant contributor to the overall rates of cirrhosis observed in this study, given the diagnostic criteria for cirrhosis in this study were based on transient elastography and ultrasound rather than through histological examination of liver biopsy. Peri-portal fibrosis and in particular branching fibrosis that can be observed with advanced schistosomiasis, could give rise to elevated liver stiffness measurement in the absence of cirrhosis (Richter et al., 2000). In this study cases of cirrhosis and HCC were drawn from a tertiary referral centre which accepts patient referrals from throughout the southern region of Malawi, whereas the control population was a discrete urban population from Ndirande in Blantyre. It is therefore difficult to exclude confounding by geographic location, since the population of Ndirande might not be expected to be exposed to the conditions for schistosomiasis transmission, relative to rural areas with significant fresh water exposure. A nationwide prevalence survey studying schistosomiasis prevalence in primary school aged children from Malawi in 2002 found a national prevalence of 0.4% (0 – 1.4) for *S. mansoni* mono-infection and 7.7% (95% CI 2.2-13.2) for mixed infection with *S. haematobium*. Infection was found to be highly geographically localised, and *S. mansoni* was not found to be generalised across any ecological area (Bowie et al., 2004). Future studies examining the contribution of schistosomiasis to cirrhosis and HCC should consider this limitation and recruit controls matching on geographic residence and origin to avoid ecological confounding and to ensure an equivalent exposure risk.

4.4.7 Cirrhosis without an identifiable cause

For a substantial proportion of patients with cirrhosis in this study, a cause could not be ascertained. A total of 60% of patients were negative for HBV and HCV and did not describe hazardous alcohol consumption. Among people with HCC, alcohol was an observable risk factor, but the prevalence of reported hazardous or harmful alcohol consumption was somewhat surprisingly not significantly increased among people with cirrhosis relative to community controls. This could be due to underreporting or social desirability bias since study staff completed questionnaires with patients on the medical wards or in clinical settings (Wynder, 1994). This hypothesis does not explain the lack of underreporting bias associated with HCC. Another explanation could be that our community controls are derived from an urban area with a higher community prevalence of alcohol disorder relative to rural areas from where most of our patients are drawn, and that if our community controls were better

geographically matched to the cases, a stronger association with alcohol could become apparent. In our study, none of the female participants with cirrhosis reported hazardous or harmful alcohol consumption whereas among males, the prevalence was 23%. This may be compared to the 2010 nationally representative population survey which estimated that 19.0% of males (95% CI 16.5 -21.5) and 2.3% of women (95% CI 1.6-3.1) reported engaging in heaving episodic drinking, which although not directly comparable due to the application of different definitions, suggests that the community control prevalence of elevated alcohol consumption we observed (16% reported hazardous consumption) may be similar to national estimates (Msyamboza et al., 2011). Ideally future studies should attempt to mitigate social desirability bias through use of non-judgemental questioning techniques and provision of improved privacy for research areas (Davis et al., 2010).

Non-alcoholic fatty liver disease (NAFLD) globally is an important and growing cause of liver disease with 20% of the general population and 70% of people with type 2 diabetes affected globally (Sattar et al., 2014). We did not systematically consider NAFLD as a cause of liver disease in this study since we lacked a specific diagnostic modality such as liver biopsy or cross-sectional imaging to provide diagnostic discrimination. Features of NAFLD can be visualised on ultrasound including globally increased echogenicity but these lack sensitivity (Sattar et al., 2014). None of the included patients with cirrhosis had obesity, 3% had grade 2 or greater hypertension and 2% of patients had a diagnosis of diabetes, suggesting that the conditions for NAFLD are largely lacking in this population making this disease less likely as a major contributor to disease in this population. Other important considerations are the prevalence of autoimmune liver disease and multisystem metabolic or genetic disorders such as Wilson's disease, haemochromatosis and alpha-1 antitrypsin deficiency (Tsochatzis et al., 2014). To elucidate the causes of cirrhosis more fully, additional diagnostic tests for other causes of cirrhosis, liver biopsy and potentially metagenomic pathogen discovery, are recommended for future studies in view of the high proportion of unattributed cases of cirrhosis.

4.4.8 Study limitations

There are limitations to the HCC case ascertainment in this study. The diagnosis of HCC was based on a pragmatic ultrasound definition of a hepatic lesion that was ultrasonographically consistent with HCC, in the absence of a characteristic appearance of metastases or alternative hepatic lesions such as haemangiomas. It is possible that lesions diagnosed as HCC in this study could represent alternative pathologies. These may include benign lesions such as focal nodular regeneration, haemangiomas, focal fat sparing, inflammatory masses such as pyogenic liver abscesses, confluent fibrosis or large

regenerative nodules. It could also represent alternative neoplastic lesions such as liver metastases or cholangiocarcinoma (Kim et al., 2015c). International guidelines recommend use of ultrasound as a screening tool and following the finding of a hepatic mass, multi-phase contrast enhanced cross-sectional imaging or contrast-enhanced ultrasound is recommended. Imaging characteristics in relation to contrast phase uptake are then used to discriminate the tumour type, usually without recourse to the need for biopsy in lesions exceeding 2cm (You et al., 2015, McEvoy et al., 2013, Sun and Song, 2015). It was not possible in this study to obtain contrast enhanced cross-sectional imaging due to the limitation of imaging resources in this setting. Ideally, contrast enhanced imaging would be used among cases to provide improved diagnostic discrimination and improve confidence in the diagnosis of HCC, and cases that remained indeterminate following cross-sectional imaging would undergo biopsy. In this study, a second opinion on ultrasound findings was obtained from expert radiologists where lesions were considered indeterminate, and we attempted to locate a primary lesion if hepatic metastases were considered in the differential diagnosis. Several considerations lend confidence to the pragmatic HCC diagnoses made in this study. First, the very poor prognosis observed among this population, with a 6-month survival of 10%, renders it unlikely that a significant misdiagnosis of benign lesions occurred. Second, repeated interval ultrasound scans were performed on individuals who had smaller or indeterminate lesions. In the case of lesions which did not significantly change in size over follow up, HCC was excluded and this procedure helped in the differentiation of a number of indeterminate lesions. Third, the majority of the hepatic masses observed in this study were large and advanced with significant mass effect on surrounding vascular structures and in many cases with portal vein invasion, providing convincing evidence of malignancy. Fourth, the strong association observed with HBV provides additional evidence of HCC, since HBV is epidemiologically strongly associated with HCC and has been only weakly associated with other forms of solid organ malignancy (Song et al., 2019).

Limitations in the representativeness of this study should be considered. This study may not be nationally applicable since patients were recruited from a single tertiary referral centre in Blantyre and the findings are subject to referral bias, and potentially to delay in diagnosis relative to patients presenting at lower tiers of the healthcare system. The PAF analysis used in this study offers a useful insight into the potential gains offered by disease control in terms of elimination of specific diseases such as HBV. There are however limitations with this approach. Our control populations were drawn from different sub-populations of the community serosurvey study in Ndirande as described above in the case of schistosomiasis. For HBsAg, anti-HBc and HCV prevalence, the community prevalence was drawn from large census-based random samples of the general population and provide representation

of community HBV prevalence for an urban township. This may therefore be an imperfect control population and may not fully represent community prevalence in rural areas from where the HCC and cirrhosis patients live. Alcohol, schistosomiasis and HIV community control prevalence estimates were drawn from the community study among HBsAg positive individuals and age-matched HBsAg negative controls. In the case-control matched populations, an association between HIV and HBV was observed and as such, community HIV prevalence was derived only from the HBsAg negative population, limiting the power of the estimates. That control populations used for community HBV, HCV and alcohol exposure estimates were not contiguous meant that opportunities to perform multifactorial analysis to explore interactions between HBV and HCV and alcohol were limited.

Transient elastography was used to diagnose cirrhosis in this study. We considered the limitations previously described in use of this diagnostic modality including the requirement for food and the need to exclude acute hepatitis, cardiac failure, cholestasis, pregnancy and hepatic neoplasia (Friedrich-Rust et al., 2016). TE was more broadly applicable in this setting since none of our included patients were obese. We observed evidence that TE was a useful predictor of outcomes, correlating in a linear fashion with mortality at 6 months. Criticisms of TE as a non-invasive marker of fibrosis have centred around its lack of applicability in certain circumstances such as obesity and extensive ascites, and reduced sensitivity for the diagnosis of intermediate stages of fibrosis eg. corresponding to METAVIR F2 (Huang et al., 2017). As described in chapter 1, the gold standard used for such evaluations is typically a liver biopsy and it is an imperfect reference test with inter-observer variability in histological grading, inconsistent evaluation based on inhomogenous fibrosis and sampling bias, and also suffers from spectrum bias whereby clinically clear-cut cases or individuals otherwise not suitable for biopsy are excluded (Friedrich-Rust et al., 2016). The ultimate purpose of fibrosis assessment is not to predict the histological appearance of the liver, but to determine who should receive antiviral treatment based on risk of development of liver related event. The focus should shift from comparison to an imperfect histological test to assessing the prognostic value for predicting liver-related outcomes and mortality. That TE was able to predict mortality demonstrates its validity and applicability in this population for assessment of antiviral therapy eligibility and is in keeping with a growing body of evidence showing a link to clinically relevant outcomes (Kim et al., 2015a).

4.4.9 Conclusions

In summary, this study of patients presenting to a tertiary hospital in Blantyre, Malawi with cirrhosis and HCC has provided several important findings. HCC was diagnosed at an advanced stage and at a younger median age relative to other African cohorts, and prognosis was very poor. The WHO recommended tool for the diagnosis of cirrhosis in low and middle income country settings, the APRI tool, performed poorly in this cohort despite advanced disease, providing further evidence that alternative approaches such as the GPR score are to be preferred. Among patients with cirrhosis, HBV was attributable as a cause in 25% and HIV was strongly associated with cirrhosis even in the absence of hepatitis B or C. A high attributable fraction of HBV to HCC was observed and this provided an impetus in the next stage of this research to go into the community, to examine HBV prevalence, the impact of the vaccine programme and to identify upstream opportunities for disease prevention and treatment to mitigate the substantial burden of preventable and treatable disease.

Chapter 5. Epidemiology, burden of disease and treatment eligibility of hepatitis B in Ndirande, Malawi: A community cross-sectional observational study

5.1. Introduction

Hepatitis B virus (HBV) is one of the most widely distributed infectious diseases, with 257 million people chronically infected (World Health Organisation, 2017a). In sub-Saharan Africa, it is the leading cause of liver cirrhosis and hepatocellular carcinoma (HCC) (Lemoine and Thursz, 2017). HCC is the second-highest incidence cancer in men and fifth among women in the region and is frequently diagnosed at an advanced stage when curative treatment is no longer possible (International Agency for Research on Cancer/ World Health Organisation, 2019, Yang et al., 2017). In 2016, the World Health Assembly agreed ambitious targets to reduce the incidence of chronic hepatitis B by 90% and mortality by 65% by 2030 (World Health Organisation, 2016a). Empirical data and modelling studies show that hepatitis B-related mortality is rising, and deaths from the disease are projected to increase beyond 2030 without the introduction of antiviral treatment programmes for adults (Nayagam et al.). Antiviral therapy for hepatitis B results in suppression of HBV DNA replication, regression and reversal of liver fibrosis, a reduction in risk of incident HCC and an increase in quality of life (Kim et al., 2015d, Marcellin et al., 2013, Younossi et al., 2018b). An evaluation in West Africa previously demonstrated that a community screen-and-treat strategy using hepatitis B surface antigen (HBsAg) rapid diagnostic tests and treatment with tenofovir was feasible and cost-effective (Nayagam et al., 2016a).

The pentavalent infant vaccine including hepatitis B delivered at 6, 10 and 14 weeks of life, was introduced into national immunisation schedules across sub-Saharan Africa between 1994 and 2014, and in Malawi in 2002. Three-dose coverage of the vaccine was a median of 86% (interquartile range (IQR) 73-92) in 2017 across sub-Saharan Africa (WHO-UNICEF, 2018). There are limited existing data on the impact of the HBV vaccine in the region and of the need at the population level for antiviral treatment. In Malawi, in a recent systematic review national estimated HBV prevalence was 8.1% based on limited data predominantly comprising samples employing convenience sampling at significant risk of bias, and largely from urban centres (Stockdale et al., 2018). There is a paucity of high quality epidemiological data and an assessment of vaccine impact has not been conducted. The community-level burden of disease and the projected need for treatment are not known in Southern Africa and are required to inform an effective public health response in line with the World Health Organisation (WHO) strategy. The aim of this study was to conduct a large census-based serological

survey of a township in Blantyre, Malawi to ascertain HBV prevalence, vaccine impact, the burden of liver disease and the population-level need for HBV treatment.

5.2. Methods

See chapter 2 for a description of the methods employed in the community HBV prevalence and treatment eligibility study.

5.2.1 Parametric bootstrapping for national treatment projections

Estimates of people with HBV eligible for HBV treatment in the national population were computed by multiplying the census population over 15 years (9,845,162 in the 2018 national census) (National Statistical Office of Malawi, 2018) with three prevalence estimates: i. the HIV negative proportion in the general population (1- national HIV prevalence estimates for the population aged 15-64) (Government of Malawi, 2018) ii. the prevalence of HBsAg among adults aged 16 years in this serosurvey, iii. the proportion of the HBsAg positive, HIV negative clinical evaluation population eligible for treatment in this study according to EASL, AASLD and WHO criteria respectively. The beta distributions for the 2.5^{th} and 97.5^{th} percentiles matching the 95% confidence intervals were derived for the three prevalence estimates: HIV negative prevalence, HBsAg prevalence and proportion eligible for treatment. Parametric bootstrapping was used to generate N=10,000,000 prevalence samples for each distribution and these were multiplied. The empirical 2.5^{th} and 97.5^{th} quantiles of this sample were used as the upper and lower bounds for the 95% CI, and the product was used to derive the central estimate. Analyses were conducted in R version 3.5.2 (R Foundation for Statistical Computing).

5.3. Results

The demographic census recorded 97,386 individuals residing in 22,364 households with median 4 (interquartile range (IQR) 3, 6) residents per dwelling (Figure 5-1). Median age was 19 (IQR 9, 31) and 49.5% were female. By comparison, the national 2018 census median age was 17 (IQR 4, 32) and 51.5% female. In the overall catchment area of $7.5km^2$, population density was 12,812 residents/km^2, and 20,940/km^2 among the $4.5km^2$ central region where 97% of the population resided (Figure 5-2). A total of 6073 individuals participated in the serological survey and were tested for HBsAg. Median age of

serosurvey participants was 18 (IQR 8-37) and 3455/6073 (56.7%) were female. The serosurvey oversampled females, younger children and older adults relative to the census distribution (Figure 5-3). A total of 160/6073 (2.6%) tested positive for HBsAg. The standardised prevalence of HBV infection in the general population was 3.1% (95% confidence interval (CI) 2.6 – 3.7). Prevalence was highest among males in the 30-39 age group, with prevalence rising from adolescence to a peak in the fourth decade and falling again among older adults (Table 5-1, Figure 5-4).

Figure 5-1 : Flowchart of census and recruitment to the serological survey and community HBV treatment evaluation study

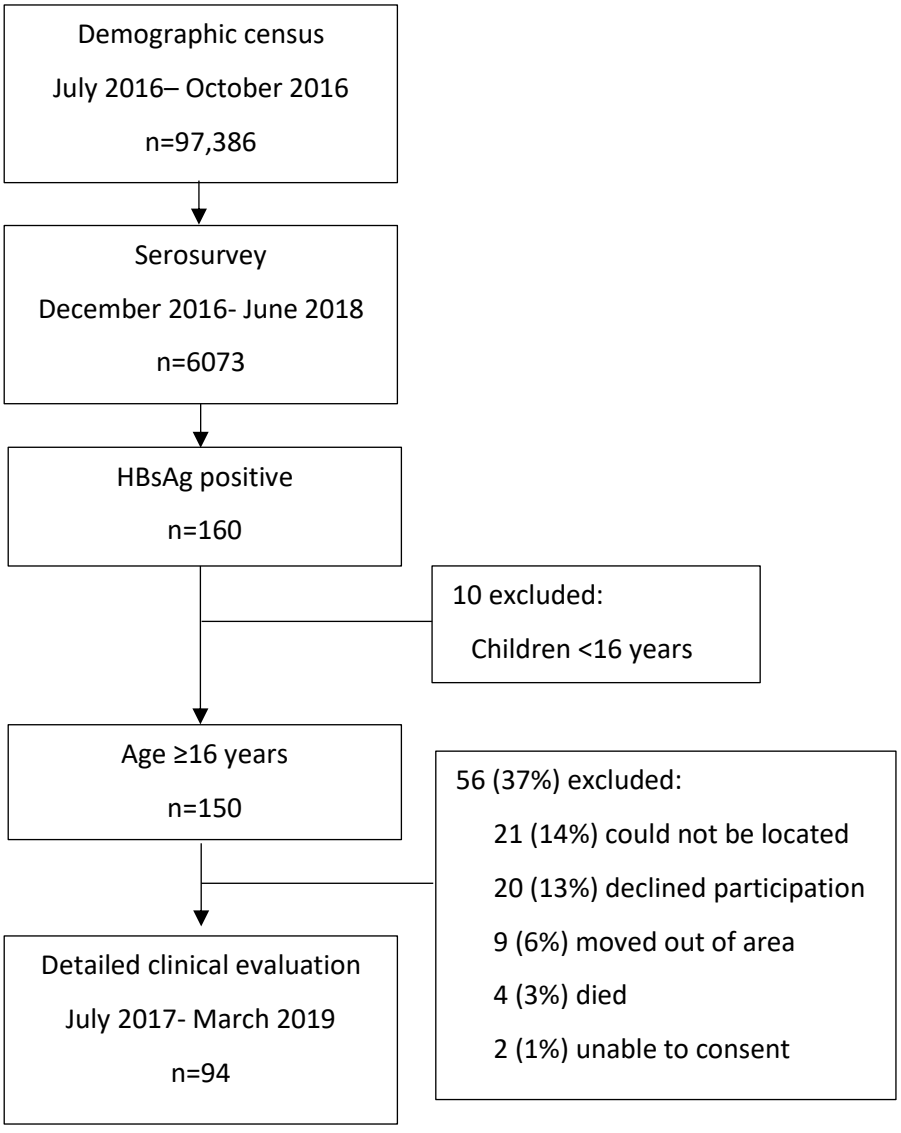
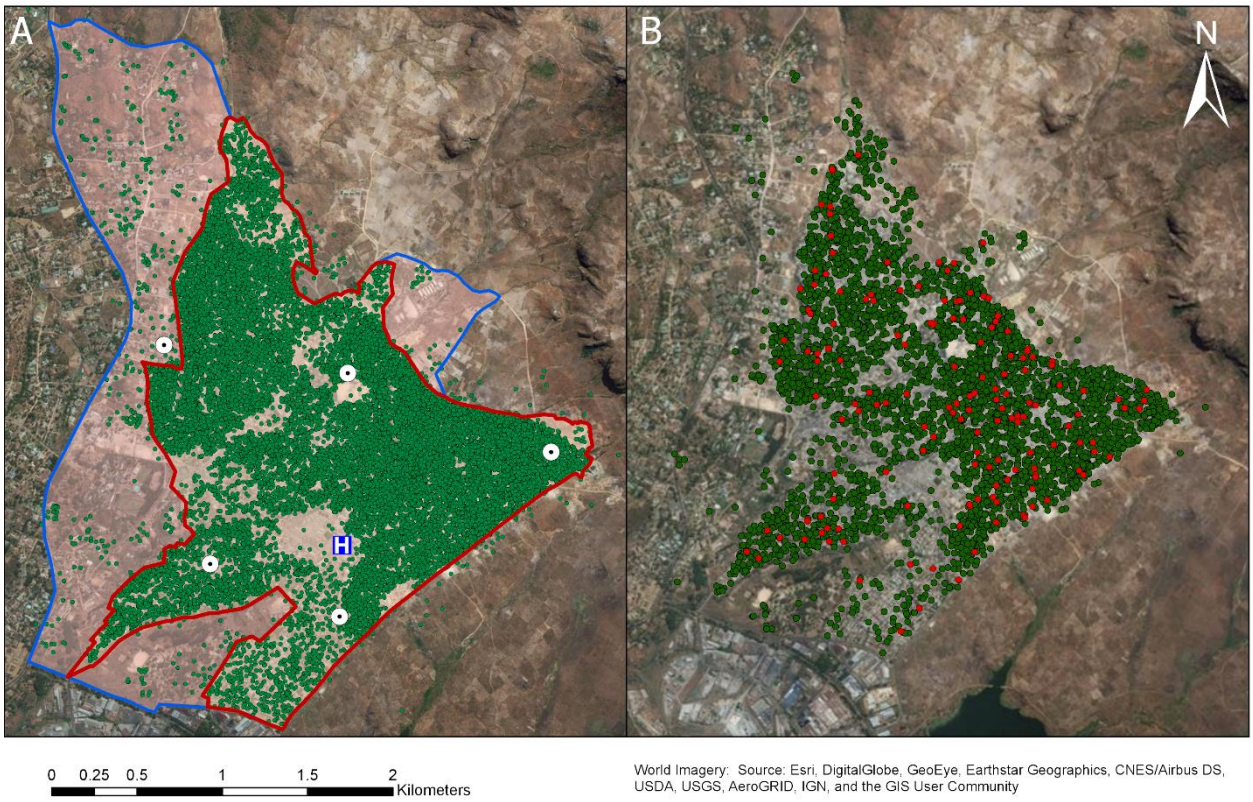


