

Study title:

Liver fibrosis in Zambian HIV-HBV co-infected patients: a long-term prospective cohort study

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Correspondence:

Dr. Michael J. Vinikoor
Centre for Infectious Disease Research in Zambia
University of Zambia School of Medicine
University of Alabama at Birmingham
5032 Great North Road
Lusaka, Zambia
mjv3@uab.edu
+260 972921285

Collaborating Institutions:

Centre for Infectious Diseases Research in Zambia (CIDRZ)
Tropical Gastroenterology and Nutrition, Department of Medicine, University of Zambia (UNZA)
University of Alabama at Birmingham (UAB)
University of Bern, Switzerland

UAB Institutional Review Board protocol #: F150819001

Key study personnel:

Principal Investigator

Dr. Michael J. Vinikoor
Investigator, Centre for Infectious Disease Research in Zambia
Honorary Lecturer, University of Zambia (UNZA)
Assistant Professor of Medicine, University of Alabama at Birmingham
mjv3@uab.edu
+260 972921285

Local Co-Investigators

Ms. Belinda Chihota
CIDRZ PhD Fellow
Epidemiology
belinda.chihota@cidrz.org
+260 964450052

Dr. Paul Kelly
Consultant Physician and Hon. Lecturer
TROPAN group, UNZA
m.p.kelly@qmul.ac.uk
+260 955295493

Dr. Samuel Bosomprah
Biostatistician and Head of Analysis, CIDRZ
Honorary Lecturer, UNZA
lbulenga@yahoo.com
+260 977344604

Dr. Edford Sinkala
Consultant Physician and Hon. Lecturer
Researcher TROPAN group
sinkalaeddie@yahoo.com
+260 955884088

Dr. Izukanji Sikazwe
CIDRZ Deputy Chief Executive Officer
Infectious Diseases Specialist
izukanji.sikazwe@cidrz.org
+260 977233829

International Co-Investigators and Advisors

Dr. Debika Bhattacharya
Associate Professor of Medicine
Univ. California at Los Angeles
debikab@mednet.ucla.edu
+1 408 838 9075

Dr. Gilles Wandeler
Attending physician
leDEA-Southern Africa
University of Bern, Switzerland
gwandeler@ispm.unibe.ch
+41 787 758 533

Dr. Michael Saag
UAB Professor of Medicine
Director, UAB Center for AIDS Research
msaag@uab.edu
+1 205934432

Dr. Lloyd Mulenga
Head, Adult Infectious Diseases Clinic
University Teaching Hospital
lbulenga@yahoo.com
+260 977 344 604

Abbreviations/Acronyms:

ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
APRI	AST-to-platelet ratio index
ART	Antiretroviral therapy
AUDIT	Alcohol Use Disorders Identification Test
BREC	Biomedical research ethics committee
cccDNA	Covalently closed circular DNA
CIDRZ	Centre for Infectious Disease Research in Zambia
EtG	Ethyl glucuronide
HBcrAg	Hepatitis B core-related antigen
HBeAg	Hepatitis B envelope antigen
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HBV DNA	Hepatitis B viral load
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HDV	Hepatitis delta virus
HIV	Human immunodeficiency virus
ICF	Informed consent form
leDEA-SA	International Epidemiology Databases to Evaluate AIDS-Southern Africa
IRB	Institutional review board
LSM	Liver stiffness measurement
MOH	Ministry of Health
Peth	Phosphatidylethanol
SAE	Serious adverse event
SSA	Sub-Saharan Africa
TE	Transient elastography
UAB	University of Alabama at Birmingham
UNC	University of North Carolina
UNZA	University of Zambia

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PART 1: BACKGROUND AND INTRODUCTION

