THE CORRELATION BETWEEN BASELINE SERUM HEPATITIS B SURFACE ANTIGEN LEVEL AND LEVELS OF OTHER BASELINE MARKERS OF VIRAL ACTIVITY IN PATIENTS WITH CHRONIC HEPATITIS B VIRAL INFECTION AT THE UNIVERSITY TEACHING HOSPITAL

BY

BRIGHT NSOKOLO

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Declaration

I declare that this dissertation represents my own work and that it has not previously been submitted for a degree, diploma or other qualifications at this or another university.

Signed……………………… Date………………………..

Candidate

Approval

This dissertation of **Bright Nsokolo** is approved as fulfilling the requirement for the award of the Master of Medicine in Internal Medicine of the University of Zambia

(External Examiner) ……………………………………….

Date:………………………………….

Prof. Paul Kelly ……………………………………….

(Supervisor) Date:…………………………………..

(Internal Examiner) ………………………………………..

Date:………………………………….

(Chairman, Board of Examiners)………………………….

Date:………………………………….

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Dedication

This study is dedicated to my wife, Dr. Jean Chirwa-Nsokolo, and my two sons, Mwezi and Musanya, for having tolerated my busy schedule and for the unwavering support during the execution of this study.

Abstract

Title: The correlation between baseline serum hepatitis B surface antigen level and levels of other baseline markers of viral activity in chronic hepatitis B viral infection at the University Teaching Hospital.

Background: Hepatitis B viral (HBV) infection rate among healthy blood donors in Zambia is about 8%, with about 100 000 patients requiring treatment. Effective monitoring of these patients requires the use of the technically difficult and expensive serum HBV DNA levels. Quantifying serum Hepatitis B surface antigen (HBsAg) which is produced by the covalently closed circular DNA (cccDNA), may be a more reliable, simple, inexpensive and non-invasive way of monitoring patients with chronic hepatitis b (CHB) infection. There is a correlation between reduction in serum HBsAg and in cccDNA and total intrahepatic HBV DNA among patients on treatment. However, it remains inconclusive whether serum HBsAg correlates with serum HBV DNA, which would make it helpful in predicting serum viral load.

Objective: To determine whether baseline serum HBsAg quantification correlates with other baseline serum hepatitis B viral markers

Methodology: This was a cross sectional study. Patients with hepatitis B infection were recruited under the STEP-HEP Study from blood donors in Lusaka, Zambia, medical wards and out-patient medical clinics at UTH over a 15 month period. We screened 49 Patients (HBeAg positive: n=14, HBeAg negative: n=35) with chronic HBV (HBsAg positive for at least 24 weeks) for other causes of liver disease and those with alternative causes of hepatitis were excluded. Blood testing was performed for baseline ALT, serum viral load, HBeAg status and serum HBsAg level. Patients with HBV DNA >2000IU/ml and ALT above the upper limit of normal (35U/L) who did not have radiological evidence of cirrhosis were included in the study. Serum HBV DNA and HBsAg were logarithmically transformed for analysis. Categorical variables were compared by the Pearson chi-square test or the Fisher exact test as appropriate. The Pearson and Spearman correlation coefficients were tested for parametric and non-parametric variables respectively. Statistical significance was defined as a *P* value of less than 0.05

Results: There was a significant inverse correlation between baseline HBsAg and serum HBV DNA (r = -0.38, P= 0.02). The correlation between serum HBsAg and ALT was not significant (p= 0.94). There was no significant difference in HBsAg level between HBeAg positive and HBeAg negative patients (p= 0.06). The correlation between serum viral load and ALT was also not significant (p=0.26). There was significantly higher ALT in HBeAg positive than in HBeAg negative patients (p=0.016). The serum viral load was significantly higher in HBeAg positive than in HBeAg negative patients (p=0.0001)

Conclusion: The inverse correlation between baseline serum HBsAg level and serum HBV DNA may reflect the inadequacy of serum HBV DNA to represent the level of intrahepatic HBV DNA which correlates with serum HBsAg. However, there was no significant correlation between baseline serum HBsAg and ALT nor was it significantly affected by HBeAg status. The correlation between baseline serum hepatitis B viral load and ALT was not significant. ALT and serum HBV DNA were significantly higher in HBeAg positive than in HBeAg negative patients. Therefore, baseline serum HBsAg quantification may not be a useful surrogate marker of other serum markers of viral activity in CHB infection, but requires further evaluation.