Figure 5-2A: Satellite image of census data, area boundaries and study locations in Ndirande township 5-2B: Map of serological survey indicating GPS location of participants

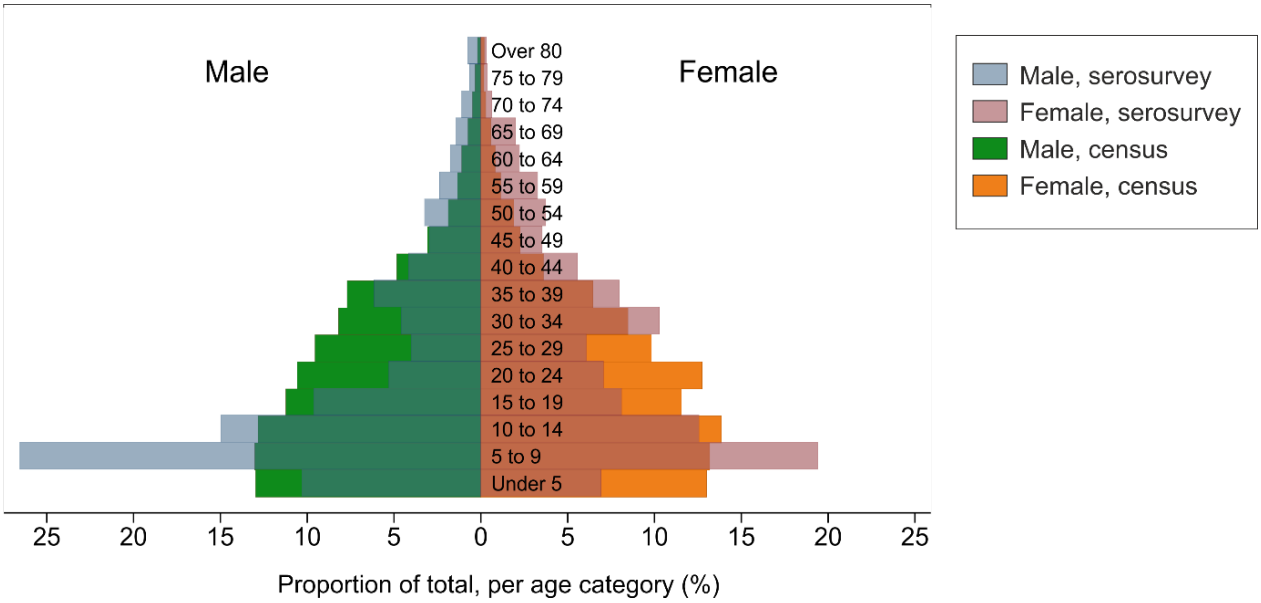


2A: Green points show GPS locations of 97386 individuals recorded on the demographic census. Blue boundaries show the 7.5km^2 outer health surveillance area and red boundaries define the 4.5km^2 central region. "H" symbol denotes the health centre. White circle markers show locations where the community evaluation study was conducted. 2B: Points show locations of 6073 participants in the serosurvey. Red markers indicate participants who tested positive for HBsAg by ELISA.

Vaccination status was available from 1172/2085 (56.2%) children aged ≤10 years and 631/931 (67.8%) of children aged ≤5 years. Data sources comprised health passport documentation for 722/1172 (61.6%) and parent/guardian report for 450/1172 (38.4%). For the remaining 913 (43.7%) children aged ≤10 years, vaccine status was unknown. Completion of 3 doses of pentavalent vaccine was reported for 1141/1172 (97.4%) children ≤10 years and 619/631 (98.1%) among children ≤5 years. Vaccine coverage was associated with data source (98.6% coverage from oral report relative to 96.5% from health passport document, p=0.03).

No HBV infection was observed among the 31 children with reported incomplete or missed vaccine. Standardised HBV prevalence was 0.6% (95% CI 0.2 – 1.4) among 913 children with unknown status, which did not differ from children who completed 3 doses (0.2%, (95% CI 0.1- 0.8), odds ratio (OR) 2.4 (95% CI 0.5 – 10.4), p=0.26).

Figure 5-3: Distribution of age and sex in the serosurvey relative to the demographic census [a]

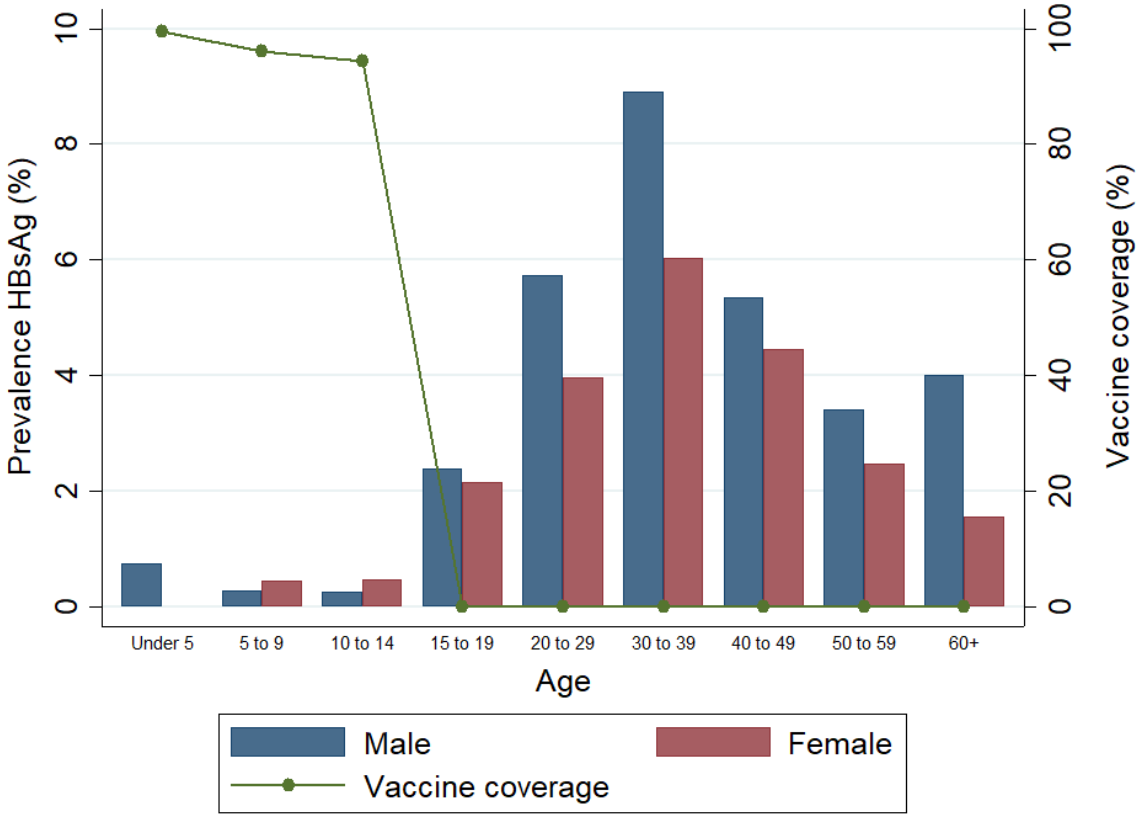


[a] Serosurvey data is layered over census data.

Table 5-1: Prevalence of hepatitis B surface antigen (HBsAg) among serosurvey participants

Age category (years)	Male		Female		Total	
	Frequency	% (95% CI)	Frequency	% (95% CI)	Frequency	% (95% CI)
0–4	2/270	0.7 (0.2 – 2.7)	0/239	0.0 (0.0 – 1.6)	2/509	0.4 (0.1 – 1.4)
5–9	2/695	0.3 (0.0 – 1.0)	2/670	0.3 (0.1 – 1.1)	5/1365	0.4 (0.2 – 0.9)
10–14	1/392	0.3 (0.0 – 1.4)	2/434	0.5 (0.1 – 1.7)	3/826	0.4 (0.1 – 1.1)
15–29	20/496	4.0 (2.6 – 6.1)	24/734	3.3 (2.2 – 4.8)	44/1230	3.6 (2.7 – 4.8)
30–39	25/281	8.9 (6.0 – 12.8)	38/630	6.0 (4.4 – 8.2)	63/911	6.9 (5.4 – 8.8)
40–49	10/187	5.3 (2.9 – 9.6)	14/314	4.5 (2.7 – 7.3)	24/501	4.8 (3.2 – 7.0)
50–59	5/147	3.4 (1.5 – 7.7)	6/242	2.5 (1.1 – 5.3)	11/389	2.8 (1.6 – 5.0)
60–69	5/84	6.0 (2.6 – 13.2)	3/146	2.1 (0.7 – 5.9)	8/230	3.5 (1.8 – 6.7)
≥70	1/66	1.5 (0.3 – 8.1)	0/46	0.0 (0.0 – 7.7)	1/112	0.8 (0.2 – 4.9
All	71/2618	2.7 (2.2 – 3.4)	89/3455	2.6 (2.1 – 3.2)	161/6073	2.7 (2.3 – 3.1)

Figure 5-4: Prevalence of hepatitis B surface antigen stratified by age and sex groups and vaccine coverage



*Individuals aged between 14 and 16 years are divided between the groups born before and born after vaccine introduction due to the 18-month duration of the serosurvey.

Among children born after the introduction of the vaccine, standardised HBsAg prevalence was 0.3% (95% CI 0.2 – 0.6), while among older children and adults born prior to the vaccine introduction, it was 5.1% (95% CI 4.3 – 6.1) (Table 5-2). Vaccine impact was 95.9% (95% CI 70.6-99.4), calculated by comparison of individuals born 5 years prior to vaccine introduction (aged 15-21 years) with those born 5 years after vaccine introduction (aged 10-16 years). In a sensitivity analysis, comparing those born 10 years before and after vaccine introduction, (ages 15-26 years vs ages 5-16 years), vaccine impact was 93.3% (95% CI 83.1-97.4). Applying post-stratification adjustment for geographic area did not affect prevalence estimates (Table 5-3).

By univariate analysis, HBV infection among individuals aged ≥16 years was associated with being in paid or self-employment, and being married, separated or divorced. No association with educational

attainment or regional SES was observed. Among the subset of participants with available household-level SES data, no association with household SES and HBV was observed. In a multivariable model incorporating age, gender and marital status, greater odds of HBV infection were observed in males and separated or divorced individuals (Table 5-4) .

Table 5-2: Prevalence of hepatitis B surface antigen stratified by birth date and vaccination status

Population	Crude HBV prevalence		Population standardised prevalence[a]	Risk ratio (95% CI)	P value
	Frequency	% (95% CI)	% (95% CI)		
All individuals	160/6073	2.6 (2.3 – 3.1)	3.2 (2.7 – 3.7)		
Birth date relative to vaccine introduction[b]					
5 yr before vaccine	15/500	3.0 (1.8 – 4.9)	2.9 (1.8 – 4.7)	Reference	
5 yr after vaccine	1/758	0.1 (0.02 – 0.7)	0.1 (0.02 – 0.8)	0.04 (0.06 – 0.29)	0.001
1 yr before vaccine	5/90	5.6 (2.4 – 12.4)	5.6 (2.4 – 12.5)	Reference	
1 yr after vaccine	1/136	0.7 (0.1 – 4.0)	0.6 (0.1 -3.9)	0.11 (0.01 – 0.84)	0.03
10 yr before vaccine	30/884	3.4 (2.4 – 4.8)	3.6 (2.5 – 5.1)	Reference	
10 yr after vaccine	5/1932	0.3 (0.1 – 0.6)	0.2 (0.1 – 0.6)	0.07 (0.03 – 0.17)	<0.0001
All before vaccine	152/3280	4.6 (4.0 – 5.4)	5.1 (4.3 – 6.0)	Reference	
All after vaccine	9/2793	0.3 (0.2 – 0.6)	0.3 (0.2 – 0.6)	0.06 (0.03- 0.12)	<0.0001
Vaccination status for age ≤10 years					
Completed 3 doses	3/1141	0.3 (0.1 – 0.8)	0.2 (0.1 – 0.8)	Reference	
Unknown status [c]	5/913	0.5 (0.2 – 1.3)	0.6 (0.2 – 1.4)	2.3 (0.5 – 10.2)	0.26
Incomplete [d]	0/31	0.0 (0.0 – 11.0)	-	-	

[a] Standardised to census age and sex distribution using post-stratification weighting. [b]Pentavalent hepatitis B vaccination was introduced on 1st January 2002. [c]Participants born after vaccine introduction for whom vaccination status could not be ascertained from parent, guardian or documentation; vaccine status was ascertained for 1172/2085 (56.2%) children ≤10 years. [d]Received 0,1 or 2 doses.

Of 160 HBsAg positive serosurvey participants, it was possible to locate 114/150 (76%) aged ≥16 years in the township; four had died, two were unable to provide consent and nine had moved outside the region (Figure 5-1). A total of 94/114 (82.5%) eligible individuals agreed to participate in an evaluation of treatment eligibility. Included individuals had a higher level of education, better socioeconomic status, and higher rate of employment or education relative to non-participants (Table 5-5). Clinical evaluation occurred a median 4.9 months (IQR 2.2, 7.6) after the serosurvey.

Table 5-3: Impact of post-stratification iterative fitting weights for geographic region on prevalence and vaccine impact estimates[a]

Population	Population standardised prevalence adjusted for age and sex % (95% CI)	Population standardised prevalence adjusted for age, sex and geographic area % (95% CI)
All individuals	3.2 (2.7 – 3.7)	3.2 (2.7, 3.8)
Birth date relative to vaccine introduction[b]		
5 yr before vaccine	2.9 (1.8 – 4.7)	2.9 (1.8 – 4.8)
5 yr after vaccine	0.1 (0.02 – 0.8)	0.1 (0.02-1.0)
10 yr before vaccine	3.6 (2.5 – 5.1)	3.5 (2.4 – 5.0)
10 yr after vaccine	0.2 (0.1 – 0.6)	0.2 (0.1 – 0.6)
All before vaccine	5.1 (4.3 – 6.0)	5.1 (4.3 – 6.0)
All after vaccine	0.3 (0.2 – 0.6)	0.3 (0.2 – 0.6)
Vaccination status for age ≤10 years		
Completed 3 doses	0.2 (0.1 – 0.8)	0.2 (0.1 -0.7)
Unknown status [c]	0.6 (0.2 – 1.4)	0.6 (0.2 – 1.4)
Incomplete [d]	-	-

[a]To assess for the potential impact of variation in geographic response rates between geographic areas in the serosurvey this sensitivity analysis examines the effect of including post-stratification iterative fitting for geographic area (based on 12 health surveillance areas in the township) to adjust prevalence for geographic variation in response rates, in addition to adjustment for age and sex.

Table 5-4: Participant characteristics associated with hepatitis B infection in the serosurvey: binomial logistic regression model

Characteristic	Univariate Odds ratio (95% CI)	P value	Multivariable model[a] Odds ratio (95% CI)	P value
Age, per year	1.03 (1.03 – 1.04)	<0.0001	[a]	
Age, years				
0-14	Reference			
15-29	11.37 (5.63 – 22.96)	<0.0001		
30-44	20.76 (10.54 – 40.80)	<0.0001		
45-59	9.22 (4.18 – 20.37)	<0.0001		
>60	8.37 (3.53 – 20.90)	<0.0001		
Birth date				
Born prior to vaccine	Reference			
Born after vaccine	0.06 (0.03 – 0.11)	<0.0001		
Sex, male vs female	1.46 (1.04 – 2.03)	0.03	1.60 (1.06 – 2.41)	0.02
Marital status[b]				
Single	Reference		Reference	
Married	1.58 (1.00 – 2.49)	0.05	1.51 (0.75 – 3.02)	0.25
Separated/divorced	2.73 (1.12 – 6.64)	0.03	2.89 (0.96 – 8.67)	0.06
Widowed	0.96 (0.39 – 2.36)	0.94	1.67 (0.53 – 5.22)	0.38
Education[b]				
Primary	Reference			
Secondary	1.07 (0.72 – 1.59)	0.74		
Vocational	0.63 (0.23 – 1.78)	0.39		
University	1.44 (0.57 – 3.61)	0.44		
None	1.36 (0.37 – 4.96)	0.64		
Employment[b]				
Unemployed	Reference			
Student	0.93 (0.40 – 2.16)	0.86		
Self-employed	2.44 (1.28 – 4.64)	0.01		
Paid employee	2.20 (1.11 – 4.38)	0.03		
Unpaid family worker	1.30 (0.62 – 2.72)	0.49		
Retired	1.65 (0.22 – 12.60)	0.63		
Socioeconomic status[c]				
Highest quintile	Reference			
2nd highest quintile	0.95 (0.48 – 1.85)	0.87		
Middle quintile	0.98 (0.48 – 2.00)	0.96		
2nd Poorest quintile	0.95 (0.49 – 1.86)	0.88		
Poorest quintile	1.16 (0.60 – 2.27)	0.66		

[a]Multivariable model considers individuals aged ≥16 years and includes a cubic spline variable for age to account for change in prevalence with respect to age. [b]Education, employment and marital status data are applicable to individuals aged >16 years. [c]Socioeconomic quintiles are derived for the 44 health surveillance areas in the serosurvey in which the participants resided.