Among HIV-infected individuals in Africa, liver disease is a neglected area of investigation but is anticipated to become increasingly common as patients live longer due to antiretroviral therapy (ART) (1). In a currently approved and active research study taking place in Lusaka Urban district – *HIV/HBV co-infection in leDEA-SA* (#010-08-12, UNZA BREC; NCT02060162, clinicaltrials.gov) – 800 consecutive HIV-infected adults were screened for possible causes of liver disease and 12% were diagnosed with chronic hepatitis B virus (HBV) co-infection. HBV co-infected patients were more likely to have evidence of liver disease; however, ascertainment of critical long-term outcomes of HIV-HBV patients was not part of the study. Hazardous alcohol consumption, another risk factor for liver disease, was also highly prevalent in this cohort (2). Building on these preliminary results and addressing the need to study HIV-HBV during a longer duration of follow-up, the current protocol will focus exclusively on Zambian HIV-HBV patients. In the proposed study, we will determine the risk factors for liver fibrosis among HIV-HBV co-infected patients in Lusaka, Zambia, and assess the long-term effectiveness of MOH-recommended antiretroviral drugs in the prevention and/or reduction of HIV-HBV liver disease. During ART for HIV-HBV, we have also noted a relatively high rate of surface antigen clearance, which is referred to as HBV ‘functional cure’. The predictors and durability of this response needs to be assessed during long-term follow-up. This study will provide useful clinical and epidemiologic information to health policymakers in Zambia and throughout the region and may lead to improvements in the care of HIV-HBV patients. Given the growing interest in designing immune modulating drugs for HBV functional cure, data on this outcome in HIV-HBV patients will be of global impact. Finally, this study will strengthen collaboration between Zambian institutions and strengthen local capacity in HBV diagnosis and management.

Rationale for the study:

An estimated 14% of Zambian adults are HIV-infected (3), of which approximately 10% are co-infected with HBV (4, 5). This translates to 1-2% adult prevalence of HIV-HBV, or among 1 million adults in Lusaka, approximately 10,000-20,000 are dually infected. Despite the burden of HIV-HBV in the country, there are scarce data on the disease or its outcomes. In upper-income settings, ART can control both HIV and HBV simultaneously, reducing liver-related mortality (6, 7). But in Africa, where HBV transmission occurs in early childhood, and environmental and genetic factors are diverse from upper income settings (8-10), the long-term outcomes of HIV-HBV need to be established. The rationale for this study is to fill the knowledge gaps around HIV-HBV in Zambia by recruiting a relatively large sample of patients and following them as they receive ART. Zambia is an ideal site for this research study because of its burden of HIV-HBV, and its capacity for this type of research, made possible by a local team of infectious disease, hepatology, and public health investigators who have the expertise and commitment to tackle this public health problem.

Significance:

Approximately 3 million HIV-infected individuals in Africa have chronic HBV co-infection (11) and are at risk for increased incidence of serum transaminase elevation (12), reduced CD4+ count recovery (13, 14), and increased mortality during ART (13). Most of the available data on HIV-HBV in Africa, including one study from Zambia, were generated from retrospective use specimens from HIV cohorts (5); therefore, liver fibrosis and liver-related end-points – critical intermediary outcomes in the natural history of the disease – were not assessed.

Prospective, longitudinal evaluation of liver fibrosis and its co-factors in HIV-HBV patients (HBV and

HIV viremia, inflammation, and immune recovery during ART) is urgently needed in SSA to better inform and optimize care for these high-risk patients (15). The timing of HBV transmission, impact of environmental exposures, and burden of other hepatotoxic co-infections are diverse in SSA compared to upper-income settings from where the vast majority of HIV-HBV treatment outcome data is currently derived. Patterns of alcohol use – an important fibrosis co-factor in upper-income settings – have not yet been characterized in African HIV-infected populations (16). As proposed in this application, a large prospective HIV-HBV cohort study in Zambia with careful evaluation of liver fibrosis would fill a critical void in the epidemiology and clinical outcomes of HIV-HBV, and could immediately inform regional and international health policy. Our results will also provide a solid epidemiologic framework for future interventional trials aimed at optimizing the care of these patients.

Aims:

- #1 To determine the prevalence of significant levels of liver fibrosis in HIV-HBV patients in Zambia using non-invasive tests.
- #2 To identify the predictors of significant fibrosis among HIV-HBV patients
- #3 To assess the impact of ART on the progression of liver fibrosis in HIV-HBV patients in Zambia
- #4 To determine the rate and predictors of HBsAg seroclearance in ART-treated HIV-HBV

Specific objectives:

- #1 Measure liver fibrosis using APRI, FIB-4, and transient elastography (TE)
- #2 Compare low-cost blood markers (APRI and FIB-4) to TE for the diagnosis of significant liver fibrosis and cirrhosis
- #3 Identify risk factors for liver disease including demographics, HIV immunologic and virologic characteristics, hazardous alcohol consumption, and HBV virologic characteristics
- #4 Determine the incidence and prevalence of liver cancer in HIV-HBV patients
- #5 Assess changes in liver fibrosis during ART
- #6 Identify factors associated with liver fibrosis regression during ART
- #7 Assess underreporting of hazardous alcohol consumption among HIV-HBV patients

Ethical issues:

The research team implementing this study will have training in the protection of human subjects and in good clinical practices. The protocol will undergo initial ethical review at a Zambian institutional review board (IRB). After the study is approved and initiated, ongoing study reports will be sent to the IRB according to guidelines, and on a yearly basis we will seek renewal of ethical approval. Full details of our plans to conduct this study in line with local and international standards of bioethics are described in the Methodology section.

PART TWO: LITERATURE REVIEW

Chronic HBV is characterized by the establishment of a genomic reservoir in host hepatocytes, and clinically defined by the persistence of HBV viremia or hepatitis B surface antigen (HBsAg) for 6+ months(17). Chronic untreated HBV infection is associated with progressive liver fibrosis, the development of hepatocellular carcinoma, and liver-related mortality. While not all patients with chronic HBV infection develop complications, 15-40% progress to cirrhosis, liver failure, or hepatocellular carcinoma (17-20). Assessment of liver fibrosis is an important part of the management of chronic HBV

as it determines which patients need treatment and when treatment should be initiated.

HIV co-infection accelerates the natural history of HBV-induced liver disease (21). HIV co-infection causes immune dysfunction and inflammation that accelerates HBV-related fibrosis progression. In addition, HIV directly infects hepatocytes (19). Although the mechanisms through which HIV infection accelerates hepatic fibrogenesis are poorly understood, HIV-HBV co-infected patients in upper-income settings have 19 times the risk of liver-related mortality compared with those with chronic HBV alone (22). World Health Organization and Zambian MOH guidelines recommend screening all HIV-infected individuals for chronic HBV co-infection using a HBsAg test, and initiation of ART in any HIV-HBV patient found to have severe liver disease regardless of CD4 count (23, 24).

There are limited data on HIV-HBV in SSA in part due to limited access to HBV assays and to tests for hepatic fibrosis. Liver biopsy – the gold standard test for hepatic fibrosis – is only available in tertiary care hospitals, requires extensive training to perform and a pathologist to interpret, and rarely results in major complications. Liver biopsy is also prone to sampling error that can lead to over- or under-staging of liver disease.

In the past decade, non-invasive fibrosis tests, including direct and indirect blood biomarkers and imaging techniques have been developed and validated which may reduce to the need for liver biopsy in the assessment of liver fibrosis. FIB-4 and AST-to-platelet ratio index (APRI) are two low cost indirect blood biomarkers that predict liver fibrosis stage, particularly at either end of the spectrum (25, 26). TE, an imaging modality that measures liver stiffness, is quick and painless, highly acceptable to patients, and produces immediate results that can guide treatment decisions(27). Liver stiffness measurements (LSM) correlate highly with fibrosis stage, and TE has been validated against liver biopsy in HBV, HCV, and other chronic liver diseases. TE has been used previously in Africa, including Nigeria, Burkina Faso, and Uganda (9, 28, 29).

Suppression of HBV may lead to reversal of liver fibrosis in upper-income settings as evidenced by a recent landmark study published in the *Lancet* (30). Among 96 HBV mono-infected patients with cirrhosis at baseline, 71 (74%) experienced regression of cirrhosis during an average of 4 years of treatment (30). In SSA where transmission, environmental, and genetic factors are diverse, the outcomes of HIV-HBV treatment may differ. In SSA, most HBV infections are acquired in early childhood unlike upper-income countries where most are percutaneous or sexually-transmitted in adulthood. Schistosomiasis, tuberculosis therapy, and the ingestion of aflatoxins or certain herbal medicines are potential causes of liver disease that are more common in SSA. Alcohol affects adherence to HIV treatment and is a potential co-factor for liver fibrogenesis in HIV-HBV but has been investigated in few studies in Africa (31, 32), in part because measuring drinking can be difficult using standard screening tests (33). Finally, incompletely understood genetic factors may affect hepatic fibrogenesis (8).