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Abbreviations

ALT…………………………………………….Alanine Aminotransferase

cccDNA……………………………………..Covalently Closed Circular DNA

CHB…………………………………………..Chronic Hepatitis B

EASL………………………………………….European Association for the Study of the Liver

ETV…………………………………………….Entecavir

HBeAg……………………………………….Hepatitis B Viral Envelope Antigen

HBsAg……………………………………….Hepatitis B Viral Surface Antigen

HBV…………………………………………..Hepatitis B Virus

HCV…………………………………………..Hepatitis C Virus

IFN…………………………………………….Interferon

NA……………………………………………..Nucleotide or Nucleoside Analogues

NIH…………………………………………….National Institute of Health

NPV……………………………………………Negative Predictive Value

p-ANCA………………………………………Perinuclear Anti-Neutrophil Cytoplasmic Antibodies

PEG-IFN………………………………………Pegelated-Interferon

PPV…………………………………………….Positive Predictive Value

STEP-HEP…………………………………..Step-Down Affordable Treatment for Chronic Hepatitis B Infection

SVR……………………………………………..Sustained Virological Response

TDF…………………………………………….Tenofovir Disoproxil Fumarate

UNZABREC……………………………….University of Zambia Biomedical Research Ethics Committee

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Chapter 1

1.0 Background

Hepatitis B virus (HBV) infection is a global public health problem. It is estimated that there are more than 350 million HBV carriers in the world, of whom roughly one million die annually from HBV-related liver diseases.[1] The prevalence of HBV carriers varies from 0.1-2% in low prevalence areas (e.g. United States and Western Europe), to 3-5% in intermediate prevalence areas (e.g. Mediterranean countries and Latin and South America), to 10-20% in high prevalence areas (Southeast Asia and Sub-Saharan Africa).[1] According to the unpublished data at the Zambia National Blood Transfusion Services, the prevalence of HBV infection in healthy blood donors in Zambia is 8%.

The predominant mode of transmission of HBV varies in different geographical areas. Perinatal infection is the predominant mode of transmission in high prevalence areas.[2,3] Horizontal transmission, particularly in early childhood, accounts for most cases of chronic HBV infection in intermediate prevalence areas, while unprotected sexual intercourse and intravenous drug use in adults are the major routes of spread in low prevalence areas.[4] The spectrum of clinical manifestations of HBV infection varies in both acute and chronic disease. During the chronic phase, manifestations range from an asymptomatic carrier state to chronic hepatitis, cirrhosis, and hepatocellular carcinoma.

Perinatally acquired HBV infection is characterized by an immune tolerance phase in which patients usually have positive hepatitis B e-antigen (HBeAg), very high HBV DNA and normal ALT levels in the initial 2–3 decades of life (refer to figure 1).[5] This is followed by an immune-clearance phase, which may lead to HBeAg seroconversion. The immune tolerance phase is characterised by minimal histologic damage.[6] Several cross-sectional studies have shown that serum HBsAg levels are generally higher in patients in the immune tolerance phase than in the immune-clearance phase.[7–11] Later in life, most of the HBV-infected population progresses to the immune active phase, loses HBeAg and seroconverts to anti-hepatitis B e-antigen antibody, with various degrees of activity and fibrosis. Then, most of this HBeAg negative chronic hepatitis B (CHB) population enters the inactive or low replicative phase, characterized by wide fluctuations in serum HBV DNA levels and transaminases.[12] The clinical spectrum of HBeAg negative CHB ranges from 'inactive carrier' status to aggressive

HBeAg negative CHB that is generally differentiated from 'inactive carriers' by serial serum ALT and HBV DNA levels.[13,14] It has been observed that in 45–65% of cases, ALT activity can fluctuate with long periods of normal ALT levels, resulting in misclassification.

Since its discovery by Blumberg in 1965, the hepatitis B surface antigen (HBsAg) is used as the fingerprint of hepatitis B infection. The relationship between intrahepatic markers of HBV infection (covalently closed circular DNA (cccDNA) and integrated HBV DNA) and serum HBsAg have been shown in several studies[7–9,15]. Differences in HBsAg levels during the different phases of the disease reflect the distribution of cccDNA during the respective infection phases. HBsAg levels are higher in HBeAg positive than in hepatitis B e-antigen-negative HBeAg negative patients.[8–10]

During the natural history of HBV, HBsAg can be used to differentiate between inactive carriers who need no treatment and HBsAg negative CHB patients who are likely to reactivate and can benefit from therapy, hence, requiring closer monitoring.[16] These results can be used to determine the best management strategy for patients. The ultimate goal of therapy is HBsAg seroconversion (loss of HBsAg and development of anti-HBs), but it is rarely observed during the natural course of chronic HBV infection, the annual incidence being 1–2% world-wide[17]. In other studies, the annual rate of delayed clearance of HBsAg has been estimated to be 0.5- 2% in Western patients and much lower (0.1- 0.8%) in Asian countries.[18–20]

Recently, quantitative serum HBsAg assays have been developed,[21,22] and the importance of HBsAg quantification has been recognized as an important marker to monitor the natural history in chronic hepatitis and predict treatment outcome.[6,11,23] Studies have clearly shown that the decline in HBsAg titers is significantly lower with nucleosides or nucleotide analogues (NA) therapy than with IFN-based treatment, but HBV DNA becomes rapidly undetectable.[24,25] Several studies have reported that baseline HBsAg levels and on-treatment HBsAg quantification are good predictive markers of the end of treatment response and SVR.[26–29]

This Study was carried out to determine whether baseline serum HBsAg quantification correlates with other hepatitis B viral activity markers in CHB patients in the Zambian population.