Table 5-5: Comparison of characteristics of participants included in clinical evaluation of HBV treatment eligibility with potentially eligible non-participants

Characteristics	Included n= 94	Non-participants N=56	P value
Age (years), median (IQR)	35 (29, 41)	34 (28, 42)	0.78
Sex (female), n (%)	49 (52)	35 (63)	0.22
Marital status[a], n(%)			0.09
Single	21 (23)	7 (14)	
Married	64 (69)	34 (68)	
Divorced/separated	3 (3)	7 (14)	
Widowed	5 (5)	2 (4)	
Education, n(%)			0.01
None	2 (2)	1 (2)	
Primary	26 (28)	25 (47)	
Secondary	60 (64)	22 (42)	
Vocational	1 (1)	4 (8)	
University	5 (5)	1 (2)	
Employment, n(%)			0.03
Unemployed	17 (18)	17 (32)	
Student	10 (11)	1 (2)	
Self-employed	47 (50)	19 (36)	
Paid employee	35 (24)	16 (30)	
Retired	1 (1)	0 (0)	
Socioeconomic status[c]			0.02
Highest quintile	10 (11)	5 (9)	
2nd highest quintile	25 (27)	6 (11)	
Middle quintile	22 (23)	12 (21)	
2nd lower quintile	12 (13)	19 (34)	
Lowest quintile	25 (27)	14 (25)	

[a]See Figure 5-1 for a list of reasons for non-participation. [b]Marital status was available for 50/56, and education and employment status for 53/56, of those excluded.

Table 5-6: Characteristics of participants and outcome of community evaluation of HBV treatment eligibility

Characteristic median (IQR) or n(%)	All participants n=94	HIV positive population[a] n=24	HIV negative population n=69
Age, years	36 (29, 41)	39 (36, 47)	34 (27, 38)
Sex, female	49 (52)	14 (58)	35 (51)
CD4 count (cells/mm^3)[b]		519 (412, 577)	
On ART		16/24 (67)	
On TDF or 3TC		16/24 (67)	
Symptoms/signs of CLD			
Signs of CLD	3 (3)	1 (4)	2 (3)
Ascites	2 (2)	1 (4)	1 (1)
Gynaecomastia	1 (1)	0 (0)	1 (1)
Past medical history			
History of tuberculosis	7 (7)	6 (25)	1 (1)
Diabetes	1 (1)	0 (0)	1 (1)
Renal disease	1 (1)	0 (0)	1 (1)
Hypertension	1 (1)	0 (0)	1 (1)
Alcohol consumption			
Abstinent	59 (63)	12 (50)	47 (68)
Low risk	23 (25)	9 (38)	14 (20)
Hazardous	10 (11)	3 (13)	6 (9)
Harmful	1 (1)	0 (0)	1 (1)
Alcohol dependence	1 (1)	0 (0)	1 (1)
Hepatitis D and C serology			
Anti-HDV	2 (2)	0 (0)	2 (3)
HCV Ag/Ab	0 (0)	0 (0)	0 (0)
Hepatitis B viral markers			
HBeAg	10 (11)	6 (25)	4 (6)
HBV DNA (IU/ml)	81 (<32, 801)	<32 (<32, <32)	215 (51, 2880)
HBV DNA> 2000 IU/ml	19 (20)	0 (0)	19 (28)
HBV DNA> 20,000 IU/ml	11 (12)	0 (0)	11 (16)
ALT (IU/L)	10 (8, 13)	12 (10, 15)	9 (8, 12)
ALT> ULN	2 (2)	0 (0)	2 (3)
ALT>2x ULN	0 (0)	0 (0)	0 (0)
Haemoglobin (mg/dl)			
Platelet count (10^{-9})			
Liver stiffness measurement (kPa)	4.8 (4.0, 6.4)	4.5 (4.0, 5.4)	5.1 (4.0, 6.9)
≥7.9kPa	10 (11)	1 (4)	9 (13)
≥9.5kPa	4 (4)	1 (4)	3 (4)

[a]One participant did not consent to HIV testing [b]CD4 count available for 21/23 (91%) of HIV positive individuals

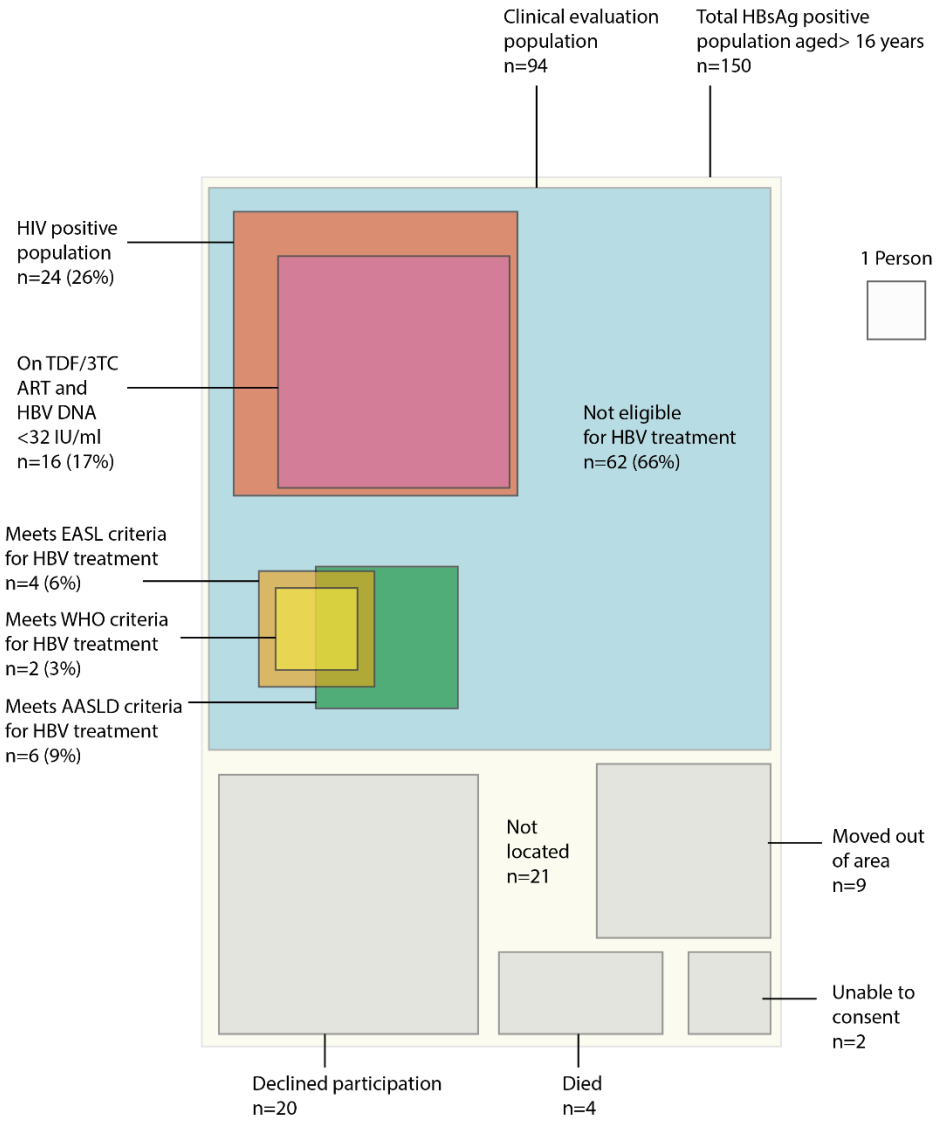
Abbreviations: IQR interquartile range; CD4 cluster of differentiation 4; ART antiretroviral therapy; TDF tenofovir disoproxil fumarate; CLD chronic liver disease; HCV hepatitis C virus; HDV hepatitis D virus; Ag/Ab antigen/antibody; HBV hepatitis B virus; ALT alanine transaminase

Characteristics of the clinical evaluation population are shown in table 5-6. Of 93/94 (99%) who agreed to HIV testing, 24/93 (26%) were HIV positive, of which 17 were previously known to be HIV positive and 7 were newly diagnosed. Median CD4 count was 519 (interquartile range (IQR) 412, 577). Of those with HIV/HBV co-infection 16/24 (67%) were on ART, all of whom were receiving tenofovir and lamivudine, and 16/16 (100%) of those had HBV DNA suppression <32 IU/ml. Anti-HDV was positive in 2/94 (2%) and HCV Ag/Ab was negative in all participants. HBeAg was positive in 10/94 (11%): 25% (6/24) among HIV positive participants and 6% (4/69) among HIV negative participants, p=0.02. Among the HIV negative population, HBV DNA was >2000 IU/ml in 28% (19/69) and >20,000 IU/ml in 16% (11/69) and 3% (2/69) had an elevated ALT >ULN. Liver stiffness consistent with cirrhosis (≥9.5kPa) was observed in 3/69 (4%) of the HIV negative and 1/24 (4%) of the HIV positive population. Clinical evaluation of the HIV negative population demonstrated that by WHO, EASL and AASLD criteria, 2.9%, 5.7% and 8.7% met eligibility criteria for HBV antiviral treatment respectively (Figure 5-5). Projected to the national population, this represents 21,234 HIV negative individuals who require HBV treatment (95% CI 5,960 –52,899) (Table 5-7).

Of the clinical evaluation population, 3/69 HIV negative individuals had a liver stiffness >9.5kPa consistent with cirrhosis. Median APRI scores among the total population were 0.27 (95% CI 0.20, -.35). Using the WHO recommended threshold of 2.0, the sensitivity of the APRI for the diagnosis of cirrhosis was 33.3% (95% CI 0.84- 90.6), specificity was 100% (95% CI 94.6 -100) and area under the receiver operating curve 0.67. Using a lower APRI threshold of 1.0 showed an identical sensitivity and specificity (Table 5-8).

Using Liu's method for defining the optimal AUROC cut-off (maximising the product of sensitivity and specificity), the optimal defined cut-off for APRI for the diagnosis of significant fibrosis was 0.52, and for cirrhosis was 0.59 (Liu, 2012). At this optimised APRI cut-off, for cirrhosis, the sensitivity was 66.7% and specificity was 95.5%. For the GPR, using the previously proposed cut offs of 0.32 for significant fibrosis (F2) the sensitivity was 33.3% and specificity was 95.0%, and for a cut off of 0.56 for cirrhosis, sensitivity was 33.3% and specificity was 98.5%. Using optimised, lowered thresholds, the sensitivity and specificity for significant fibrosis with a cut-off of 0.16 was 77.8% and 71.7% and for cirrhosis with a cut-off of 0.30 was 100% and 92.4% respectively (Table 5-8).

Figure 5-5: Outcomes of community clinical evaluation for HBV treatment eligibility[a]



Box marked "1 Person" indicates the area representing one participant. Disposition of study participants and outcome of evaluation is demarcated in colour and by description. Abbreviations: TDF tenofovir disoproxil fumarate ; 3TC lamivudine ; HBsAg hepatitis B surface antigen; HBV hepatitis B virus; WHO World Health Organisation; EASL European Association for Study of the Liver; AASLD American Association for the Study of the Liver. [a] Area is proportional to the number of people in each group. Treatment eligibility criteria are considered only for HIV negative individuals, as all HIV positive people should receive ART containing TDF/3TC.

Table 5-7: Parametric bootstrapping procedure to estimate confidence intervals for national estimates of treatment eligibility[a]

Metric	Prevalence (95% CI)		Number 95% CI	
HIV prevalence in national population aged 15-64	10.6%	(9.9 - 11.2)	1,043,587	(974,671 – 1,102,658)
Estimated HIV negative population aged ≥15 years	89.4%	(88.8 – 90.1)	8,801,575	(8,742,504 – 8,870,491)
HBV prevalence in general population aged ≥15 years	5.1%	(4.3 – 6.1)	502,103	(423,342 – 600,555)
Eligible for treatment according to EASL 2017 criteria	5.7%	(1.6-14.2)	25,586	(7,172 – 65,519)
Eligible for treatment according to AASLD 2018 criteria	8.7%	(3.3-18.0)	39,053	(14,737 – 83,426)
Eligible for treatment according to WHO 2015 criteria	2.9%	(0.7-10.0)	13,018	(3,142- 46,030)

Table 5-8: Diagnostic performance evaluation of APRI and GPR tools for diagnosis of significant fibrosis and cirrhosis relative to transient elastography reference test

Characteristics	Significant fibrosis (LSM ≥7.9kPa)		Cirrhosis (LSM ≥9.5kPa)		
	Proposed	Optimised	Proposed		Optimised
APRI					
Cut off	0.5		1.0	2.0	0.6
AUROC	0.86		0.67	0.67	0.81
Sensitivity (%)	77.8 (40.0-97.2)		33.3 (0.8- 90.6)	33.3 (0.8- 90.6)	66.7 (9.4 -99.2)
Specificity (%)	95.0 (86.1- 99.0)		100 (94.6- 100)	100 (94.6- 100)	95.5 (87.3- 99.1)
PPV (%)	70.0 (34.8- 93.3)		100 (2.5- 100)	100 (2.5- 100)	40 (5.3- 85.3)
NPV (%)	96.6 (88.3- 99.6)		97.1 (89.8- 99.6)	97.1 (89.8-99.6)	98.4 (91.6 -100)
Positive LR	15.6 (4.9- 49.5)		-	-	14.7 (3.8 – 57.4)
Negative LR	0.23 (0.07- 0.80)		0.67 (0.3 -1.4)	0.67 (0.3 -1.4)	0.35 (0.07- 1.73)
GPR					
Cut off	0.32	0.16	0.56		0.30
AUROC	0.64	0.75	0.66		0.96
Sensitivity (%)	33.3 (7.5- 70.1)	77.8 (40.0-97.2)	33.3 (0.8- 90.6)		100 (29.2- 100)
Specificity (%)	95.0 (86.1- 99.0)	71.7 (58.6- 82.5)	98.5 (91.8 – 100)		92.4 (83.2- 97.5)
PPV (%)	50 (11.8- 88.2)	29.2 (12.6- 51.1)	50 (1.3- 98.7)		37.5 (8.5- 75.5)
NPV (%)	90.5 (80.4- 96.4)	95.6 (84.9- 99.5)	97.0 (89.6- 99.6)		100 (94.1- 100)
Positive LR	6.67 (1.61- 28.1)	2.75 (1.61- 4.68)	22.0 (1.77- 273)		13.2 (5.7- 30.7)
Negative LR	0.70 (0.44- 1.12)	0.31 (0.09- 1.06)	0.68 (0.3- 1.51)		-

5.4. Discussion

Fifteen years after implementation of the hepatitis B vaccination programme in Malawi, vaccine impact of 96% was observed, by comparing prevalence among people born 5 years before and after vaccine introduction. Vaccination coverage exceeding 97% was observed among children for whom vaccination status could be ascertained. These encouraging data show that with in an urban township with high coverage, even without additional preventative interventions such as birth dose vaccination, use of hepatitis B immunoglobulin or maternal antiviral therapy, HBV prevalence was 0.3% among all children aged 5-10 years and 0.2% among those known to have completed a 3-dose vaccination course. High vaccine coverage may be due to community acceptance and enthusiasm for vaccination and the accessibility of the health centre.

There are limited existing data evaluating community HBV vaccine effectiveness or impact from sub-Saharan Africa. In the Gambia, among 753/2670 young adults who could be linked to vaccination status data, vaccine effectiveness was 94% (95% CI 77-99) (Peto et al., 2014). In a cross-sectional evaluation of a 4-dose HBV vaccination programme in rural Edo State, Nigeria which included a birth dose, and where vaccine coverage was 55%, vaccine effectiveness was 85% (95% CI: 68, 93%), among children with a mean age of 7 years (Odusanya et al., 2011). Follow up of children aged 9-12 who participated in a HBV vaccination study in Senegal observed vaccine efficacy of 94.5% (95% CI 77.4-98.7) (Coursaget et al., 1994). Four cross-sectional or cohort studies from South Africa found HBV prevalence ranging from 0 to 0.1% among vaccinated children relative to an estimated prevalence of 7.8% in the pre-vaccination era (Spearman and Sonderup, 2014). Collectively these studies show HBV vaccination in sub-Saharan Africa has had a significant impact in reducing HBV prevalence among children but also highlight significant data gaps, with a paucity of community-level programme assessments across most of the continent.

A quarter of HBV patients at the community level had HIV co-infection in this study and two-thirds were receiving HBV-active ART at the time of cross-sectional evaluation, all with HBV DNA suppression. The finding is consistent with results of a national population HIV survey in which 68% of individuals had HIV virological suppression, in keeping with the remarkable progress Malawi is making towards the 90:90:90 goals for awareness of HIV status, use of ART and HIV virological suppression (Government of Malawi, 2018). HIV treatment programmes may represent a platform for HBV due to significant similarities such as the need for lifelong antiviral therapy, use of tenofovir and opportunities for collaboration such as the potential to share HIV clinic staff, facilities and laboratory equipment including molecular platforms to quantify HBV DNA (Lemoine and Thursz, 2017, Desalegn et al., 2018).

The lack of association we observed between HBV and socioeconomic status is in contrast to most infectious diseases, where poverty has a causal role. There are several potential explanations. The township has among the highest poverty levels in Blantyre and limited intra-sample variation could be the cause of lack of observable association. Interventions to prevent mother-to-child transmission (MTCT) of HBV, beyond routine pentavalent vaccination have not been introduced in Malawi and it is not clear that higher socioeconomic status would confer improved access to routine vaccination as overall coverage is high in Malawi. The association observed between HBV infection and being divorced or separated may be related to risk of sexual transmission. Male gender was an independent risk factor for HBV infection among adults, in keeping with other studies from sub-Saharan Africa (Bwogi et al., 2009, Martinson et al., 1998). Causal mechanisms may include unsafe circumcision, shared razor blades or use of barbershops (Spengane et al., 2018).

Low HBV prevalence was observed in children, rising through adolescence before peaking in the 30-40 year group and falling again among older adults. This finding of falling prevalence beyond 40 years may be due to spontaneous functional loss of HBsAg, death due to HBV-associated complications such as cirrhosis or HCC, or death from other associated blood-borne infections that share transmission routes with HBV (Mendy et al., 2008). Increasing prevalence among adults between adolescence and 40 years may be caused by adult HBV transmission, or due to a cohort effect with impact of HIV prevention strategies such as promotion of condom use among successive age groups. In keeping with this second possibility, the HBV prevalence patterns with a single peak at 40 years mirrors national HIV prevalence data (Government of Malawi, 2018). Given the limitations of the cross-sectional study design, a detailed understanding of transmission dynamics is limited and only pseudo-longitudinal inference is possible. A longitudinal cohort study or repeated cross-sectional surveys are needed to understand changes in transmission patterns over time.