Our understanding of the HBV natural history has improved in recent years due to better characterization of the replication and persistence of the HBV covalently closed circular DNA (cccDNA) in the infected liver cell nucleus.(34) cccDNA can only be directly measured through liver biopsy and even with biopsy tissue, procedures are not standardized. A number of new biomarkers have been developed that can be measured in peripheral blood and appear to be good surrogates of cccDNA transcription and the host immune response to HBV.(35, 36) These markers may be able to predict liver fibrosis and cancer end points. In Asia, levels of hepatitis B core-related antigen was associated with the risk of developing HCC, independent from other factors.(37, 38) At the same time, new drugs are

being developed for HBV functional or complete cure, which can reverse or prevent liver disease.(39)
Some of these biomarkers may become end-points for novel therapies since current end-points, the ALT and HBV DNA, are less usefully once a person is on therapy.(40)

PART THREE: METHODOLOGY

Study design:

In this study, a prospective observational cohort design will be used.

Study population:

The study will take place in Lusaka Urban district where there is large burden of both HIV and HBV. We will identify potential participants at public-sector HIV care facilities in the district.

Inclusion criteria:

- (1) Age 18 years or older
- (2) HIV-infected
- (3) HBV-infected, defined as any single positive HBsAg assay
- (4) Naïve to antiretroviral therapy or currently participating in *HIV/HBV co-infection in leDEA-SA*

Exclusion criteria:

- (1) Unable or unwilling to provide informed consent
- (2) Planning to relocate out of Lusaka district

Sample size:

We aim to enroll 400 HIV-HBV patients into a prospective observational cohort study. Of note, we will recruit HIV-HBV patients who are currently participating in *HIV/HBV co-infection in leDEA-SA* to co-enroll in this study (currently 105 HIV-HBV patients are participating). Our target sample size is justified as follows. The prevalence of significant liver fibrosis among HIV-HBV patients ranged from 20-40% in published studies in Europe and the US (41, 42). We aim to determine the predictors of significant fibrosis; therefore, small differences in the prevalence of each potential predictor will be sought. To detect a 7-10% difference in the proportion with fibrosis, with $\geq 80\%$ power (assuming two-tailed alpha 0.05), we estimate the need for a sample size of 400 participants. This sample size will also provide adequate power to evaluate changes in the proportion of patients with significant fibrosis before and after treatment and to identify the variables that are associated with fibrosis, assuming 70% retention.

Study visits:

Study visits will take place every 6 months for up to 10 years. Long-term follow-up is justified because the development of liver fibrosis and cirrhosis can take years to become apparent. Plus, serological endpoints like loss of surface antigen (now called a 'functional cure') and liver cancer are rarely ascertained in shorter duration of follow-up. If possible, these visits will take place at the same time as scheduled ART follow-up visits. At enrollment, informed consent will be obtained and a physical examination will take place. A summary of visit procedures and data that will be collected is listed below.

Schedule of study investigations and data collected:

Following enrollment, participants will be followed for up to 10 years, in order to ascertain their long-term outcomes of antiretroviral treatment. The following table lists the planned investigations throughout the study.

Table 1. Schedule of study visits and investigations																					
	Study visit month																				
	0	6	12	18	24	30	36	42	48	54	60	66	72	78	84	90	96	102	108	114	120
Socio-demographic data ^a	X																				
HIV treatment data ^b	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical examination	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
ART monitoring laboratory tests per MOH guidelines ^c	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
ART adherence ^d		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Urine spot protein and creatinine ratio	X		X		X		X		X		X		X		X		X		X		X
AUDIT-C (43, 44)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Alcohol biomarker (urine ethyl glucuronide)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Diabetes screening (fasting glucose, HBA1C)	X		X		X		X		X		X		X		X		X		X		X
Quantitative HBsAg	X	X	X		X		X		X		X		X		X		X		X		X
HBV DNA	X		X		X		X		X		X		X		X		X		X		X
HBV sequencing	X																				
HCV antibody	X																				
Schistosoma antibody	X																				
HDV antibody	X																				
Surrogate biomarkers of cccDNA (HBV RNA, core-related/specific antigen)	X	X	X		X		X		X		X		X		X		X		X		X
HBV serological markers (surface, core, and e antigen and antibody)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
APRI and FIB-4	X		X		X		X		X		X		X		X		X		X		X
Liver stiffness ^e	X		X		X		X		X		X		X		X		X		X		X
Liver ultrasound for HCC	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Alpha fetoprotein	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