1.1 Statement of the Problem

With the 8% HBV infection rate among healthy blood donors in Zambia (ZNBTS unpublished data), about 100 000 patients need treatment. Effective monitoring of these patients requires the use of the technically difficult and expensive serum HBV DNA levels. In addition, even with negative serum HBV DNA after a course of peg-IFN and NAs such as lamivudine combination treatment, a significant proportion of patients still have hepatitis reactivation.[30] This observation may reflect the inadequacy of serum HBV DNA to represent the level of cccDNA and total intrahepatic HBV DNA, which have been shown to predict sustained virologic response.[31] Previous data among patients on adefovir dipivoxil suggested a correlation between changes in serum HBsAg and reduction in cccDNA inside the liver.[32] However, the invasiveness of liver biopsy makes the routine use of cccDNA and intrahepatic HBV DNA assays in routine clinical practice unfavourable

1.2 Study Justification

Quantifying serum HBsAg which is produced by the cccDNA, may be a more reliable, simple, inexpensive and non-invasive way of monitoring treatment response in patients with CHB infection than cccDNA and total intrahepatic DNA that require liver biopsy, and serum HBV DNA which is expensive. Quantification of this marker has recently been standardised by automated assays leading to an increased interest in its clinical utilisation. The development of these immunoassays has made it possible to quantitate HBsAg in a robust, reproducible, and sensitive manner. This justifies the need for further clarification of the use of HBsAg quantification in monitoring CHB patients on therapy.

1.3 Objectives

1.31. General Objective

i. To determine whether baseline serum HBsAg level correlates with other serum markers of hepatitis B viral activity.

1.32. Specific Objectives

i. To determine the correlation between baseline serum HBsAg level and serum HBV DNA.
ii. To determine whether serum baseline HBsAg level correlates with ALT.
iii. To determine the effect of HBeAg status on the baseline serum HBsAg level.

iv. To determine whether baseline ALT correlates with serum HBV DNA.
v. To determine the effect of HBeAg status on the baseline ALT.
vi. To determine the effect of HBeAg status on the baseline serum HBV DNA.

Chapter 2

2.0 Literature Review

The HBV is a small DNA virus. Once it enters the hepatocyte, the virus sheds its protein coat and the partially double-strand genome is transported into the nucleus of the cell where it is transformed into a fully double-strand cccDNA. The cccDNA resides in the nucleus of infected hepatocytes, where it acts as a template for transcription of the viral gene and recycles in the nucleus to renew the cccDNA pool.[17,33] HBsAg is one of the viral proteins of clinical importance. It is an envelope protein whose synthesis during the HBV viral life cycle is complex. The production of HBsAg exceeds that required for virion assembly, and excess surface envelope proteins are covalently linked and secreted as empty non-infectious filamentous or spherical sub-viral particles.[7] These empty particles may co-exist with anti-HBs-antibodies as part of circulating immune complexes.[34] Serum HBsAg is a result of the combination of these proteins (complete virion, filamentous or spherical sub-viral particles). HBsAg quantification measures all three forms of systemic HBsAg. The production of HBsAg by the cccDNA is independent of the replication of the virus, hence, the antiviral agents that suppress the activity of polymerase will promptly reduce the serum HBV DNA but may not directly affect the production of HBsAg.[15]

Inactive carriers have no or mild histological lesions in the liver with an excellent prognosis for survival, while patients with HBeAg negative CHB with fluctuating activity have a more severe disease progression with frequent cirrhosis.[14,35,36] Differentiating the latter from the former group is very important as these patients could benefit from therapy. According to National Institute of Health (NIH) and European Association for the Study of the Liver (EASL) guidelines, the differentiation between inactive and active phases of HBeAg negative CHB is based on an HBV DNA cut-off of 2000 IU/ml.[37,38].