While our study indicates that HBV infection has become infrequent among children with the use of the pentavalent vaccine as the sole preventative strategy, modelling data indicates that adult HBV antiviral treatment is needed to begin to reduce HBV associated mortality (Nayagam et al.). In the absence of adult antiviral treatment, mortality is projected to rise until 2040 and eradication would not be achieved by 2100. In this community, by use of WHO, EASL or AASLD criteria, 3, 6 and 9% respectively were eligible for HBV treatment. There is only one previous community study assessing HBV treatment eligibility in a representative population sample in sub-Saharan Africa, in the Gambia. This showed 4.4% of community participants were eligible for treatment whereas 9.7% of HBsAg positive blood donors were eligible, based on EASL 2012 criteria (Lemoine et al., 2016b). In a hospital-based sample in Ethiopia with a mixture of referral sources, 20% had cirrhosis and 25.2% required

treatment by EASL 2012 criteria (Desalegn et al., 2018). Among female commercial sex workers and men who have sex with men in Cote d'Ivoire, 7.7% required treatment whereas 2.1% and 21.6% of prisoners in Senegal and Togo, were eligible respectively based on WHO 2015 criteria (Jaquet et al., 2017). Based on Malawi's 2018 census population of 17.6 million people, and based on the assumption that this population is representative of the national population, we estimate that 25,586 HIV negative people (95% CI 7,172 – 65,519) will require HBV treatment by EASL criteria. This compares to an estimated 970,000 adults living with HIV of whom 810,000 are receiving ART, with 28,000 new cases annually (UNAIDS, 2018).

Within the clinical evaluation population, the biomarker APRI had poor sensitivity, particularly for the diagnosis of cirrhosis, which is the intention of the tool as implemented in the WHO 2015 HBV treatment guidelines (World Health Organisation, 2015). Applying the WHO recommended cut-off of 2.0, the sensitivity for diagnosing cirrhosis was only 33%, and this increased to 67% with a lower threshold of 0.8. By contrast, the GPR performed well in this cohort and at an optimised lower threshold of 0.3, identified all patients with cirrhosis with only a modest trade off in terms of specificity. These findings should be interpreted with caution since only 3 and 9 HIV negative HBsAg positive patients in this sample had liver stiffness consistent with significant fibrosis or cirrhosis, respectively and these optimised thresholds require external validation with larger samples. These data act as further evidence that the WHO guidelines in the current format are not suitable for use in Africa, and add further weight to the proposed alternative use of GPR to classify cirrhosis.

There are several limitations to this analysis. First, it was not possible to estimate vaccine effectiveness. This was due to high vaccine coverage and consequent lack of ascertainment of HBV prevalence among unvaccinated children despite the large sample size. Instead vaccine impact was estimated, comparing birth cohorts relative to the timing of national vaccine introduction. This approach relies upon an assumption that exposure risk is equivalent between the two groups, which may not hold if significant HBV transmission occurs in early adolescence. Baseline pre-vaccination prevalence data among young children were not available to compare identical age groups in a before-after comparison(Stockdale et al., 2018), although overlap between pre- and post-vaccination groups did occur due to the 18 month duration of the serosurvey. Second, these findings may not be generalisable nationally or to other sub-Saharan African countries since this sample was from an urban township where vaccine uptake exceeds national coverage (WHO-UNICEF, 2018). Our sample is urban, yet in the 2018 population census, 84% of Malawians resided in rural areas. However, Ndirande is a common location for inward migration and settlement for those moving from rural areas to Blantyre city and may provide a broader representation of Malawian citizens than most urban samples. Third,

reasons for refusal were not recorded during the serosurvey to facilitate assessment of nonresponse bias. Attempts to mitigate this bias included using post-stratification weighting to adjust estimates to the population age and sex distribution and use of a sensitivity analysis to examine for geographic variation in responses. No influence of geographic non-response was observed. Fourth, due to the interval between conducting the serological survey and the clinical evaluation, and due to a high rate of migration from the township, a fifth of HBsAg positive individuals could not be located, who had participated in the serosurvey and experienced additional attrition due to death or relocation. This may have led to an underestimation of population disease burden and treatment requirements since non-participants were observed to have had lower educational and socioeconomic status and were less likely to be in employment, and clearly among those who died an opportunity to ascertain liver disease may have been missed.

In conclusion, in an urban township in Malawi, fifteen years after pentavalent HBV vaccination was introduced HBV prevalence has markedly declined from 5.1% among adults to 0.3% among young children, and 0.2% among children known to have completed a 3-dose immunisation course, with an estimated vaccine impact of 96%. At the population level, HIV co-infection is present in a quarter of adults with HBV infection, two thirds of whom are already on antiretroviral therapy with HBV virological suppression. Among HIV negative individuals, antiviral therapy was required in 3, 6 and 9% of HBsAg positive adults based on WHO, EASL and AASLD criteria respectively, which related to the national 2018 census population of 17.6 million, represents an estimated 25,586 adults who are eligible for HBV treatment in Malawi.

Chapter 6. Epidemiology, burden of disease and treatment eligibility of hepatitis C in Ndirande, Malawi: A community observational cross-sectional study

6.1. Introduction

In 2016, the World Health Assembly approved a plan to reduce mortality from viral hepatitis by 65% and reduce incident cases by 80% by 2030 (World Health Organisation, 2017a). To achieve these goals, epidemiological estimates representative of the general population and specific risk groups are necessary to inform an effective public health response. In sub-Saharan Africa, an estimated 1% of the population, representing 11 million people, have hepatitis C virus (HCV) infection (World Health Organisation, 2017a). Global HCV prevalence estimates were recently revised downward as a result of data from West Africa showing lower prevalence relative to previous estimates, and the incorporation of confirmatory HCV RNA testing (Polaris Observatory, 2017). Prevalence estimates based on anti-HCV detection have been associated with overestimation due to low specificity (Mullis et al., 2013, Polaris Observatory, 2017, Twagirumugabe et al., 2017). Line immunoassay data provide evidence that anti-HCV results in the majority of patients who are negative for HCV RNA are false positives, with evidence of spontaneous clearance in a minority of cases (King et al., 2015, Rouet et al., 2015). In a pooled analysis of 35 studies from sub-Saharan Africa, HCV RNA PCR testing confirmed active infection among 51% of anti-HCV positive people (Rao et al., 2015).

A recent systematic review did not identify any high-quality general population HCV prevalence data for southern Africa, and included only a single study from the region, from South Africa (Polaris Observatory, 2017). In Malawi, previous prevalence estimates ranged from 0.5-12%, based on anti-HCV testing from convenience samples of diverse populations including antenatal, blood donor, occupational or HIV clinic populations (Stockdale et al., 2018). Three studies that used confirmatory HCV RNA PCR in blood donors, HIV-positive pregnant women and HIV positive adults estimated prevalence of 0-0.7% (Stockdale et al., 2018, Andreotti et al., 2014, Demir et al., 2016, Candotti et al., 2001). HCV treatment programmes are not yet widely available in the region, although there are unprecedented opportunities for disease control with the advent of directly acting pan-genotypic antivirals associated with sustained virological response (SVR) rates exceeding 90%, and point of care molecular tests. SVR refers to the absence of detectable viraemia following the completion of HCV treatment, is usually assessed at 12 weeks post-completion of treatment, and represents HCV cure. Achieving HCV elimination will require elucidation of key populations at risk of infection and specific risk factors for transmission (Heffernan et al., 2019).

The aim of this study was to ascertain HCV prevalence in the general population based on a demographic census and single-stage probability sampling serosurvey of an urban township in Blantyre. HCV testing was conducted using a fourth generation combined antigen and antibody (HCV Ag/Ab) ELISA and confirmatory HCV RNA PCR. Whole genome next generation sequencing with probe-based target enrichment was used to identify the molecular epidemiology of HCV genomes among identified samples from the community serosurvey, adding sequences obtained from the hospital study (as described in Chapter 4). Secondary objectives were to ascertain rates of concomitant hepatitis B (HBV) and HIV infection and prevalence of hazardous alcohol use, and to identify risk factors for HCV infection.

6.2. Methods

The methods for the community serosurvey, analysis of HCV epidemiology and HCV sequencing are described in chapter 2.

6.3. Results

The demographic census recorded a population of 97,386 individuals residing in 22,364 households with median 4 (IQR 3, 6) residents per dwelling. The median age was 19 years (interquartile range (IQR) 9, 31) and 49.5% were female. A total of 1661 serosurvey participants aged over 16 years were randomly selected from the total serosurvey population (n=3258 individuals aged ≥16 years) for HCV testing, with median age 30 years (IQR 22, 38.8) and 52.7% (876/1661) female (Figure 6-1). The serosurvey under-sampled men aged 20-35 and oversampled men under 20 and aged 35-45 years relative to the census distribution (Figure 6-2).

Among the serosurvey population, crude HCV Ag/Ab prevalence was 13/1648 (0.78% (95% CI 0.46 – 1.33)) and population standardised prevalence was 0.80% (95% CI: 0.47 – 1.38). HCV RNA was detected in 3/13 HCV Ag/Ab positive individuals (23.1%, 95% CI 8.2 – 50.3) with HCV RNA concentrations of 5.94, 6.63 and 7.09 $\log_{10}$ IU/ml respectively. Population standardised HCV RNA prevalence was 0.18% (95% CI 0.06- 0.53). HCV RNA detection was associated with the HCV Ag/Ab ELISA sample to cut off ratio (S/CO), with a median S/CO among HCV RNA positive people of 13.6 (standard deviation (SD) 0.6) and of 1.7 (SD 0.3) among HCV RNA negative participants, $p<0.0001$ (Figure 6-3).

Figure 6-1: Flowchart of study recruitment

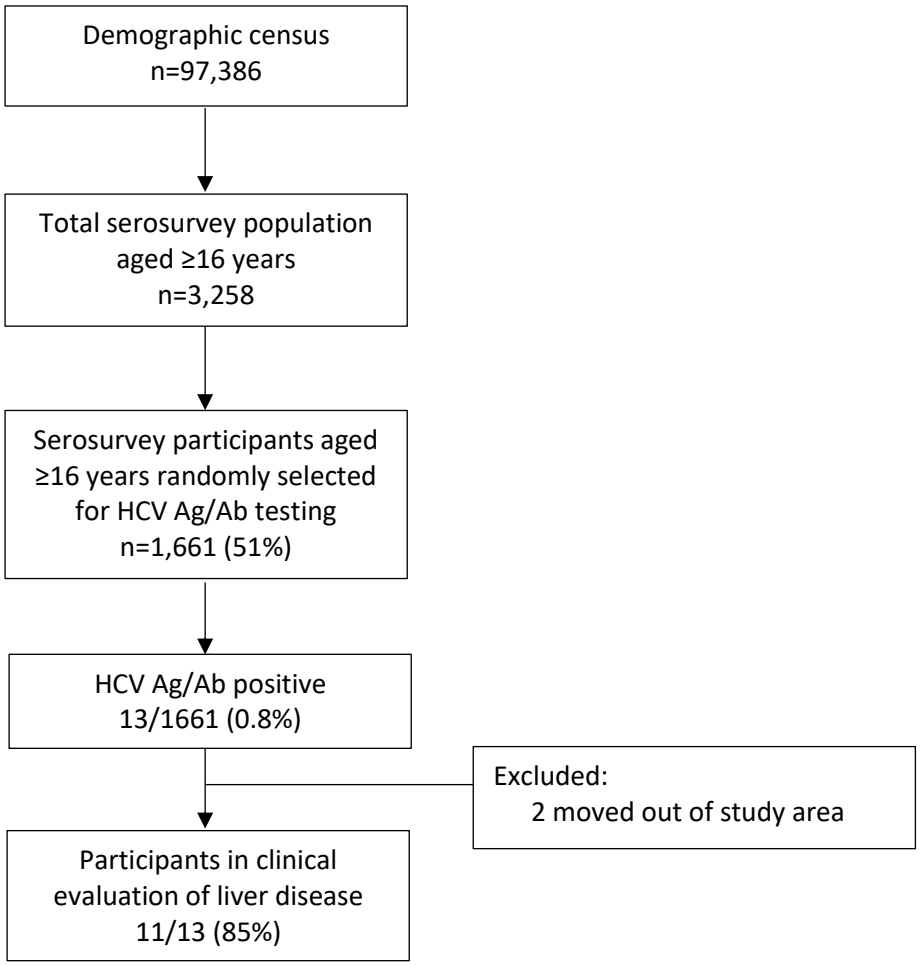


Figure 6-2: Comparison of census and serosurvey age and sex distribution

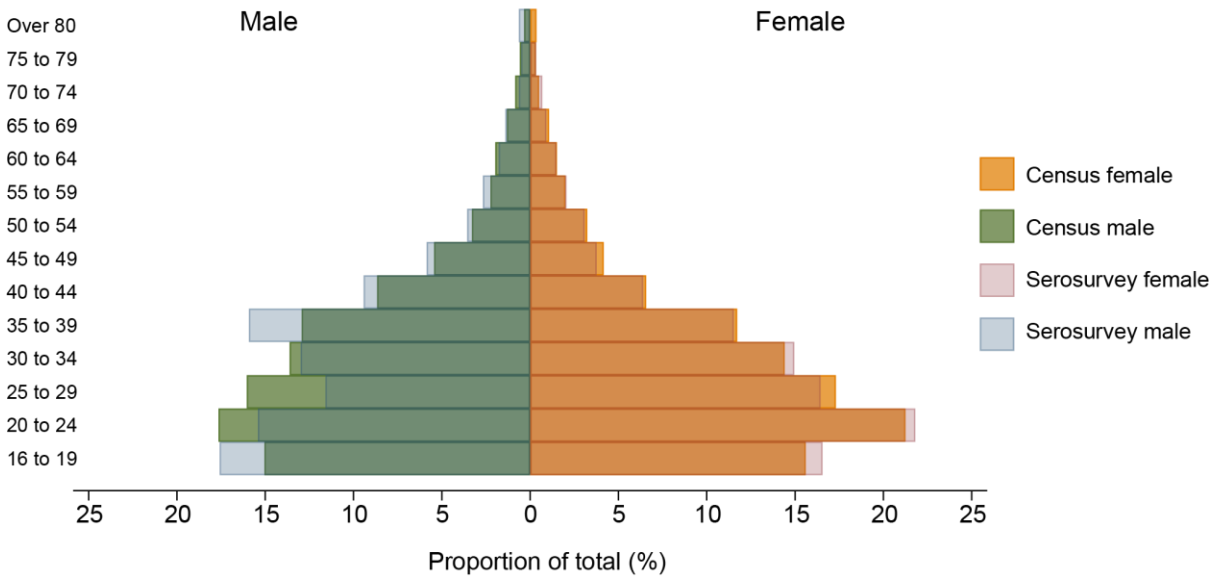
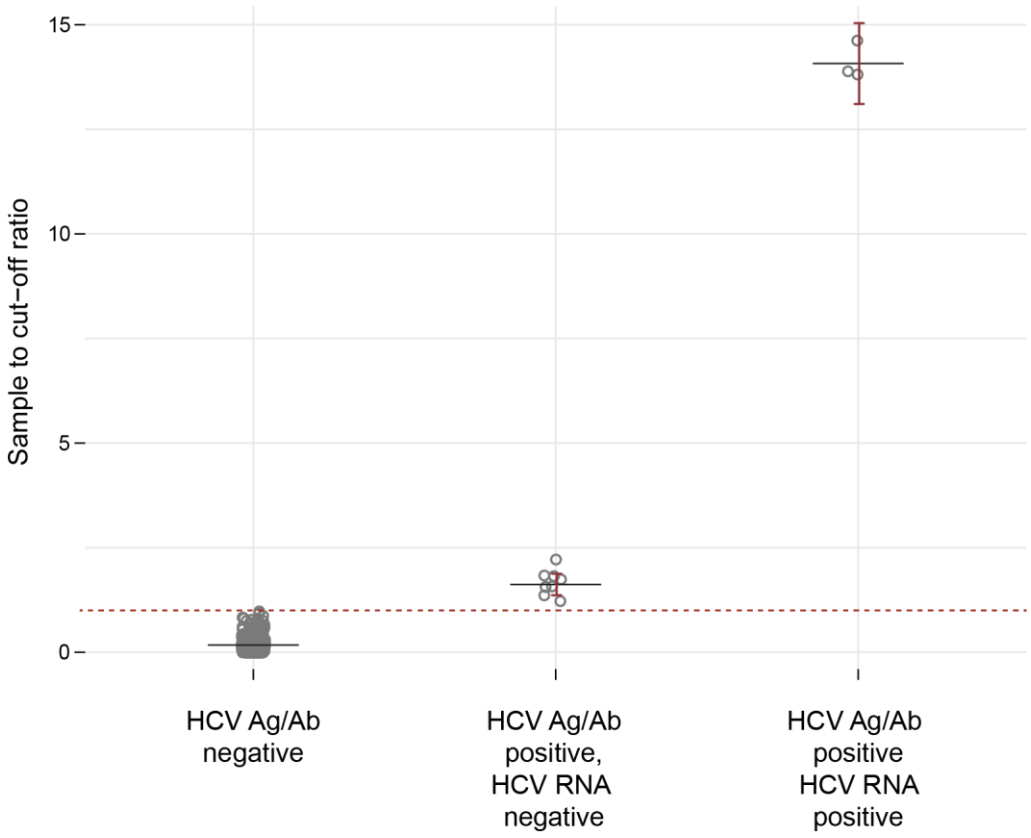


Figure 6-3: Association between HCV Ag/Ab ELISA sample to cut-off (S/CO) ratio and HCV RNA status[a]



[a] S/CO >1 is interpreted as positive (red dotted line). Mean S/CO results for HCV Ag/Ab positive individuals (n=13) (comprised of 3 HCV RNA positive and 10 HCV RNA negative) and 1648 HCV Ag/Ab negative individuals are shown. Black horizonal lines indicate mean value and red error bars show 95% confidence interval..

Relative to HCV negative serosurvey participants, the 13 HCV Ag/Ab positive individuals were older, but no differences in sex, educational level, employment or socioeconomic status were observed (Table 6-1). No cases of co-infection with HBV were observed. A total of 11/13 (85%) HCV Ag/Ab positive serosurvey participants could be located in the township and consented to evaluation of liver disease.

In a nested case-control analysis with nine age-matched negative controls per HCV Ag/Ab positive case, no association was observed between HCV Ag/Ab and gender, HIV status, schistosomiasis (assessed by urinary CCA detection), alcohol consumption, or sexual risk factors (Table 6-2). HIV prevalence of HCV Ag/Ab positive people was 18.1% (95% CI 5.1 – 47.7), relative to 10.1% (95% CI 6.5-

17.6) among the matched control population, p=0.66. This compares with an estimated HIV prevalence for Blantyre city of 17.7% (95% CI 16.0- 19.5) in a national prevalence survey (Government of Malawi, 2018).