^aIncludes age, sex, and socioeconomic status; ^bIncludes dates of HIV diagnosis, enrollment in an HIV clinic, and ART initiation, the regimen type, WHO stage, and ART drug regimen; ^cAdherence will be assessed by self-report and pharmacy refill records (45); ^dMOH guidelines recommend complete blood count, creatinine, ALT, HBsAg, CD4 count and urinalysis at ART initiation and creatinine, and ALT every 6 months thereafter; ^eLiver stiffness measurements will be delayed until 6 weeks post-partum for any pregnant patients

Specimen collection, storage, and exportation:

Blood specimens will be collected during this study for both routine MOH-recommended monitoring of ART and for study-specific investigations (see Table 1). Only trained and experienced clinicians and/or

nurses will perform phlebotomy, and sterile technique will be used at all times to reduce the risk of complications. Routine tests such as CD4 count and ALT will be performed at primary healthcare facilities whenever possible. When necessary blood samples will be transported to a reference laboratory for testing. Blood samples may be stored temporarily for batch testing or for quality control purposes, but no samples will be stored after the end of the study. Local capacity for HBV diagnostic testing will be strengthened through this study, and our intention is to perform all blood testing in-country. In the current protocol, we propose several new assays (HBV genotyping/resistance testing, HDV antibody, urine EtG, surrogate biomarkers of cccDNA and serological markers of the HBV immune response; see Table 1) that have not been validated in Zambia. As part of the informed consent form, we will request participants' voluntary permission to ship up to 5ml of their blood abroad for testing only if we cannot establish and validate these new assays in-country. If necessary, the samples would be shipped to a collaborating laboratory at the following address: Dr. Gavin Cloherty, Head of Infectious Disease Research, Abbott Diagnostics, 11 Abbott Park Road, Abbott Park, Illinois, USA, Zipcode 60064. Prior to shipping any specimen abroad, we will also seek a material transfer agreement from the National Health Research Authority at MOH according to Zambian National Health Research Bill (46).

Measurement of liver stiffness using TE:

TE is a relatively novel non-invasive technique to measure liver stiffness and to reduce the need for liver biopsy. The test is highly acceptable to patients because it takes less than 5 minutes to perform, is painless, and results are available immediately (27, 47). Two Zambian nurses and two physicians have been trained to perform the test. TE is relatively contraindicated in pregnancy because of the manufacturer's lack of experience using the device in that patient population. Pregnant women will be allowed to participate in this study but we will not perform TE during pregnancy. For pregnant participants, we will delay scheduled TE until at least 6 weeks post-partum.

Data analysis:

Participant characteristics including demographics (age, sex), HIV-related characteristics (WHO clinical disease stage, CD4+ T-cell count), HBV-related characteristics (HBV viral load, HBeAg, and serum aminotransferases), APRI and FIB-4, and alcohol use will be analyzed using descriptive statistics. Alcohol use disorders will be investigated with the 3-question version of the World Health Organization's Alcohol Use Disorders Identification Test (AUDIT-C), which has been validated in resource-constrained settings (43, 44). In addition, we will measure alcohol consumption using a biomarker, urine ETG (33, 48, 49). We will assess underreporting on the AUDIT-C as those who reported no/moderate recent drinking and have detectable levels of the two alcohol markers. We will analyze laboratory and clinical variables as both continuous and categorical data using accepted cut-offs. We will calculate the prevalence of significant fibrosis as the number of patients with LSM >7 kPa divided by the total number of participants. Using log linear models, we will assess the bivariable and multivariable relative risk of significant fibrosis for potential co-factors. Multivariable models will be created including possible correlates of fibrosis based on the literature and based on having a P-value <0.01 in bivariable analysis. This model will then be reduced until only factors associated with the outcome at a P-value <0.05 remain. These factors will be considered independent predictors of having significant fibrosis. To assess the proportion with significant fibrosis during ART, we will use a multivariable logistic regression model, with generalized estimating equations to account for within-subject correlation as a result of repeated measures. Covariates in the model will include HIV and HBV viral loads, CD4+ T-cell count, adherence (categorized according to established thresholds), and AUDIT-C result. Secondary outcomes from the cohort study will include changes in APRI and FIB-4, conversion from HBeAg positive to negative, normalization of serum aminotransferases, and HIV

outcomes (change in CD4+ T-cell count and HIV viral suppression). Additional liver outcomes, including the incidence rate of hepatocellular carcinoma, hepatic decompensations, hepatic encephalopathy, and esophageal varices will be reported descriptively. The incidence of HBV functional cure will be the number of patients experiencing confirmed loss of HBsAg divided by person-time. Using a multivariable piecewise exponential survival model we will identify predictors of functional cure.