Several recent studies on serum HBsAg monitoring show that HBsAg levels change during the natural course of CHB and during on-going therapy. In Italian genotype D patients, the HBsAg levels were higher in patients with HBeAg negative CHB than in 'inactive carriers' and

a single point quantification of HBV DNA <2000 IU/ml and HBsAg <1000 IU/ml identified inactive carriers with a positive predictive value (PPV) of 88%, although this observation must be validated across all HBV genotypes.[39] Chan et al[40] in a study of 68 HBeAg-negative CHB patients predominantly infected with genotype C reported that the patients with inactive disease tend to have lower HBsAg levels than those with active disease; 2.24 ± 1.61 $\log_{10}$ IU/ml vs. 2.98 ± 0.88 $\log_{10}$ IU/ml respectively ($P = 0.054$). However, they noted that no cut-off value can confidentially differentiate 'inactive carriers'. In another long-term follow-up study (median 10 years). In another study, it was reported that HBsAg <1000 IU/ml predicts seroclearance (91% specificity: 75% sensitivity).[41] Martinot-Peignoux et al[42] reported similar results in a study from France in 165 patients with HBeAg negative CHB (genotypes A–E). The authors reported that HBsAg levels were lower in the 76 'inactive carriers' than in the 89 patients with an HBeAg negative CHB; 3.25 ± 0.96 $\log_{10}$ IU/ml vs. 3.67 ± 0.70 $\log_{10}$ IU/ml respectively ($P < 0.001$). They concluded that a combination of a single measurement of HBsAg <1000 IU/ml and HBV DNA <2000 IU/ml identifies 'inactive carriers' with a PPV of 86%.

In another more recent study, it has reported that the combination of a single measurement of HBsAg >1000 IU/ml and HBV DNA >2000 IU/ml identifies patients with a 'high risk of reactivation' with a negative predictive value (NPV) of 96%, and sensitivity 92%.[43] The authors concluded that a combination of HBsAg and HBV DNA levels at a single time point may accurately identify HBeAg negative CHB patients during remission with a high probability of reactivation and who are good candidates for treatment.

In 1994, Janssen et al[44] considered HBsAg quantification to be a promising, simple and inexpensive method to monitor viral replication in chronic hepatitis B patients who were receiving IFN treatment. More recently, the availability of well-standardized commercial assays has renewed interest in the quantitative serum HBsAg as a biomarker for treatment response in chronic hepatitis B.[45,46] Current indicate that on-treatment HBsAg quantification could help identify either patients with a high probability of sustained virological response (SVR) or non-responders to pegylated-IFN in HbeAg positive patients.[47–52] The response rate to PEG-IFN is low (<20%) in HBeAg negative patients.[53–56] These patients are difficult to monitor. Although most of these patients achieve undetectable serum HBV DNA at the end of therapy, they relapse after treatment is stopped.[55–57] Therefore, HBsAg quantification,

which recent evidence suggests is a worthwhile marker for monitoring PEG-IFN therapy, can be used as a predictive factor of on-treatment response and may help define more appropriate treatment strategies in certain patients.[16,58,59]

Recent studies have clearly shown that the decline in HBsAg titers is significantly lower with NA therapy than with IFN-based treatment, but HBV DNA becomes rapidly undetectable.[60,61] In a study of HBsAg kinetics in patients who were successfully treated with long-term entecavir (ETV) or tenofovir (TDF), Zoutendijk *et al*[62] used linear mixed regression analysis of individual HBsAg declines to estimate the duration of therapy required to achieve an HBsAg decline of 1 log_{10} UI/ml from baseline and HBsAg clearance. They showed that the median durations of therapy to achieve a 1 log_{10} IU/ml decrease were as follows: 6.6 [1.7–18] years and 8 [0.5–15] years in HBeAg positive and HBeAg negative patients respectively. Median durations for HBsAg clearance were as follows: 36 [10–93] years and 39 [1.3–90] years in HBeAg positive and HBeAg negative patients respectively. These results show the importance of determining HBsAg cut-offs to discontinue NA therapy with lowest risk of reactivation.

Marcellin et al[63,64] in two studies of TDF; study 102 (HBeAg negative) and study 103 (HBeAg positive) patients, they confirm that HBsAg kinetics is steeper in HBeAg positive patients than in HBeAg negative and in patients receiving TDF monotherapy. The only patients with HBsAg loss were HBeAg positive patients with a ≥2 log_{10} IU/ml HBsAg decrease from baseline at 24 weeks of therapy, a higher baseline HBsAg level and genotypes A or D. Recent studies including treatment-naive patients receiving NA report that low baseline HBsAg levels and an early decline in HBsAg (24-week therapy) are good predictors of SVR.[26–29] In addition, studies suggest that an HBsAg cut-off ≤2–3 log_{10} IU/ml could be used for treatment discontinuation.[65–68]

It is evident that the role of HBsAg monitoring during NA therapy must be clarified. The development of rules for stopping these life-long therapies should be determined. Several studies suggest that baseline and on-treatment HBsAg levels might help identify patients who can stop therapy with no risk of reactivation. Baseline HBsAg level, decline in HBsAg level, baseline HBV genotype, and baseline HBV DNA are predictors of HBeAg but not of HBsAg loss, however, further studies are required to clearly define the possible uses of

HBsAg quantification in monitoring the management of CHB patients treated with NAs.[27] It is evident that Studies to determine the role of HBsAg quantification have not been conclusive for CHB patients and require further clarification.