Table 6-1: Characteristics of serosurvey participants tested for HCV, stratified by HCV status

	HCV RNA positive n=3	**HCV Ag/Ab positive n=13**	**HCV Ag/Ab negative n=1648**	**Odds ratio of HCV Ag/Ab positive[a]**	**P value**
Sex, female (%)	2 (67)	7 (54)	869 (53)	0.99 (0.33 – 2.98)	0.99
Age, median (%)	53 (47, 55)	35 (33, 50)	30 (22, 39)	1.03 (1.01 – 1.06)	0.02
Hepatitis B surface antigen positive n(%)	0 (0)	0 (0)	90 (5.5)	-	1.00
Education, n (%)					0.31
Primary	2 (67)	7 (58)	482 (31)	Reference	
Secondary	1 (33)	5 (38)	942 (60)	0.41 (0.13 – 1.30)	
Technical	0 (0)	1 (8)	61 (4)	1.22 (0.15 – 9.97)	
University	0 (0)	0 (0)	55 (4)	-	
None	0 (0)	0 (0)	27 (2)	-	
Employment, n (%)					0.75
Unemployed	0 (0)	2 (15)	218 (14)	Reference	
Student	0 (0)	1 (8)	313 (20)	0.38 (0.03 – 4.28)	
Self-employed	1 (33)	4 (33)	469 (30)	0.77 (0.14 – 4.34)	
Paid employment	1 (33)	3 (25)	348 (22)	0.84 (0.14 – 5.19)	
Unpaid family work	1 (33)	3 (25)	218 (14)	1.25 (0.20 – 7.72)	
Retired	0 (0)	0 (0)	4 (0.3)	-	
Marital status, n (%)					0.31
Single	0 (0)	3 (23)	530 (35)	Reference	
Married	2 (67)	8 (62)	889 (58)	1.24 (0.33 – 4.67)	
Separated	0 (0)	1 (8)	42 (3)	3.07 (0.32 – 29.78)	
Widowed	1 (33)	1 (8)	69 (5)	2.12 (0.23 – 20.95)	
SE status, n(%)					0.34
Highest quintile	0 (0)	1 (8)	133 (8)	Reference	
2nd highest quintile	0 (0)	2 (15)	352 (21)	0.81 (0.72 – 9.14)	
Middle quintile	1 (33)	0 (0)	391 (23)	-	
2nd poorest quintile	0 (0)	7 (54)	385 (23)	1.71 (0.21 – 13.83)	
Poorest quintile	2 (67)	3 (23)	387 (23)	0.97 (0.10 – 9.34)	

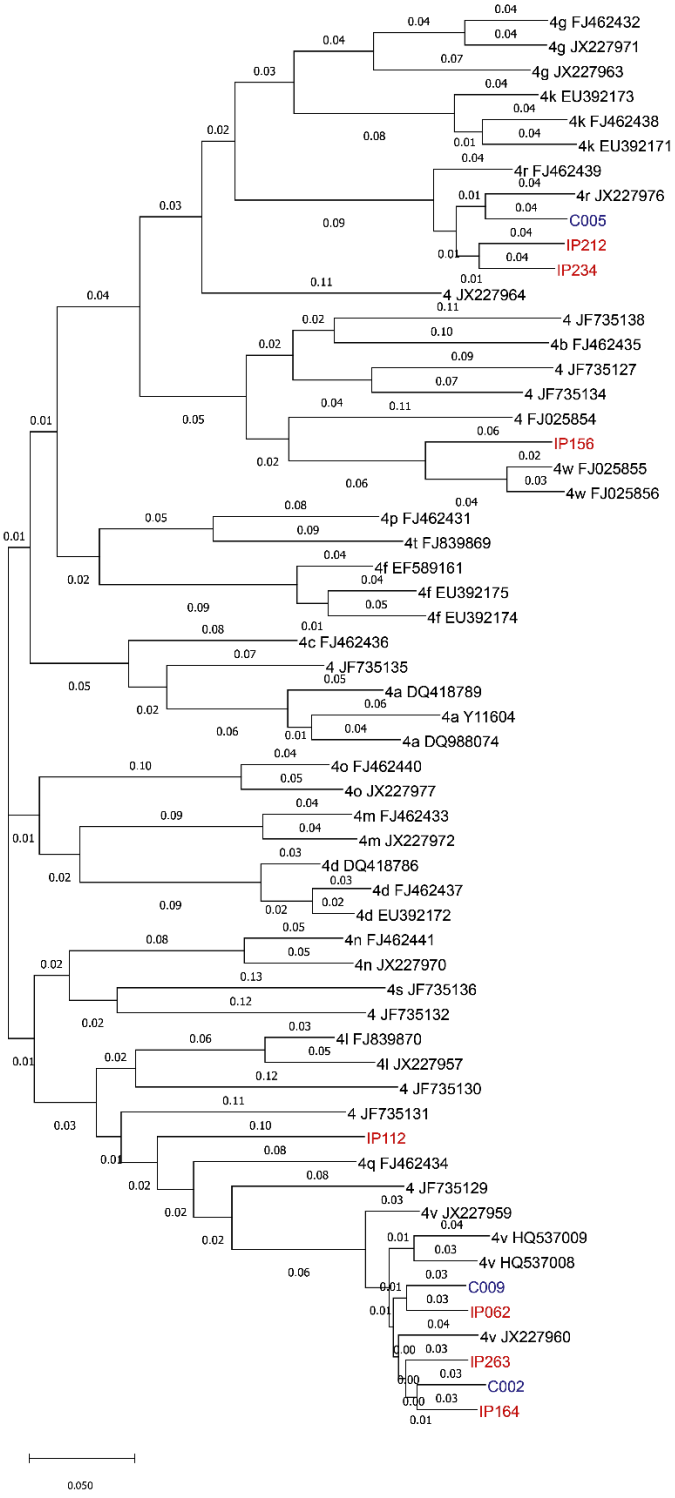
[a]Bivariate logistic regression, adjusted to census age and sex distribution by survey post-stratification proportional fitting weights. SE= socioeconomic

Table 6-2: Characteristics of HCV RNA positive and HCV Ag/Ab positive individuals relative to 9:1 age-matched controls: conditional logistic regression

Characteristic	HCV Ag/Ab positive		HCV Ag/Ab negative age-matched controls n=99	Odds ratio for HCV Ag/Ab positive[a] (95% CI)		P value
	HCV RNA+ n=3	HCV RNA- n=8				
Sex, female, n(%)	2 (67)	5 (63)	57 (58)	1.24	(0.30 – 5.11)	0.77
HIV positive, n(%)	1 (33)	1 (13)	10 (10)	1.52	(0.24 – 9.61)	0.66
Schistosomiasis urinary CCA positive, n(%)	0 (0)	0 (0)	3 (3)	-	-	-
Use of condom, n(%)						
Never	1 (33)	5 (63)	29 (29)	Ref		
Sometimes	2 (67)	3 (38)	65 (66)	0.36	(0.11 – 1.15)	0.09
Usually /always	0 (0)	0 (0)	5 (5)	-	-	-
Number of lifetime sexual partners, n(%)				1.01	(0.94 – 1.07)	0.84
0-4	2 (67)	6 (75)	76 (77)	Ref		
5-10	0 (0)	1 (13)	19 (19)	0.50	(0.56 – 4.41)	
>10	1 (33)	1 (13)	4 (4)	6.37	(0.73 – 55.47)	
Ever paid for sex, n(%)	0 (0)	1 (13)	10 (10)	0.64	(0.07- 6.27)	0.70
Alcohol						
Abstinent/low risk	3 (100)	7 (88)	87 (89)	Ref		
≥Hazardous	0 (0)	1 (13)	11 (11)	0.82	(0.09 – 7.34)	0.86
Liver stiffness (kPa), median (IQR)	7.7 (7.1, 8.3)	5.1 (4.1, 5.8)	4.6 (3.6, 5.3)	1.03	(0.93 – 1.13)	0.63
Liver stiffness >13kPa (cirrhosis), n(%)	0 (0)	0 (0)	5 (5)	-	-	-

[a]Conditional logistic regression comparing 11 HCV Ag/Ab positive cases with age-matched controls. Abbreviations: n, number (frequency); CCA, circulating cathodic antigen; HCV, hepatitis C virus; Ref, reference; IQR, Interquartile range;

Figure 6-4: Maximum likelihood phylogenetic tree: complete genome sequences aligned to genotype 4 subtype reference sequences from the International Committee on Taxonomy of Viruses



The tree was constructed using a General Time Reversible model with neighbour-join and BioNJ algorithms applied to a pairwise distance matrix, using Mega-CC. The evolutionary rate difference were estimated using a discrete Gamma distribution with invariant sites. Numbered branch lengths represent number of substitutions per site. IP sequences (in red) are 7 HCV RNA positive patients from the hospital study with cirrhosis or HCC (Chapter 4) and 3 C sequences (in blue) are from this community study.

Table 6-3: Resistance associated substitutions (RAS) observed in HCV NS5A sequences: prevalence of predicted amino acid variants at NS5A positions by deep sequencing

			NS5A RAS (%)				NS5B RAS (%)	
N	Source	GT	28	30	31	93	282	321
IP112	H	4	M (29)	R(70) S(30)	M (99)	Y (99)	S (99)	V (100)
C005	C	4r	M (98)	R (100)	L (99)	Y (99)	S (99)	I (99)
IP212	H	4r	V (99)	R (99)	L (99)	Y (98)	S (99)	I (99)
IP234	H	4r	V (99)	R (100)	L (99)	Y (99)	S (99)	I (100)
C002	C	4v	L (99)	R (99)	M (99)	Y (99)	S (99)	V (98)
C009	C	4v	L (99)	R (99)	M (99)	Y (99)	S (99)	V (98)
IP062	H	4v	L (100)	R (99)	M (99)	Y (99)	S (99)	V (99)
IP164	H	4v	M (66) L (34)	R (99)	M (99)	Y (97) H (2)	S (99)	V (99)
IP263	H	4v	L (100)	R (99)	M (99)	Y (99)	S (99)	V (99)
IP156	H	4w	V (100)	S (99)	M (98)	F (98)	S (98)	V (99)

Abbreviations: GT genotype, H, Hospital cohort study (described in chapter 4); C, Community study, NS non-structural, RAS, resistance associated substitution

Three HCV RNA positive patients from the community serosurvey and seven from the hospital study with cirrhosis or HCC underwent whole genome sequencing. Median coverage using subtype-specific reference genome alignment was 99.5% of the reference genome (IQR 99.1, 99.7), with a mean depth of 33,177 reads (SD 24,957). Five of the sequences aligned to genotype 4v (three hospital and two community), three aligned to genotype 4r (two hospital and one community), one aligned with 4w, and one was assigned genotype 4 without alignment to a subgenotype (Figure 6-4)

Analysis of deep sequencing data showed among genotype 4r patients, two of three patients had NS5A resistance associated substitutions (RAS) L28V + L30R, associated with high level NS5A inhibitor resistance (Table 6-3). Among the five patients with subtype 4v, one had a majority (66%) L28M mutation with L30R. All remaining 4v patients had L30R + L31M. In the patient with genotype 4w, L29V + L31M was observed and in the patient with GT4 without subtype classification, the L28M + L31M combination was observed. No patients were observed to have mutations at position 93 or in NS5B at the S282 site.

6.4. Discussion

This is the first census-based community random probability sampling HCV prevalence survey in Southern Africa. Previous community studies from the region have suffered from important biases that may limit sample representativeness. A study from Zimbabwe, nested within a community schistosomiasis prevalence study, recruited individuals volunteering at community meetings, without using a random sampling framework. In the study, 269 out of 2281 participants were tested for anti-HCV and the subset selection method was not described (Kallestrup et al., 2003). A study from the highveld region of Zimbabwe sampled members of a church, which may not share the same level of risk for acquisition of blood borne viruses as the general population and did not include confirmatory testing (Moyo et al., 2009). We consulted three systematic reviews of HCV prevalence and supplemented by searches of the literature, we did not identify any other community prevalence studies for the Southern Africa region (Riou et al., 2016, Polaris Observatory, 2017, Rao et al., 2015).

Our estimated HCV prevalence rates are significantly lower than estimates for the wider WHO Africa region and from previous modelling estimates for Southern Africa. These data suggest that Malawi has a concentrated HCV epidemic. Further prevalence surveys are now required to identify specific population groups at increased risk of HCV such as commercial sex workers or men who have sex with men. Estimates among HIV-positive populations attending for routine HIV care in Malawi and recent data from Zambia, Mozambique, Uganda and Kenya similarly found low prevalence with anti-HCV prevalence ranging from 0.2-1.1% and HCV RNA from 0 to 1% (Demir et al., 2016, Andreotti et al., 2014, Loarec et al., 2019, Wandeler et al., 2016). Our results contrast with previous estimates from Malawi which relied upon anti-HCV testing of convenience samples from diverse populations with estimates ranging from 0.5 to 12% (Stockdale et al., 2018). The discrepancy may be a function of improved diagnostic performance of more recent HCV assays. We observed that the magnitude of the HCV Ag/Ab ELISA S/CO ratio predicted HCV RNA positivity, in accordance with previous data (Rouet et al., 2015). In an HIV clinic study in Gabon, a S/CO of 1.7 was found to be optimal for ascertainment of positive HCV RNA, using the same assay we used in this study. The majority of participants with HCV Ag/Ab+/HCV RNA- had results from line immunoassay consistent with false positive HCV Ag/Ab reactivity, with only a small proportion (13%) with evidence of spontaneous clearance (Rouet et al., 2015). Similar findings were observed in a study of HIV positive individuals in Ghana and blood donors in Rwanda (Twagirumugabe et al., 2017, King et al., 2015). Our study adds further evidence that adoption of an increased S/CO ELISA or CMIA threshold might reduce unnecessary confirmatory testing. The optimal threshold should be specific to the assay and population pre-test probability of HCV RNA, according to the clinical setting and local epidemiology.

In this study employing both population-level analysis and a nested case-control design, specific risk factors associated with HCV infection could not be identified, though due to lower-than-expected HCV prevalence there was limited statistical power. Although our sample size was sufficient to estimate HCV prevalence with precision of 0.5%, only three HCV RNA positive individuals were identified, precluding analyses of risk factors based on HCV RNA detection. HCV Ag/Ab positive/ RNA negative cases in our sample could represent false-positive cases rather than previous cleared infection. In contrast to previous data from Uganda and Egypt we did not observe an association between anti-HCV and schistosomiasis in this study although larger samples and more extensive schistosomiasis diagnostics such as serology and PCR are required to validate this finding in Malawi (Mullis et al., 2013, Helal et al., 1998).

This study highlights the importance of obtaining representative population-level data and the limitations of using convenience samples as proxies for community prevalence estimation. Innovations in patient sampling including the use of dried blood spot sampling to mitigate logistical issues around cold storage and use of point of care oral fluid based antibody testing may facilitate future community level sampling (Geretti et al., 2017, Peeling et al., 2017). Population-level representative HIV viral load assessments represent a model, and potential opportunity for future collaboration with viral hepatitis surveys (Justman et al., 2018).

Among the community and hospital-based HCV sequences, all sequences were classified as genotype 4, with the majority grouped as 4w. Three sequences were classified as genotype 4r. This subtype is common in central and East Africa, and has been observed in Uganda, the Democratic Republic of Congo, Gabon and Central African Republic (Kamal and Nasser, 2008, Iles et al., 2014). Genotype 4r has been associated with low SVR rates when using empirical sofosbuvir/ ledipasvir in Rwanda (Gupta et al., 2019) and France (Fourati et al., 2019). HCV sequences showed a combination of NS5A RAS (resistance associated substitutions) associated with high level resistance to NS5A inhibitors, which have been associated with treatment failure with other regimens including velpatasvir and daclatasvir, with frequent evidence of majority populations of L28M/V, L30R and L31M. Data from the UK HCV treatment programme showed that among genotype 4 patients, the combination of NS5A RAS L30R + L31M was associated with treatment failure with sofosbuvir + ledipasvir regimens in both pre- and post-treatment sequencing, with additional emergence of NS5B RASs associated with treatment failure. For subtype 4r, the single pre-treatment L30R was associated with failure (da Silva Filipe et al., 2017). In a French study of virological failure among patients with genotype 4r, all of whom were of African origin, the most common pre-treatment polymorphism observed was L28V + L30R (Fourati et al., 2019) and failure, L28A/C/I/M/V mutations together with L30R were frequently observed. Among

13 patients who were treated with sofosbuvir with a NS5A inhibitor (daclatasvir, ledipasvir or velpatasivir), 8 had L28V + L30R, and 10 patients had L31M/V at failure while Y93H was found in only one patient. The sofosbuvir mutation S282C/T was observed after treatment failure more commonly in genotype 4r relative to other genotypes. Among those with failure, S282 mutations were observed at very low levels pre-treatment and emerged in the presence of high level NS5A mutations L28V + L30R. The presence of RAS associated with high level NS5A resistance among the HCV sequences in these treatment naïve patients are cautionary and highlight the need for observational treatment studies with pre- and post-treatment sequencing to ascertain rates of SVR among patients treated with pan-genotypic regimens in Malawi. Sofosbuvir and ledipasvir is one of the WHO-recommended first line regimens for adolescents aged 12-17 years (World Health Organisation, 2018). Outcomes of treatment of other genotype 4 subgenotypes besides 4a are sparse, despite their widespread distribution in Africa (Davis et al., 2019). These finding may have implications for HCV pangenotypic treatment programmes and further data of treatment efficacy with these subgenotypes are needed.

In conclusion, in the first census-based community probability sample in Southern Africa, HCV infection was uncommon and lower than regional estimates or previous convenience samples, amounting to 0.2% at the population level. HCV Ag/Ab positive participants were older, but no association with gender, socioeconomic status or sexual risk factors was observed, although this finding should be tempered due to limited statistical power resulting from lower-than-expected HCV prevalence. Genotype 4 predominates with subgenotypes 4w, 4v and 4r prevailing and data on treatment efficacy using pangenotypic regimens is needed. Representative probability community samples from the general population and specific risk groups in the region are now required to inform an effective public health response.

Chapter 7. Diagnostic performance evaluation of hepatitis B e antigen rapid diagnostic tests in Malawi

7.1. Introduction

Ascertainment of hepatitis B e antigen (HBeAg) status is fundamental to the clinical classification of chronic HBV. Guidelines from the World Health Organisation (WHO) and hepatology associations recommend use of HBeAg to determine whether to start antiviral treatment (World Health Organisation, 2015, European Association for the Study of the Liver, 2017, Sarin et al., 2016, Terrault et al., 2018b). Determination of HBeAg status is central to a recently proposed simplified treatment protocol for Africa (Shimakawa et al., 2018). HBeAg may also be used to select which pregnant patients will require antiviral therapy to prevent mother-to-child transmission, as a surrogate marker of high HBV DNA concentration in settings where HBV DNA quantification is unavailable (Dionne-Odom et al., 2018) although it is an imperfect correlate (Shimakawa et al., 2016b).