Local capacity building:

This study will build capacity in HBV diagnosis and management. Blood samples for HBV tests that have been historically shipped abroad (5) will be tested in local laboratories. This study will provide training to a Masters in Epidemiology student at UNZA (Ms. Kalo Musukuma, co-investigator). The student will propose an independent study that builds on this study, and will submit a separate protocol to UNZA BREC. Finally, implementation of this study will build local clinical and epidemiology experience in the area of HBV across Zambian institutions, providing a foundation for future research and health programs in this area.

Ethical issues:

We will require study participants to provide written informed consent. We will make informed consent forms (ICFs) available in English and in the two most commonly spoken Zambian languages in Lusaka. In the participant's chosen language, we will read the ICF out loud to participants, after which time the participant will be given adequate time to ask questions or ask for clarifications. Then, if willing to participate in the study, the participant will be asked to sign the ICF. Participants who are unable to physically sign their name and/or are illiterate will be allowed to thumbprint the ICF instead of signing. If the participant is providing a thumbprint, an impartial witness will be present during the entire consent procedure and will co-sign the ICF.

Participants will be provided a transportation reimbursement of K50.00 per study visit, in line with local standards. If necessitated by increasing cost of transportation in Lusaka, this amount may be increased following communication with and approval from the IRB.

We will maintain strict participant confidentiality in various ways during the study. We will collect data using an identification number that is only linkable to the patient's name, phone number, and address using a linkage file. Files will be maintained in a locked filing cabinet in a locked office and the linkage file will be locked separately. Only a member of the study team will keep the keys and be able to open the file cabinets containing participant records. Access to the files will be restricted to members of the study team. If necessary, the IRB, MOH, or other regulatory body will be granted access to study files. At the end of the study, linkage files and personal identifying information will be destroyed, leaving only de-identified data for ongoing analysis and dissemination.

Patients will receive clinical care and treatment according to MOH guidelines. Antiretroviral drugs will be free of charge and dispensed by public-sector clinics in Lusaka district. Throughout their participation in the study, patients will have access to study physicians (including infectious diseases and liver disease specialists) for consultations and referrals. A study team member will be available to participants by phone at all times. We will refer patients who require hospitalization or additional medical attention to University Teaching Hospital where key study personnel can ensure that optimal care is delivered. During this observational study, we will ascertain all serious adverse events (SAEs), defined as deaths, hospitalizations, or serious medical conditions that result in major disability. SAEs will be reported to the IRB on an annual basis. Unanticipated problems, defined as unexpected medical

events that are possibly related to participation in the research and suggest that other participants may also be at greater risk of harm, will be reported to the IRB within 7 days.

PART FOUR: BUDGET

The below table summarizes the budget for the study, stratified by category.

Table 2. Budget summary		
	Zambian Kwacha	US dollars
Personnel	900,000	150,000
Supplies and materials	240,000	40,000
Travel	60,000	10,000
Other	180,000	30,000
Indirect costs	120,000	20,000
Totals	1,500,000	250,000

PART FIVE: TIME FRAME

Once ethical approval has been obtained, the protocol will be submitted to the Ministry of Health for authorization. Once authorization to conduct the research is granted, sensitization will take place at the district and primary health center level, and with community leaders and stakeholders. Below we list our tentative time frame for the study activities.

Table 3. Study Timeline						
	2014-2015	2016-2017	2018-2019	2020-2021	2022-2023	2024-2025
Protocol development	X					
Ethical approval and government authorization	X					
Staff hiring and training	X					
Sensitization	X					
Enrollment	X	X				
Follow-up visits		X	X	X	X	X
Data analysis and dissemination		X	X	X	X	X

PART SIX: REFERENCE LIST

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PART SEVEN: APPENDICES

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