Chapter 3

3.0 Methodology

This was a sub-study of the STEP-HEP Study (appendices 1, 2 and 3). It was a cross-sectional study. Patients with chronic HBV (HBsAg positive for at least 24 weeks) who consented were screened for other causes of liver disease and patients with alternative causes of hepatitis were excluded. Participants had blood testing performed for baseline ALT, viral load, HBeAg status and HBsAg quantification. Patients with HBV DNA >2000IU/ml and ALT above the upper limit of normal (36U/L) who did not have radiological evidence of cirrhosis were included in the study.

3.1 Recruitment

Patients were recruited under the STEP-HEP Study from blood donors in Lusaka, Zambia and from medical wards and out-patient medical clinics at UTH over a 15 month period. (January, 2014 to March, 2015)

3.2 Sample Size

49 patients were recruited (HBeAg positive: n=14, HBeAg negative: n=35). 40 were males and 9 were females. The median age was 30 years. By applying the Sample Size for Correlation Program from StatsToDo, using alpha (α) of 0.05 and 80% power, a sample size of 49 participants detects a minimum correlation coefficient (r) of 0.36.

3.3 Inclusion criteria

i. 18 years of age and above
ii. Serum HBV DNA >2000IU/ml
iii. ALT higher than the upper limit of normal (>36U/L)

3.4 Exclusion Criteria

i. Radiological evidence of cirrhosis
ii. HIV infection

iii. History of alcohol abuse
iv. History of any long-term drug ingestion
v. Serological evidence of metabolic liver disease (haemochromatosis, Wilson's disease, a1-antitrypsin deficiency) or autoimmune liver disease (antibodies to M2, p-ANCA, nuclear antigens, microsomes or smooth muscle) or other cause for liver inflammation
vi. Serological evidence of schistosomiasis
vii. Virological evidence of HCV

3.5 Serum HBsAg quantification

HBsAg quantification was done by using the Hepanostika HBsAg Ultra microelisa system. Specimens where diluted to the ratio of 1 in 500. The standard curve was determined by making 10-fold dilutions of a 237 000 000IU/ml HBsAg standardised sample and the serum HBsAg levels determined from the standard curve.

3.6 Testing for serum HBV DNA

Determination of serum HBV DNA was done using a Roche Taqman Assay with a linear reportable range of 20 -170 000 000IU/ml.

3.7 Testing for ALT

Testing for ALT was done as routinely done at UTH using the automated AU400 Olympus machine which has an upper limit of normal ALT of 36IU/L.

3.8 Testing for HBeAg

The HBeAg was tested by using the DiaSorin HBeAg Enzyme Immunoassay kit. The assay is a direct, non-competitive test based on the use of polystyrene microwells coated with mouse monoclonal antibodies to HBeAg. An enzyme tracer containing horseradish perioxidase-labelled mouse monoclonal antibody to HBeAg detects any captured HBeAg.

3.9 Outcome Measures

3.9.1 Primary outcome measure

i. The correlation between baseline serum HBsAg level and serum HBV DNA.

3.9.2 Secondary outcome measures

i. The correlation between baseline serum HBsAg level and ALT.
ii. The effect of HBeAg status on the baseline serum HBsAg level.
iii. The correlation between baseline ALT and serum HBV DNA.
iv. The effect of HBeAg status on the baseline ALT.
v. The effect of HBeAg status on the baseline serum HBV DNA.

3.10 Statistical Analysis

Statistical tests were performed by Stata, version 12. In event where the subgroup had a small number of participants, continuous variables were expressed as a median (range) because of our small sample size. Serum HBV DNA and HBsAg were logarithmically transformed for analysis. Categorical variables were compared by the Pearson chi-square test or the Fisher exact test as appropriate. The Pearson and Spearman correlation coefficients were tested for parametric and non-parametric variables respectively. Statistical significance was defined as a *P* value of less than 0.05

3.11 Ethical Considerations

This study was a sub-study of the STEP-HEP study which was approved by the UNZABREC. The amendments to the parent study protocol that were required for conducting this subsection were made and approved by UNZABREC. However, further consent for this study was obtained from participants. There was no significant risk to the participants.

The data obtained from participants Data was anonymized. There were no outcomes from this research that were misused for harmful purposes.

The study protocol and consent forms that were used had been approved by UNZABREC under the STEP-HEP Study and were adequate for this study. However, further approval was sought and approved by UNZABREC.