In low- and middle-income countries, there are several potential barriers to accessing enzyme immunoassay (EIA), chemiluminescence immunoassays (CLIA) or electrochemiluminescence assays (ECA) for HBeAg detection. These include cost, limited laboratory capacity, the need for cold supply chain for reagents and a reliable electricity supply, and that laboratory equipment is frequently unavailable in rural areas where the majority of the population lives (Easterbrook et al., 2017). In many hospitals in sub-Saharan Africa, immunochromographic rapid diagnostic tests (RDTs) are routinely and widely used for diagnosis of infectious diseases, both at the point of care and in hospital laboratories. They offer many advantages including the lack of requirement for cold storage or electricity supply, minimal training requirements, rapid time to a result, usually within 15 minutes, and healthcare worker familiarity with RDT devices (Chevaliez and Pawlotsky, 2018). WHO has recently listed the HBeAg rapid diagnostic test as an essential diagnostic test that should be available at the primary health care level (World Health Organisation, 2019b).

A recent report from Senegal showed low sensitivity of HBeAg RDTs (Seck et al., 2018). This finding might have several implications for HBV management guidelines and further diagnostic performance evaluation studies in sub-Saharan Africa are warranted. The aim of this study was to assess diagnostic performance characteristics of three commercially available HBeAg RDTs in two settings in Malawi: in a community observational study in an urban township (described in Chapter 5) and in patients with cirrhosis or hepatocellular carcinoma in a tertiary hospital in Blantyre (Chapter 4), using a laboratory ELISA as a reference test.

7.2. Methods

Methods for the conduct of the demographic census, serological survey and community study, and inpatient study are set out in Chapter 2.

7.2.1 Assessment of rapid diagnostic tests

Three commercial rapid diagnostic tests (RDTs) for HBeAg were assessed: i) SD BIOLINE HBeAg (Product code 01FK30, Alere, Kempton Park, South Africa), ii) HBeAg Serum Rapid Test (Cassette), (Catalogue DTS382, Creative Diagnostics, Shirley, NY, USA) and iii) HBeAg Rapid Test (Catalogue RAPG-HBeAg-001, Biopanda Reagents, Belfast, United Kingdom). Test characteristics are described in table 7-1. All kits and reagents were shipped to the testing facility in Blantyre, Malawi using temperature-controlled shipments and stored in a temperature-monitored cold room at 4°C until use, within the expiry date of the kits. Following collection and processing, serum from HBsAg positive participants was stored at -80°C and brought to room temperature prior to testing. Two investigators (AS and NS) tested serum from each participant using each of the three RDTs in accordance with the manufacturer instructions. The results of each kit were recorded by each investigator independently, after which a result was ascertained by consensus.

7.2.2 Statistical analysis

Performance characteristics were calculated using exact binomial confidence intervals. Inter-rater agreement was calculated using the Cohen and Conger method (Conger, 1980). HBeAg concentration was calculated using a five parameter logistic (5PL) regression curve, plotting concentrations of the WHO standard against absorbance at 450/620nm (Gottschalk and Dunn, 2005). Samples exceeding the range of the standard were diluted and repeated until within range of standard dilutions. Analyses were performed using Assay Fit Pro (AssayCloud, Nijmegen, The Netherlands), *diagt*(Seed, 2010) and *kappaetc* packages in Stata release 14.2 (Statacorp, College Station, TX, USA).

7.3. Results

Three HBeAg RDTs were evaluated in HBsAg positive individuals: 100 community study participants and 94 inpatients with cirrhosis or HCC. Characteristics of the RDTs and reference ELISA are shown in Table 7-1. By qualitative ELISA, 11/100 (11%) community and 36/94 (39%) inpatients were HBeAg

positive. Baseline characteristics of study participants is shown in Table 7-2. Good inter-observer agreement was observed for all RDTs with the κ statistic ranging from 0.71 to 1.0. Diagnostic sensitivity was consistently poor on evaluation of all three assays ranging from 36 to 64% in community samples and 25 to 75% in inpatient samples. Specificity was good for all assays in both cohorts (Table 7-3).

Table 7-1: Comparison of characteristics of HBeAg rapid diagnostic tests and the reference ELISA test

Characteristic	**SD BIOLINE HBeAg (Alere)**	**HBeAg serum rapid test (Creative Diagnostics)**	**HBeAg Rapid Test (Biopanda Reagents)**	**Monolisa HBeAg Plus (Bio-Rad)**
Assay type	IC RDT	IC RDT	IC RDT	ELISA
Analyte	Serum or plasma	Serum or plasma	Serum or plasma	Serum or plasma
Sample volume	100ul	120µl	3 drops (approx. 75µl)	100µl
Regulatory approval	None	None	CE marked	CE marked
Wait time	5-20 minutes	10-20 minutes	15 minutes	4 hours incubation, 1.5 hours pipetting time
Kit storage conditions	2-30°C	2-30°C	2-30°C	2-8°C
Cost per unit[a]	2.1 USD	4.5 USD	0.4 USD	3.1 USD
Sensitivity and specificity (%, (95% CI)) according to manufacturer data	95.5(88.9-98.2) 98.6(96.1-99.5)	96.3(92.1-98.6) 97.9(96.1-99.1)	99.9 (97.7-100) 98.8(97.0-99.7)	99.5(97.3, 100) 100 (98.5- 100)

Abbreviations: IC RDT Immunochromographic rapid diagnostic test; ELISA Enzyme linked immunosorbent assay; CE Conformité Européenne; USD United States Dollars. [a] Price per test in 2018 United States Dollars, excluding shipping.

HBeAg detection rate by the RDTs was associated with HBeAg concentration (Figures 7-1 and 7-2). For the SD Bioline, Creative Diagnostics and Biopanda HBeAg RDTs, the minimum HBeAg concentration at which all HBeAg positive samples was detected was 3.1, 2.2 and 2.6 $\log_{10}$ IU/ml respectively.

Table 7-2: Baseline characteristics of HBsAg positive study participants

Characteristic (median (IQR) or n(%))	**Community study (n=100)**	**Inpatient study (n=94)**
Age (years)	36 (29, 41)	40 (34, 45)
Sex (female)	52 (52)	30 (32)
HIV positive	24/99 (24)	27/93 (29)
CD4 count (cells/mm^3)[a]	519 (412, 577)	247 (149, 346)
On ART[a]	16/24 (66.7)	12/27 (44.4)
HBV DNA concentration (log_{10} IU/mL)	1.92 (1.23, 3.12)	4.18 (2.44, 6.33)
Liver stiffness measurement (kPa)	4.9 (4.0, 6.5)	66.6 (27.4, 75)

Abbreviations: IQR Interquartile range; ART antiretroviral therapy; CD4 cluster of differentiation 4; HBV hepatitis B virus. [a] CD4 count and proportion on ART are described for HIV positive participants

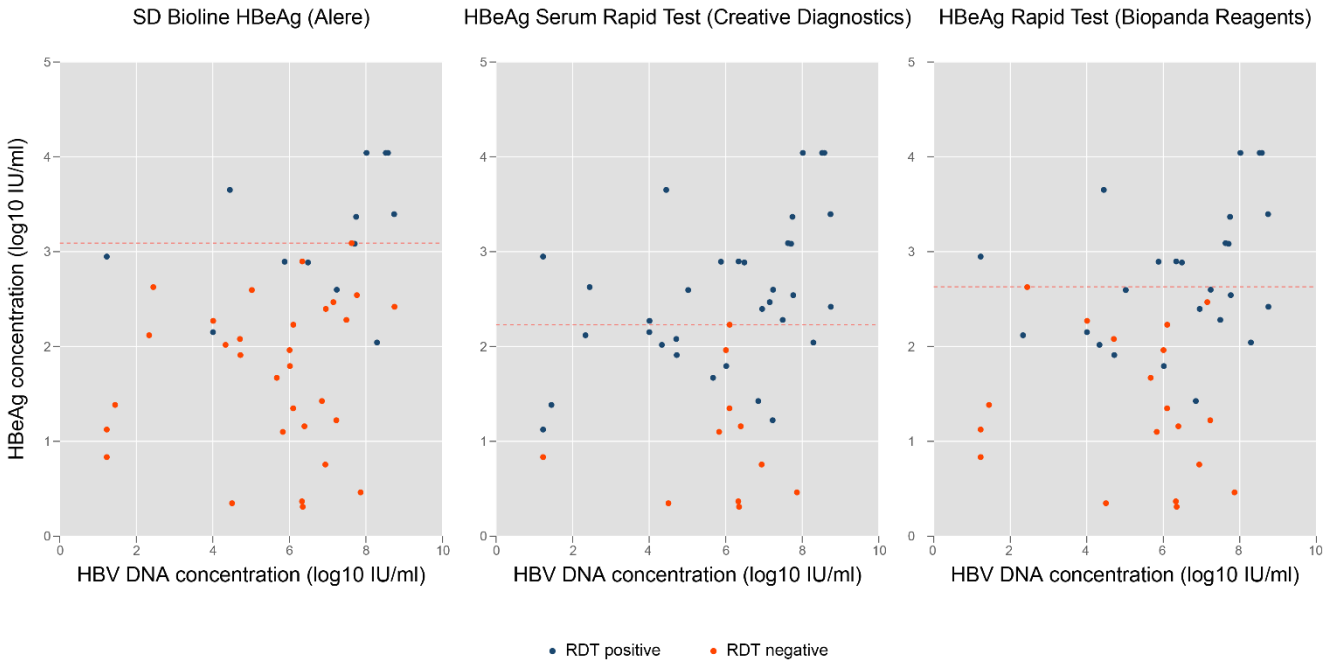
The application of HBeAg as a surrogate for identifying individuals with a HBV DNA >200,000 (5.3 log_{10}) IU/ml was considered, in alignment with the threshold applied for use of maternal antiviral therapy for prevention of mother to child transmission.(Nayagam et al., 2019, Jourdain et al., 2018) A weak correlation was observed between HBeAg concentration and HBV DNA concentration among HBeAg positive individuals (Spearman's ρ =0.35, p=0.02). The sensitivity for detection of HBV DNA above 200,000 IU/ml for the HBeAg RDTs was 22.2% (95% CI 11.2, 37.1) for SD Bioline, 53.8% (95% CI 37.2, 69.9) for Creative Diagnostics and 48.7% (95% CI 32.4, 65.2) for Biopanda RDTs. By comparison, the sensitivity for the reference test HBeAg ELISA for the detection of HBV DNA >200,000 IU/ml was 76.9 (95% CI 60.7, 88.9).

Table 7-3: Results of diagnostic performance evaluation of commercial HBeAg RDTs using a laboratory ELISA reference test

	Diagnostic performance characteristics (95% CI)		
HBeAg RDT	SD BIOLINE HBeAg (Alere)	HBeAg serum rapid test (Creative Diagnostics)	HBeAg Rapid Test (Biopanda Reagents)
Community study (n=100)			
κ statistic (inter-rater agreement)	0.71 (0.41- 1.0)	1.0 (1.0– 1.0)	1.0 (1.0- 1.0)
Sensitivity (%)	36.4 (10.9- 69.2)	63.6 (30.8- 89.1)	36.4 (10.9- 69.2)
Specificity (%)	100 (95.9- 100)	100 (95.9- 100)	98.9 (93.9- 100)
AUROC	0.682	0.818	0.676
Positive predictive value (%)	100 (39.8- 100)	100 (59 – 100)	80.0 (28.4- 99.5)
Negative predictive value (%)	92.7 (85.6- 97.0)	95.7 (89.4- 98.8)	92.6 (85.4- 97.0)
Diagnostic accuracy (%)	93.0 (86.1- 97.1)	96.0 (90.1- 98.9)	92.0 (84.8- 96.5)
Inpatient study (n=94)			
κ statistic (inter-rater agreement)	1.0 (1.0- 1.0)	0.95 (0.88- 1.0)	0.92 (0.83-1.0)
Sensitivity (%)	25.0 (12.1- 42.2)	75.0 (57.8- 87.9)	58.3 (40.8- 74.5)
Specificity (%)	100 (93.8- 100)	98.3 (90.8- 100)	91.4 (81.0- 97.1)
AUROC	0.749	0.866	0.749
Positive predictive value (%)	100 (66.4- 100)	96.4 (81.7- 99.9)	80.8 (60.6- 93.4)
Negative predictive value (%)	68.2 (57.2- 77.9)	86.4 (75.7- 93.6)	77.9 (66.2- 87.1)
Diagnostic accuracy (%)	71.3 (61.0- 80.1)	89.4 (81.3- 94.8)	78.7 (69.1- 86.5)

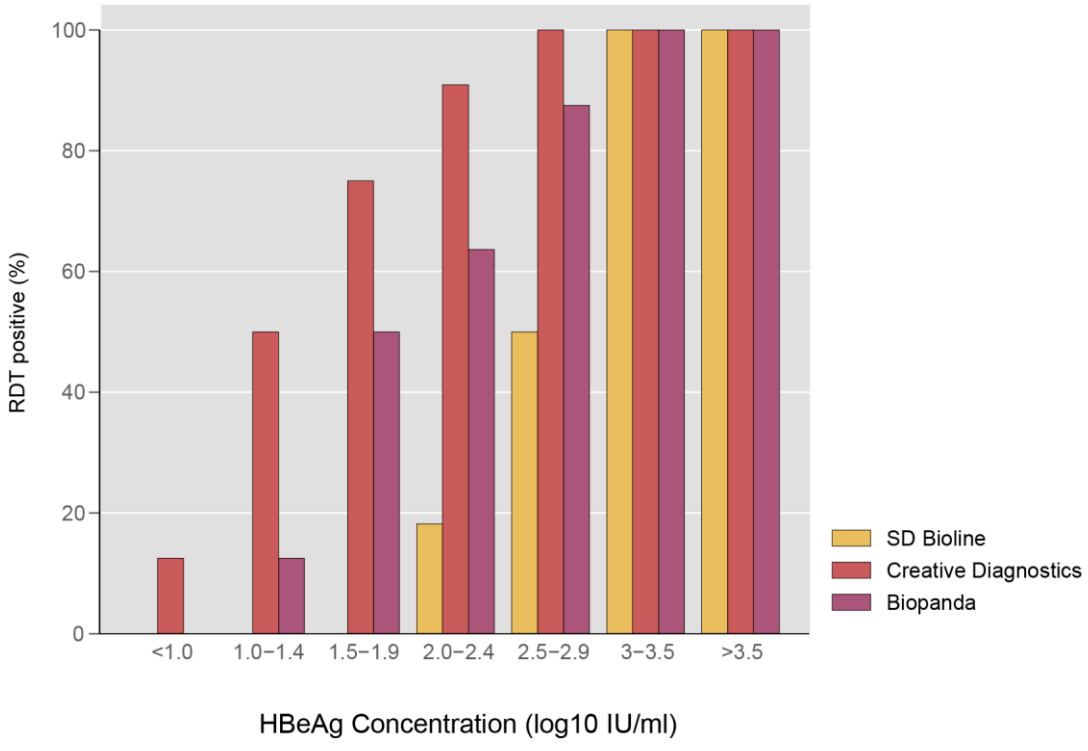
Abbreviations: AUROC Area under the receiver operating curve; RDT rapid diagnostic test; ELISA enzyme linked immunosorbent assay

Figure 7-1: Detection of HBeAg with RDTs relative to HBeAg and HBV DNA concentrations among HBeAg positive samples



All HBeAg positive results (by ELISA) are plotted according to the concentration of HBeAg (y-axis) and HBV DNA (x-axis). Red dotted lines refer to the minimum HBeAg concentration at which all samples are RDT positive for each RDT assay. Results of HBeAg rapid diagnostic tests are shown by the colour of the points: orange dots are positive and blue are negative.

Figure 7-2: RDT HBeAg results according to HBeAg concentration among HBeAg positive participants



7.4. Discussion

Rapid diagnostic tests in routine use in low resource settings had low sensitivity in Malawi for the detection of HBeAg, compared to a reference ELISA test. The lower limit of consistent detection varied from 2.2- 3.1 $\log_{10}$ IU/ml between RDTs. The assays also performed poorly at identification of patients with HBV DNA above 200,000 IU/ml and thus were observed to be unsuitable for use as a surrogate marker of high viral load. The findings are in keeping with recent evidence from West Africa (Seck et al., 2018).

This finding has substantial implications. The WHO recently added HBeAg RDTs to the list of essential medical diagnostics and RDTs are widely used in hospital laboratories in sub-Saharan Africa for HBeAg ascertainment (World Health Organisation, 2019b). Furthermore, HBeAg is a central component of a recently proposed treatment eligibility scoring tool for Africa (Shimakawa et al., 2018). HBeAg RDTs may therefore be misclassifying patients and potentially incorrectly denying patients access to antiviral therapy for chronic HBV treatment and for prevention of mother-to-child transmission.

The HBeAg is an important marker of HBV replicative status and is considered fundamental to classification of the clinical stage of disease, according to international guidelines for the management of HBV (European Association for the Study of the Liver, 2017, Terrault et al., 2018b). A soluble 25kDa protein encoded from the pre-C transcript of the HBV core open reading frame, HBeAg is an immunomodulatory protein acting as a T cell tolerogen(Milich and Liang, 2003, Kramvis et al., 2018). After translation from pre-genomic RNA, it is post-translationally modified and secreted into the serum via the endoplasmic reticulum (Kramvis et al., 2018). Its role in HBV immunology is to attenuate the adaptive response, favouring Th2 over Th1 activation and downregulating toll like receptor expression (Visvanathan et al., 2007, Milich and Liang, 2003). Seroclearance of HBeAg and seroconversion to anti-HBe is associated with a HBeAg-negative chronic HBV infection “inactive carrier” phase with decreased HBV DNA, and reduced hepatic inflammation such that HBeAg is associated with high HBV DNA and HBV disease activity (European Association for the Study of the Liver, 2017).

RDTs have many advantages over alternative laboratory techniques and are widely used for the detection of many infectious diseases in low and middle income countries (Chevaliez and Pawlotsky, 2018) where in many settings access to ELISA and CLIA is unavailable. The HBeAg ELISA reference test used in this study has a total incubation period of four hours and hands on laboratory time of one hour, requiring substantial skilled laboratory technician time and batching of samples. Diagnostic platforms based on CLIA are expensive and seldom available outside of central hospitals. An

alternative approach would be to quantify HBV DNA, which has recently become more feasible with the advent of point of care cartridge based systems such as the HBV DNA viral load GeneXpert (Lemoine and Thursz, 2017) and other systems currently in development. HBV treatment programmes in Africa may also share the infrastructure developed for HIV care including laboratory systems since molecular platforms currently used for HIV RNA quantification may also be used to support HBV DNA quantification (Easterbrook et al., 2017). Dried blood spot sampling may also be used to facilitate sample storage and transport (Lange et al., 2017).

These findings highlight the importance of ensuring that diagnostic tests undergo evaluation in the environment where they will be used, to reflect local epidemiology, population and viral genetic characteristics. Performance evaluation data from the manufacturer suggested sensitivity in excess of 95% for each of the evaluated assays. There is a pressing need to develop HBeAg RDTs with improved sensitivity adapted for use in sub-Saharan Africa. In the interim, alternative methods of HBeAg classification or HBV DNA quantification methods are recommended.

Chapter 8. Concluding remarks and future research priorities

8.1. Summary of research findings

In the Global Health Sector Strategy for Viral Hepatitis 2016-2021 from the World Health Organisation and endorsed by the World Health Assembly, the first Strategic Direction is to generate epidemiological data for focused action (World Health Organisation, 2016a). Priorities include to assess the national hepatitis burden, the number of people with HCC and cirrhosis attributable to HBV and HCV, and to monitor access to viral hepatitis treatment services. The aim of this thesis was to answer some of these questions of strategic importance for Malawi, in terms of defining the epidemic and providing evidence to support public health interventions, including a viral hepatitis treatment programme for HBV and HCV in Malawi and regionally. A summary of the main research questions and principal findings is shown in Figure 8-1.

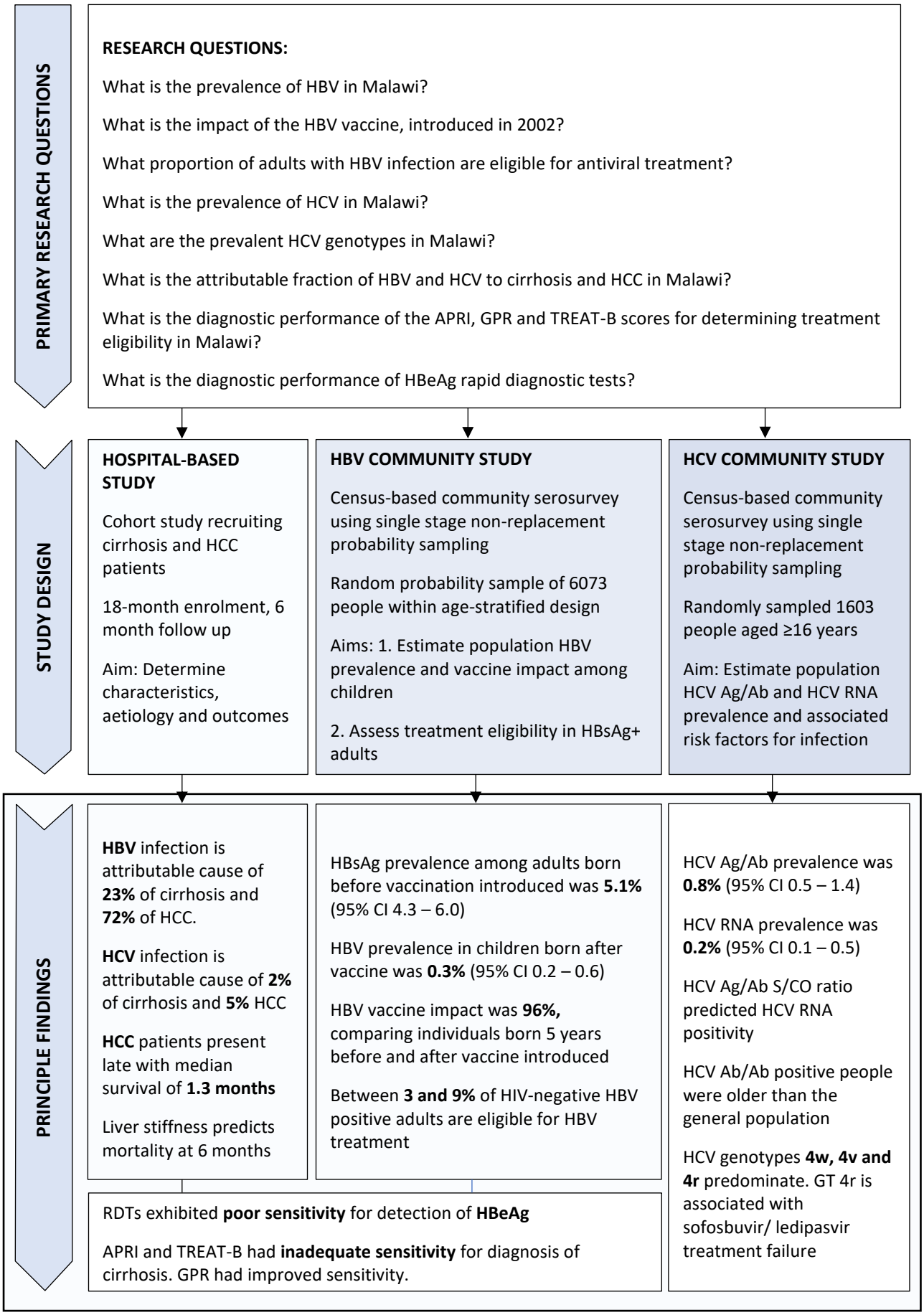
In this chapter, the findings of this research will be summarised, followed by an assessment of the future priorities for research to build on these findings.

8.1.1 Systematic review of HBV, HDV and HCV epidemiology

In Chapter 3 a systematic review was conducted to describe previous published epidemiological data on HBV, HDV and HCV prevalence in Malawi. Existing epidemiology data were observed to be predominantly convenience samples from blood donors, antenatal clinics, HIV clinics or occupational samples with considerable risk of bias and low representativeness of the general population, with no previous community data employing probability sampling.

A pooled prevalence from these previous data estimated HBV prevalence in Malawi of 8.1% (95% CI 6.1 – 10.3) (Stockdale et al., 2018). A single prior study of HDV prevalence was identified among people with HIV/HBV co-infection in Blantyre, which reported anti-HDV of 1.5% and HDV RNA prevalence of 0% (Stockdale et al., 2017). Among studies reporting HCV prevalence, estimates varied widely from 0.7 to 18.0% based on anti-HCV detection across a diverse range of general populations. Among three samples which used confirmatory HCV RNA PCR testing, prevalence estimates were <1%. The review illustrated the paucity of data from the Northern region and from rural areas and highlighted the need to obtain community based prevalence data employing representative probability sampling techniques, and data from children born after the HBV vaccination was introduced, to define the prevalence, impact of HBV vaccination, and disease burden associated with HBV and HCV in Malawi.

Figure 8-1: Summary of principle research questions, method and findings



8.1.2 Hospital based study of patients with cirrhosis and HCC

Patients with clinical features suggestive of cirrhosis or HCC were prospectively recruited from the Medical, Surgical and endoscopy departments of Queen Elizabeth Central Hospital, as described in Chapter 4. Transient elastography and abdominal ultrasound were used to assess eligibility, and participants were followed for six months after admission to ascertain outcomes. Patients with HCC presented at an advanced stage at the time of diagnosis with median tumour size of 12.4cm and with a median survival of 1.3 months. The population attributable fraction of HBV infection to cirrhosis was 23% and to HCC was 72%. HDV was an uncommon finding with anti-HDV observed in none of the patients with cirrhosis and among 3% of HCC cases. A strong association between HIV and cirrhosis and HCC was observed, even in the absence of HBV or HCV co-infection. Potential explanations include an association with tuberculosis or its treatment, enhanced case ascertainment and referral for diagnosis among people receiving HIV care, and a direct effect of HIV in promoting hepatis fibrosis. HCV was less important as a cause of liver disease with a PAF of 2% for cirrhosis and 5% for HCC. Alcohol was associated with HCC but not with cirrhosis. Schistosomiasis was associated with cirrhosis but we lacked geographically matched community controls to estimate a population attributable fraction. A significant proportion of cases of cirrhosis (60%) in this cohort were not explained by reference to hepatitis B, C or alcohol exposure. The main finding from this hospital-based study was that HBV is associated with almost three quarters of HCC cases and a quarter of cirrhosis cases, and patients with HCC present at an advanced stage with a poor prognosis. This presents both a challenge and great opportunity for disease prevention and control through the potential for additional MTCT interventions and for adult HBV treatment programmes.

8.1.3 Community study of HBV prevalence, vaccine impact and treatment eligibility

In Chapter 5, a census-based probability sampling community serosurvey was conducted in a township in Blantyre to estimate the prevalence of HBV and HCV and vaccine impact from introduction of the HBV vaccine in 2002. The population standardised prevalence of HBsAg among adults born prior to the introduction of the HBV vaccine in 2002 was 5.1% (95% CI 4.3- 6.0), and among children born after introduction was 0.3% (95% CI 0.2- 0.6). Vaccine coverage was obtained from 1172/2085 (56.2%) of children aged ≤10 years and complete 3-dose vaccination was reported for 1141/1172 (97.4%). The estimated vaccine impact, calculated by comparison of children born 5 years prior to, and after introduction of the HBV vaccine was 95.9% (95% CI 70.6 -99.4). Factors associated with HBV infection included marital status, with an increased odds of HBV infection among separated or divorced

individuals, and males. Among individuals born before vaccine implementation, HBV prevalence increased from adolescence and peaked at 30-39 years, before falling again in older people. This may represent a cohort effect of falling transmission among successive generations due to changing risk factors for transmission or alternatively that adult HBV transmission is more important than previously recognised. The falling prevalence among older individuals may represent spontaneous HBsAg clearance or death from HBV, or epidemiologically associated diseases sharing risk factors from transmission such as HIV.

Among the 160 HBsAg positive individuals in the serosurvey, 150 were aged 16 years or older and 94 were located in the community and consented to clinical evaluation of treatment eligibility. A quarter of cases (26%) were HIV positive and two thirds (66.7%) of HIV positive individuals were on ART with HBV DNA suppression below 32 IU/ml. Among HIV negative individuals 3, 6 and 9% met eligibility criteria for antiviral therapy according to WHO, EASL and AASLD guidelines respectively.

This study showed that the HBV vaccine, delivered to infants at 6, 10 and 14 weeks of life, has been highly effective in Malawi with very low prevalence in children. It also showed that a high proportion of individuals were co-infected with HIV and were already receiving HBV-active antiretroviral therapy. Among HBV positive HIV negative individuals, based on a cross-sectional evaluation a small proportion required antiretroviral therapy. The appropriateness of these criteria for use in this setting, particularly in view of the origin of the derivation data from Western or Asian cohorts, and the substantially younger age of people with incident HCC in the region, remains a pressing research question. Potential research methodologies to tackle this question are discussed in section 8.2.

8.1.4 HCV community study

Among participants in the serosurvey, HCV prevalence was tested among a random selection of 1661 individuals aged ≥16 years from the census, as described in Chapter 6. Population standardised HCV Ag/Ab prevalence was 0.8% (95% CI 0.5 -1.4) and HCV RNA prevalence was 0.2% (95% CI 0.1 – 0.5). HCV Ag/Ab positive individuals were older but no differences in sex, education, employment or socioeconomic status were noted. Limited statistical power to ascertain specific risk factors was available due to lower than expected HCV prevalence.

Estimated HCV RNA prevalence for southern Africa was 0.7% (95 CI 0.4-0.9) from a recent systematic review and modelling study based on convenience sampling, and the authors did not identify and include any probability sampling community survey data. This new data from Malawi indicates lower

HCV RNA prevalence than previously estimated and demonstrates that in Malawi the HCV epidemic is concentrated. Additional sampling among specific population groups such as people who inject drugs, men who have sex with men, commercial sex workers, as well as individuals from other regions and rural areas of Malawi are required to better characterise the epidemiology of HCV and formulate recommendations for a targeted public health response.

8.1.5 Evaluation of HBeAg rapid diagnostic tests

A recently proposed simplified diagnostic algorithm for establishing eligibility for HBV treatment in sub-Saharan Africa is based on the use of ALT and HBeAg determination. The World Health Organisation recently listed rapid diagnostic tests as essential diagnostic in vitro devices that should be available to all tiers of the health system down to local health centres, in view of their utility for classification of HBV disease and to their potential to determine eligibility for HBV antiviral therapy in pregnant women for MTCT prevention. Three commercial HBeAg RDTs were evaluated on sera from community evaluation and hospital study HBsAg positive participants using a laboratory ELISA as the reference test (Chapter 7).

In community evaluation participants and hospital patients with HCC or cirrhosis who were HBsAg positive, the performance of HBeAg RDTs was poor, with sensitivity of 36-64% in community patients and 25-75% in hospital patients. Specificity was consistently good, exceeding 90% in each kit in both settings. The concentration of HBeAg was shown to correlate with the sensitivity of the test with the lower limit of detection varying from 2.2 to 3.1 $\log_{10}$ IU/ml for each kit. The sensitivity of the RDTs for detecting patients with HBV DNA >200,000 IU/ml, the threshold recommended for antiviral therapy to prevent MTCT, was 77% (95% CI 61, 89).

These findings highlight that existing commercial HBeAg RDTs have insufficient sensitivity for detection of HBeAg in Malawi and fail to adequately classify patients, or identify patients with high HBV DNA who could benefit from antiviral therapy. The poor sensitivity may be a result of the kits being developed and optimised for patients with a different HBV genotype and in a different clinical setting. The findings may act as a reminder that diagnostic tests must be evaluated in the population in which they will be used that reflects local epidemiology, population and viral genetic characteristics (Whiting et al., 2011). The main recommendation stemming from these results are that commonly used HBeAg RDTs cannot be currently recommended and alternative classification methods such as HBV DNA quantification or ELISA should be used in this setting.

8.2. Future research priorities

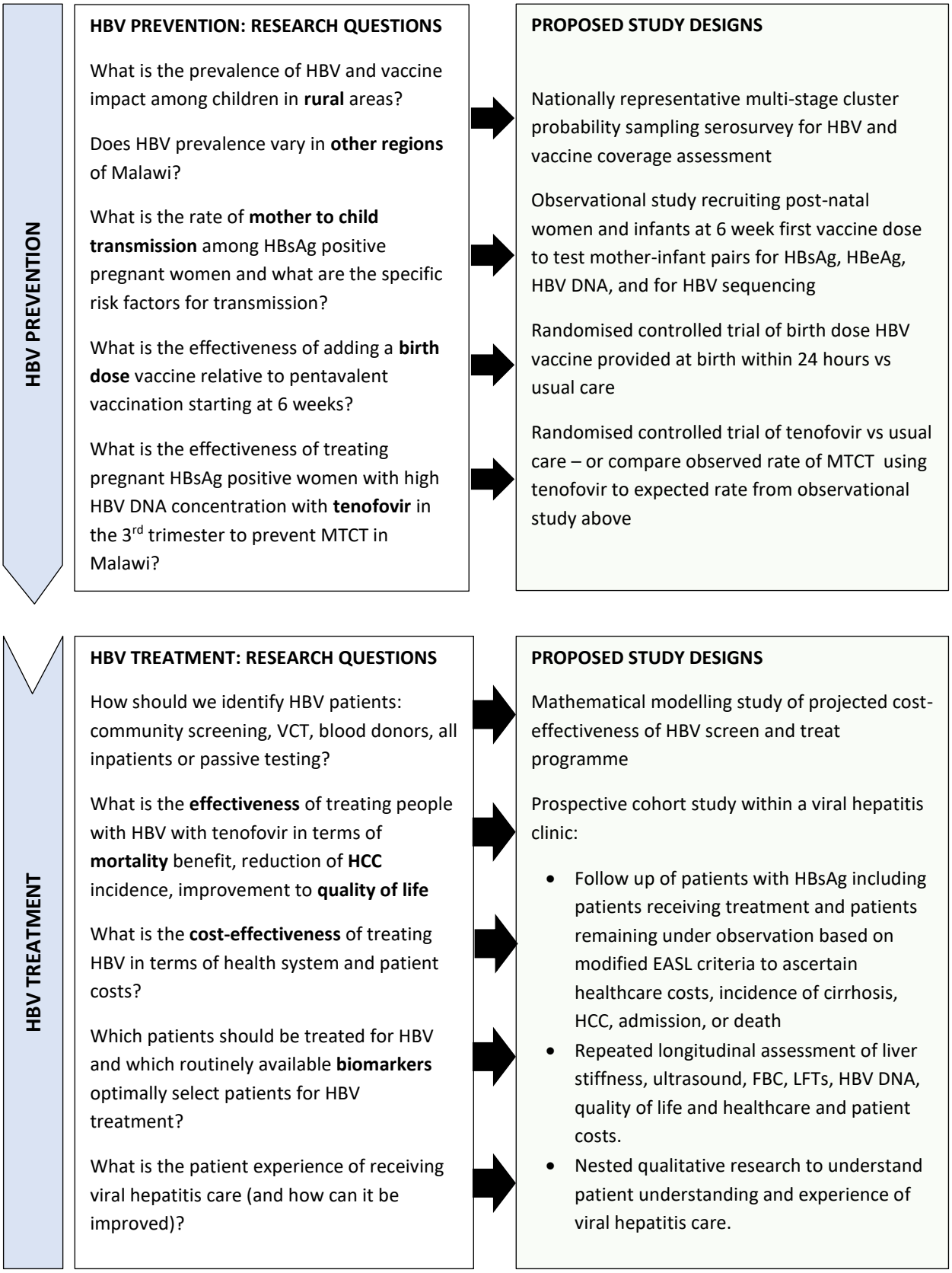
Based on the findings of the research presented in this thesis, I have formulated focused research questions that represent barriers to control of HBV and HCV associated liver disease in Malawi and attempted to identify optimal study designs to answer these questions (Figure 8-2). These questions are likely to be applicable not only to Malawi but regionally to neighbouring countries in Southern and Eastern Africa and elsewhere in sub-Saharan Africa.

8.2.1 Epidemiological data gaps for HBV

The HBV community survey observed peak HBsAg prevalence among men aged 30-39. The median age of onset of cirrhosis and HCC in the hospital cohort was 40 years. It is expected that the burden of cirrhosis and HCC will increase over the next decade as the population with peak HBV prevalence age and begin to develop HBV-associated liver disease, in keeping with findings from a recent mathematical modelling study (Nayagam et al., 2016b).

Remaining epidemiological data gaps for HBV in Malawi relate to the limitations of our serological study data. These data represent an urban township which experiences a high rate of inward migration from rural areas but is not likely to be fully representative of the population of Malawi. In particular, the vaccine coverage observed in this study at 97%, exceeds the national coverage of 92% for 2018 (World Health Organisation and United Nations Children's Fund (UNICEF), 2019). The township is served by a health centre, centrally located such that the population is no more than 2km from a vaccination centre. These factors limited the representativeness of the sample with respect to vaccine impact. To better understand HBV prevalence and vaccine efficacy nationally, a nationally representative sampling framework will be required, similar to the nationally representative HIV prevalence survey (Government of Malawi, 2018, Justman et al., 2018). Indeed, colleagues engaged in the national HIV prevalence study have recently secured funding to retest samples for HBsAg (Personal Communication, Dr Rabson Kachala, Malawi Ministry of Health) and this will be critical to understand regional variation in prevalence.