Chapter 4

4.0 Results

4.1 General description of the results

Between 29th January, 2014 and 17th March 2015, 186 HBsAg patients were evaluated, and 49 of them met the criteria for enrolment into the study (i.e. ALT >35 and HBV DNA >2000IU/ml). 17 (34.7%) were recruited from the ZNBTS and 32 (65.3%) from the UTH medical wards and out-patient medical clinics. 40 (81.6%) were males and 9 (18.4%) were females. The median age for the enrolled patients was 30 years, the age range was 18-57 years. Out of the 49 patients enrolled in the study, 14 (28.6%) were HBeAg positive and 35 (71.4%) were HBeAg negative. All the patients were negative for the Delta virus antibodies.

The median for the baseline hepatitis B viral load (Log IU/ml) for all the enrolled patients was 3.9, an interquartile range of 2.8-5.7 and a range of 1.6-8.3. The baseline ALT (IU/ml) range was 35-435, a median of 61 and an interquartile range of 44-84. The median for the baseline HBsAg (Log IU/ml) was 11.5, a range of 11.2-11.6 and an interquartile range of 0-12.5.

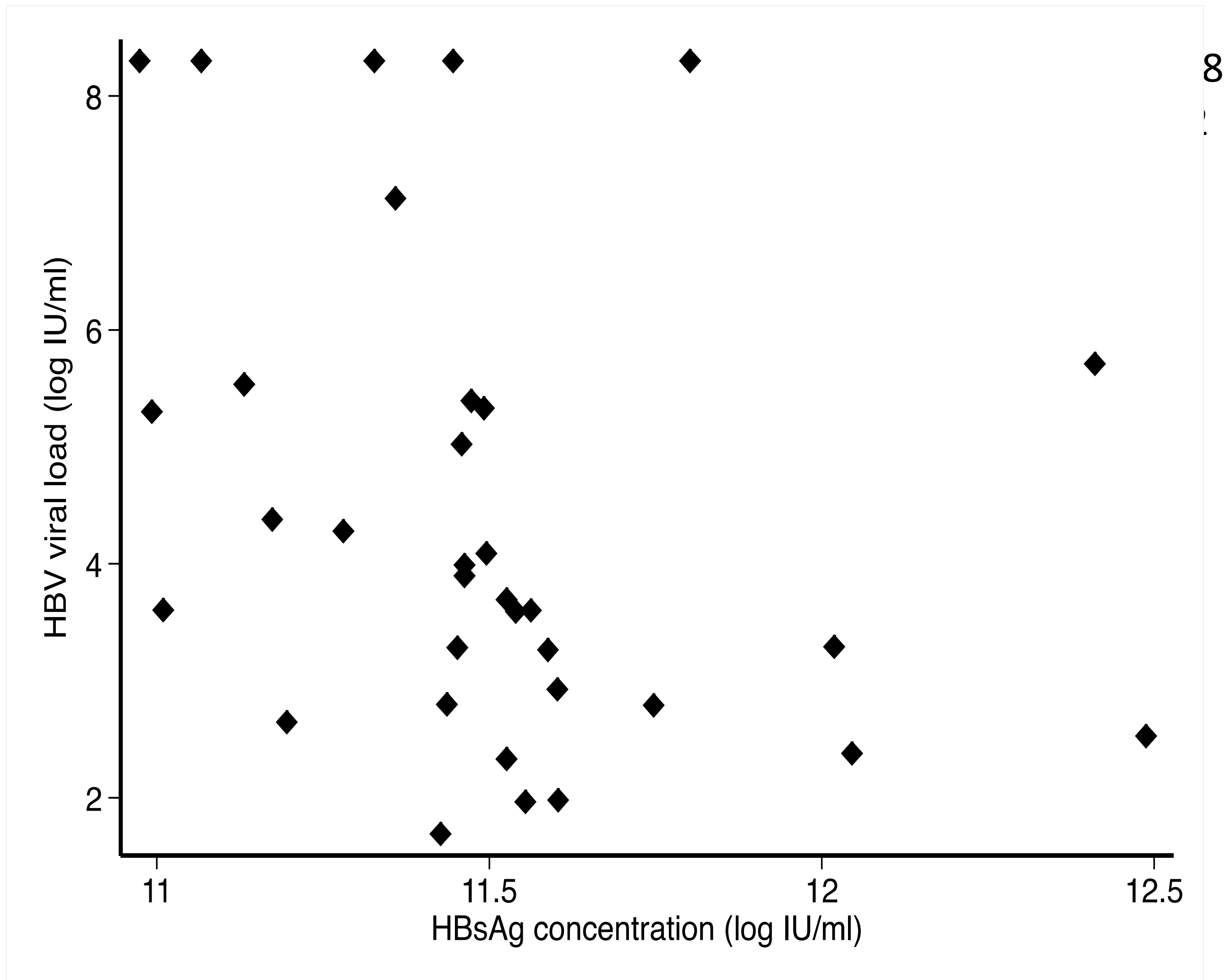
Table 1: Characteristics of Chronic Hepatitis B Patients from UTH and ZNBTS

	N (%)	Median	IQR	Range
Males	40 (81.6)			
Females	9 (18.4)			
Age		30		18-57
Blood	17 (34.7)			
Clinic/Ward	32 (65.3)			
ALT (median, IQR) (IU/ml)		61	44-84	35-435
Viral Load (median, IQR) (Log IU/ml)		3.9	2.8-5.7	1.6- 8.3
HBeAg Positive	14 (28.6)			
HBeAg Negative	35 (71.4)			
HBsAg (median, IQR) (Log IU/ml)		11.5	11.2-11.6	0-12.5
Delta Ab	0			
Liver Biopsy Performed	21			

4.2 Correlation between HBsAg and serum HBV DNA

There was a significant inverse correlation between baseline HBsAg and plasma hepatitis B viral load. (ρ=-0.38) and (p= 0.02)

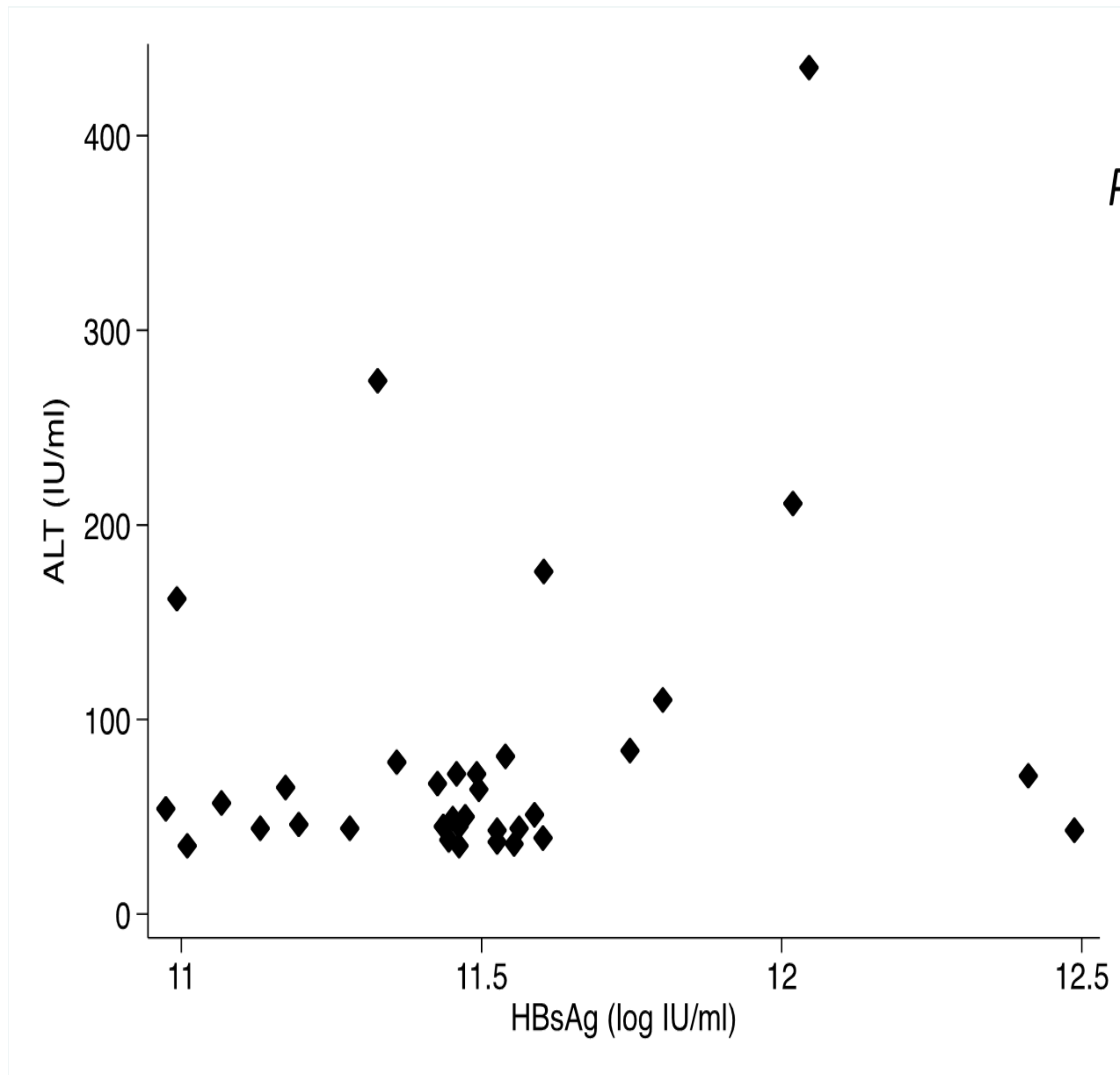
Graph 1: Correlation between HBsAg and serum Hepatitis B Viral Load



4.3 Correlation between serum HBsAg and ALT

There was no significant correlation between serum HBsAg and ALT (p= 0.94).