Figure 8-2: Summary of research questions relating to hepatitis B stemming from this research and proposed study designs to answer these questions



This thesis presents the first random probability community sampling study of HBV prevalence in Malawi and due to the single time point cross-sectional nature of the study, only pseudo-longitudinal interpretations can be made about a cohort effect with respect to changing exposure or risk behaviour over time, relative to the importance of adult transmission. A repeated survey will be required within the same population to better understand the change in risk, to add temporal resolution to interpretation of the age period cohort effect (Lee et al., 2009, Tu et al., 2012).

The hospital cohort study studied patients with cirrhosis and HCC at a single tertiary centre. To better understand the burden of disease associated with HBV and HCV, a study that is more representative of the health system should be recommended, with case ascertainment based on a multi-stage random probability sampling design to sample from lower tiers in the health system such as district and rural hospitals to obtain better estimates of disease incidence using standardised surveillance case definitions. This will also require quality control assessment and training of ultrasound capability to ensure case ascertainment is adequately standardised.

8.2.2 Which additional HBV prevention activities should be recommended in Malawi?

The World Health Organisation recommends implementation of a birth dose vaccine in sub-Saharan Africa (World Health Organization, 2019), but there are limited data on its efficacy in the region, with only one non-randomised observational study identified in a recent review (Nayagam et al., 2019). Similarly data on the efficacy for use of tenofovir among women with a high viral load are derived from Western and Asian cohort and the efficacy of an additional birth dose should be demonstrated in this setting (Jourdain et al., 2018, Chen et al., 2017).

To gather the data required to support introduction of additional MTCT interventions, in a low-income country such as Malawi, efficacy and cost-effectiveness must be demonstrated due to high rates of preventable mortality and a broad range of competing public health priorities. First, observational evidence should be sought to understand the natural history of MTCT by recruiting mothers and infants attending for the first 6-week vaccination, to understand rates of MTCT with use of the pentavalent vaccine alone to quantify the “vaccination gap” and to ascertain the risk factors for transmission including the maternal HBV DNA concentration at which additional interventions are required. To improve understanding of HBV transmission further, ideally a birth cohort would perform serial repeated testing of infants over time to better understand cumulative risk and the timing of HBV transmission in children, combining longitudinal observation and a traditional epidemiological risk factor-based analysis with phylogenetic and phylogeographic approaches to understand residual

transmission in the infant vaccination era. This would be a substantial undertaking requiring a large sample size and would ideally be performed within the context of a larger birth cohort to maximise the value of the research.

A randomised trial or an observational study to evaluate the effect of the birth dose HBV vaccine and tenofovir to prevent MTCT of HBV is recommended. In the latter study design, observed MTCT rates with use of the intervention should be compared with those expected based on prior observational evidence. An RCT would provide a higher standard of evidence but might be difficult to justify on ethical grounds, given that a network meta-analysis of data from Western and Asian studies showed clear evidence of efficacy relative to infant vaccination beginning at 6 weeks (Chen et al., 2017). Since tenofovir (TDF) is not currently recommended by the WHO, it would be more acceptable to use a RCT design to study the efficacy of TDF in this setting (World Health Organisation, 2015). An alternative approach would be to use a stepped-wedge design to monitor the effects of TDF introduction in a phased approach among randomly selected treatment clusters (Hemming et al., 2015). An additional intervention, use of hepatitis B immunoglobulin for passive immunoprophylaxis might be more difficult to study in view of the high intervention cost and limited scope for application outside of the context of research (Howell et al., 2014).

8.2.3 How should a HBV treatment programme be implemented in Malawi?

This research has demonstrated that Malawi has intermediate prevalence of HBV (5.1%) among unvaccinated adults. To begin to tackle the problem of cirrhosis and in particular HCC, a public health approach similar to that used for HIV will be needed (Nayagam et al., 2016b). As explored in this thesis, there are several opportunities to leverage the successes of the HIV programme in the process of implementation of an HBV antiviral programme (Figure 8-2).

Based on the community serosurvey, the 2018 national census, national HIV prevalence data, the results of our treatment eligibility assessment, and projected to the national population using a parametric bootstrapping approach, an estimated 25,586 (95% CI 7,172 – 65,519) individuals require HBV treatment in Malawi. This compares to an estimated 970,000 adults living with HIV of whom 810,000 are receiving ART (UNAIDS, 2018).

This study has identified several important research questions regarding implementation of a public health based HBV treatment programme. First, how should these HBV patients be identified? The first and easiest approach might be to accept referrals from patients referred from hospitals identified in

clinical settings as a result of routine or targeted testing. This strategy alone will be insufficient and too late for many patients given the advanced disease observed in the hospital study at the point of HBV diagnosis. A second tier might be to start with blood donors who experience donation refusal due to testing HBsAg positive. In the third successive grouping, HBV testing could be added to voluntary counselling and testing which is conducted for HIV in a decentralised fashion throughout the country and is based on use of point of care rapid diagnostic tests that could be amenable to the simultaneous testing of HBsAg (Kania et al., 2013). The fourth tier would be community outreach to promote testing with community engagement combined with testing drives to encourage widespread testing and to promote public awareness of HBV. In the Gambia the latter strategy was found to be cost-effective but with a lower HBV prevalence and a lower gross domestic product, the cost-effectiveness of community screen and treatment needs to be assessed in a model specific to Malawi (Nayagam et al., 2016a). In the inpatient and community studies with a combined sample size of 481 participants, only 8% and 5% of participants respectively had heard of HBV prior to involvement in the study.

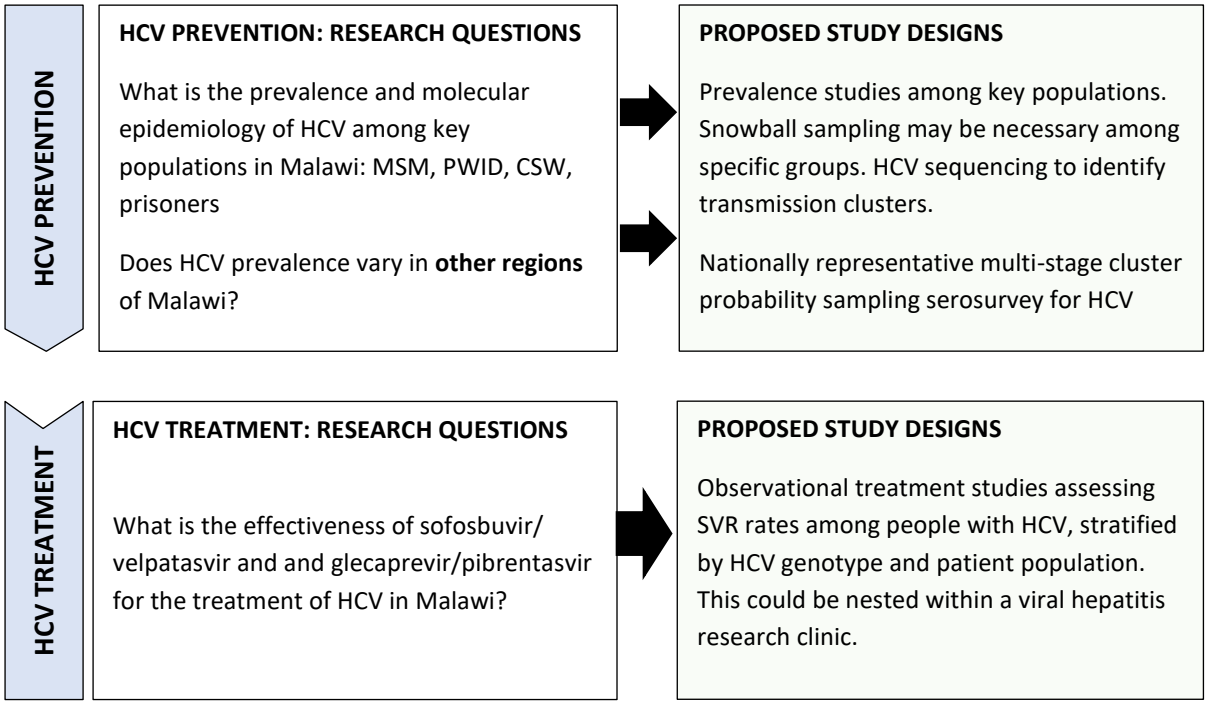
A number of creative methods of community engagement have been adopted in the promotion of health science messaging and this has been particularly observed for HIV where effective health communication has been associated with dramatic shifts in treatment uptake and destigmatisation (Vermund et al., 2017). In the community where this community study was conducted, a typhoid vaccine trial was conducted with implementation of an extensive science communication programme. This included engagement with community leaders and local chiefs acting as trusted information channels, attempting to identify and address misconceptions through community engagement meetings, use of a variety of different channels of communication such as music, art, social media, radio, and engagement with multiple relevant stakeholders such as government, charities, the private sector, religious leaders and community based organisations (Meiring et al., 2019). It is clear that significant work remains to increase public awareness and understanding of HBV and to empower citizens to press government to fulfil commitments to address viral hepatitis incidence and mortality (Spearman et al., 2017).

The second major question concerns how to optimally assess eligibility for HBV treatment. Current WHO guidelines are based on Western and Asian data, and data from this study together with existing evidence show that the eligibility criteria recommended by WHO are not suitable in sub-Saharan Africa, with poor sensitivity for diagnosis of cirrhosis, resulting in misclassification and denial of antiviral treatment both in the hospital and community cohorts to those who would benefit (Bonnard et al., 2010, Lemoine et al., 2016a, Lemoine et al., 2017, Johannessen et al., 2019, Desalegn et al., 2017). The GPR classification tool performed well in both cohorts for the classification of fibrosis,

particularly with a modified lower cut off threshold. Descriptive data of ultrasound findings from the hospital based cohort also showed the potential utility of ultrasound-based diagnostic criteria which could complement biomarker-based tools. This would benefit from external validation in other cohorts. Further work in this area would involve recruitment of a larger cohort to improve precision of diagnostic performance estimates and collaborative assessment of these tools together with other centres in sub-Saharan Africa.

The third key research question is to determine the effectiveness of a HBV treatment programme. This would involve ascertainment of outcomes on treatment, rates of HBV suppression, changes in liver stiffness and rates of incident cirrhosis and HCC. This would require comparison of the observed to the expected rate or comparison of treated and untreated populations. Currently, long term follow up data of an untreated HBV cohort are lacking for disease ascertainment and natural history data in sub-Saharan Africa, apart from a single study from the Gambia (Shimakawa et al., 2016a). For this purpose, a repeated survey of the clinical evaluation population would be useful, although the population size is small and ascertainment of incident HCC would be limited. Those who do not meet current eligibility for treatment should be monitored to assess outcomes for the purposes of optimising criteria to the local population and cost effectiveness of the programme should be assessed. This would involve use of mathematical modelling to compare programme costs with estimated health system costs of untreated HBV, and could support a case for programme implementation. Finally, patient-reported outcomes such as changes in quality of life should be evaluated, and a qualitative study on the understanding and experience of patients with HBV participating in a hepatitis treatment programme should be undertaken to better understand how to improve the care of HBV patients.

Figure 8-3: Summary of research questions relating to hepatitis C stemming from this research and proposed study designs to answer these questions



Abbreviations: HCV, hepatitis C virus; MSM, men who have sex with men; PWID, people who inject drugs; CSW, commercial sex workers; SVR, sustained virological response

8.2.4 Data gaps for HCV

In this thesis, the prevalence of HCV was estimated in a community prevalence study using random probability sampling for the first time in Southern Africa (Chapter 6) and revealed a lower than expected prevalence based on previous modelling data (Polaris Observatory, 2017). The community HCV prevalence study both in a whole-population and case-control analysis was unable to identify specific risk factors for infection due to limited power, although HCV RNA positive individuals were significantly older, potentially pointing towards a historical and changing transmission source such as nosocomial infection, and a trend was observed towards sexual risk factors. Future work should aim to study HCV prevalence among specific populations such as commercial sex workers, men who have sex with men, people who inject drugs, prisoners to identify population groups important in the transmission of HCV to optimise case finding and to facilitate a future HCV treatment study (Figure 8-3). Because SVR is associated with HCV cure, and spontaneous HCV clearance is uncommon, HCV treatment trials do not require a control group. The study also demonstrated that among the first

length HCV sequences from Malawi and among the few available from sub-Saharan Africa(Niebel et al., 2017), that genotypes 4r, 4w and 4v were most common. Among these subtypes, 4r has been associated with poor response to sofosbuvir/ ledipasvir with evidence of intrinsic resistance and NS5A substitutions associated with NS5A inhibitor resistance at positions 28, 30 and 31 were frequently identified (Gupta et al., 2019). In light of poor SVR rates observed with genotype 4r and limited data on outcomes of treatment with other genotype 4 subgroups, a study of HCV treatment using alternative WHO recommended pangenotypic regimens such as sofosbuvir/velpatasvir and glecaprevir/pibrentasvir is warranted in Malawi (World Health Organisation, 2018).

8.3. Conclusion

There are currently unprecedented opportunities to prevent HBV transmission and to implement treatment programmes for HBV and HCV in Malawi to reduce viral hepatitis incidence and mortality in line with WHO targets, which the Government of Malawi have endorsed. Although the data in this thesis have indicated a substantial burden of liver-related disease associated with viral hepatitis, particularly hepatitis B, this also presents an opportunity for implementation of treatment programmes. Together with colleagues and collaborators, I aim to continue to build upon the findings in this thesis, to improve understanding of HBV and HCV transmission and to translate these findings into policies and services that will reduce the burden of disease and mortality among the current generation of young adults in Malawi and Southern Africa.

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Appendix 1: Ethical permission from the National Health Sciences Research Ethics Committee of Malawi

Telephone: + 265 789 400
Facsimile: + 265 789 431

All Communications should be addressed to:

The Secretary for Health and Population

In reply please quote No.

.....

MINISTRY OF HEALTH AND POPULATION
P.O. BOX 30377
LILONGWE 3
MALAWI

18th April, 2017

Alexander Stockdale
MLW
Blantyre

Dear Sir,

RE: PROTOCOL # 16/11/1698: HEPATITIS B IN MALAWI: DETERMINANTS OF FIBROSIS AND VIROLOGICAL EXPRESSION (HEP-FIVE)

Thank you for the above titled proposal that you submitted to the National Health Sciences Research Committee (NHSRC) for review. Please be advised that the NHSRC has **reviewed** and **approved** your application to conduct the above titled study.

- **APPROVAL NUMBER : 1698**
- The above details should be used on all correspondences, consent forms and documents as appropriate.
- **APPROVAL DATE : 18/04/2017**
- **EXPIRATION DATE**
  This approval expires on **17/04/2018**. After this date, this project may only continue upon renewal. For purposes of renewal, a progress report on a standard form obtainable from the NHSRC Secretariat should be submitted one month before the expiration date for continuing review.
- **SERIOUS ADVERSE EVENT REPORTING:** All serious problems having to do with subject safety must be reported to the NHSRC within 10 working days using standard forms obtainable from the NHSRC Secretariat.
- **MODIFICATIONS**: Prior NHSRC approval using forms obtainable from the NHSRC Secretariat is required before implementing any changes in the protocol (including changes in the consent documents). You may not use any other consent documents besides those approved by the NHSRC.
- **TERMINATION OF STUDY**: On termination of a study, a report has to be submitted to the NHSRC using standard forms obtainable from the NHSRC Secretariat.
- **QUESTIONS**: Please contact the NHSRC on phone number *+265 888 344 443* or by email on mohdoccentre@gmail.com.
- **OTHER**: Please be reminded to send in copies of your final research results for our records (Health Research Database).

SECRETARY FOR HEALTH
2017 -04- 18
P.O BOX 30377, CAPITAL CITY, LILONGWE 3

Kind regards from the NHSRC Secretariat.

.....
For: **CHAIRPERSON, NATIONAL HEALTH SCIENCES RESEARCH COMMITTEE**
Promoting Ethical Conduct of Research[1]

Executive Committee: *Dr B. Chilima (Chairperson), Dr B. Ngwira (Vice-Chairperson)*
Registered with the USA Office for Human Research Protections (OHRP) as an International IRBIRB Number IRB00003905 FWA00005976

Appendix 2: Ethical permission from the University of Liverpool Research Ethics Committee

Research Integrity and Ethics

18 May 2017

Dear Professor Gordon,

I am pleased to inform you that your application for research ethics approval has been approved. Details and conditions of the approval can be found below:

Reference:	1954
Project Title:	Hepatitis B in Malawi (HEP-FIVE)
Principal Investigator/Supervisor:	Professor Melita Gordon
Co-Investigator(s):	Dr Alexander Stockdale, Professor Anna Geretti
Lead Student Investigator:	-
Department:	Clinical Infection, Microbiology and Immunology
Reviewers:	Professor Graham Kemp
Approval Date:	18/05/2017
Approval Expiry Date:	Five years from the approval date listed above

The application was **APPROVED** subject to the following conditions:

Conditions

- All serious adverse events must be reported via the Research Integrity and Ethics Team (ethics@liverpool.ac.uk) within 24 hours of their occurrence.
- If you wish to extend the duration of the study beyond the research ethics approval expiry date listed above, a new application should be submitted.
- If you wish to make an amendment to the research, please create and submit an amendment form using the research ethics system.
- If the named Principal Investigator or Supervisor leaves the employment of the University during the course of this approval, the approval will lapse. Therefore it will be necessary to create and submit an amendment form using the research ethics system.
- It is the responsibility of the Principal Investigator/Supervisor to inform all the investigators of the terms of the approval.

Kind regards,

Research Integrity and Ethics

794-8290

Appendix 3: Participant consent forms

HEP-FIVE Document 13: Consent Form (English)

Hepatitis B in Malawi

Hepatitis B in Malawi: Determinants of Fibrosis and Virological expression

Consent Form

Study ID No:

		Initial or mark
1	I have read the information or had the information read to me	
2	I have been given a copy of the information to keep	
3	I also had a chance to discuss with study staff	
4	I understand that I am free to withdraw from this study at any time without giving a reason and this will not affect normal medical care	
5	I agree that the information collected about me will be shared with other researchers and used for future medical research	
6	I agree that genetic tests on my blood and saliva samples can be performed	**Y / N**
7	I agree for my blood and saliva samples to be stored and also shipped to researchers overseas for up to 10 years	**Y / N**
8	I agree to have an HIV test done	**Y / N**
9	I would like to know the result of the HIV test	**Y / N**
10	I agree that you may contact me again in future for a follow up study	**Y / N**
11	Female participants: I agree to have a pregnancy test	**Y / N**
12	I voluntarily agree to take part in this study	**Y / N**

Signature of participant, parent or guardian:

Signature/Thumbprint	Full name:	Signed Date:
x_____	x_____	____/____/___

I, the undersigned, have fully explained the relevant information of this study to the person named above and will provide her/him with a copy of this signed and dated informed consent form.

Signature of the study staff	Full Name	Signed Date
x_____	x_____	___/____/_____

If the person giving consent cannot read the form themselves, a witness must be present and sign here: I was present throughout the entire informed consent process with the participant. This form was read accurately to the participant, all questions from the participant were answered and the participant has agreed to take part in the research.

Witness Signature	Full Name	Signed Date
x_____	x_____	___/____/_____