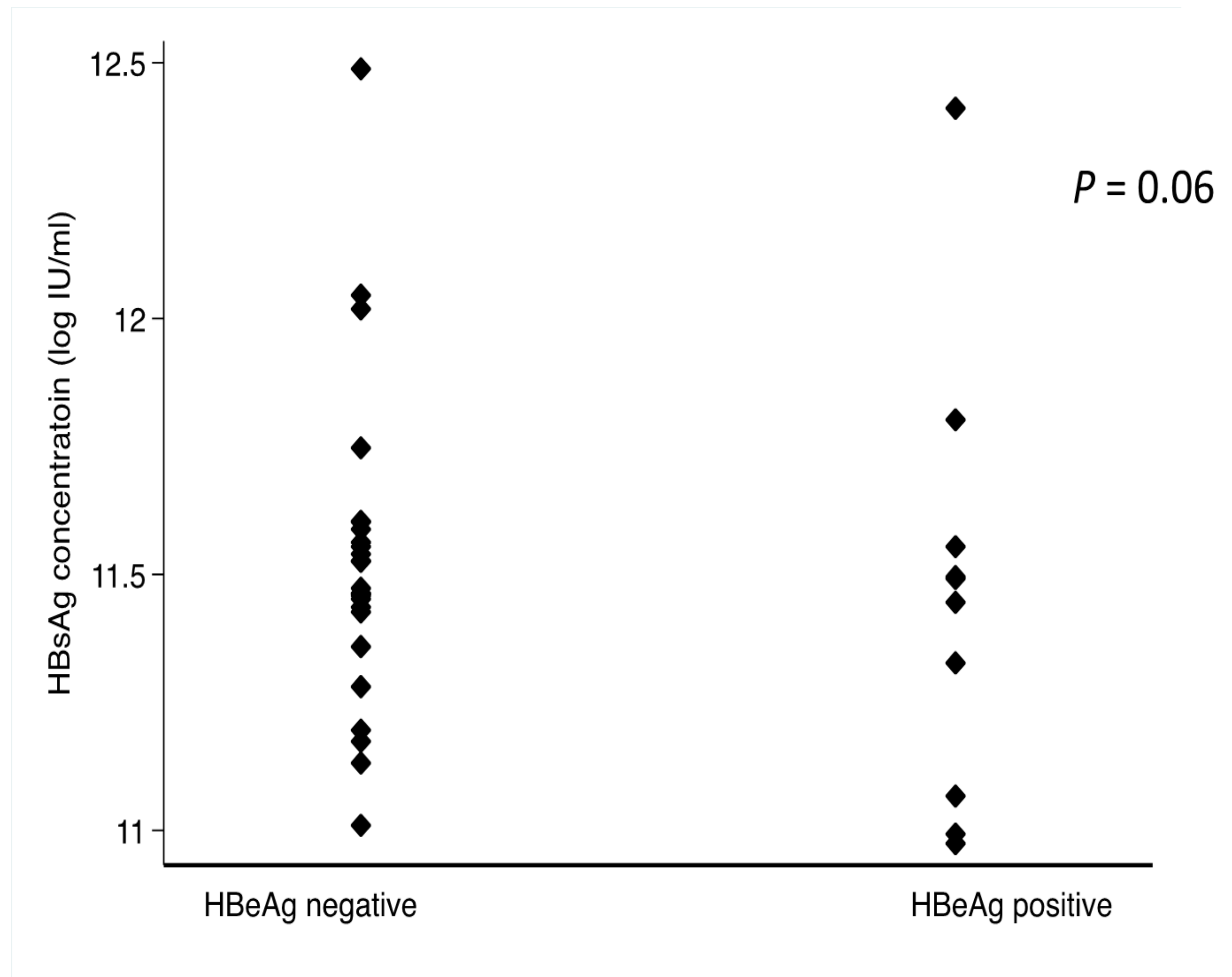
Graph 2: Correlation between serum HBsAg and ALT



4.4 HBsAg levels in HBeAg positive and HBeAg negative patients

There was no significant difference in HBsAg level between HBeAg positive and HBeAg negative patients. (ρ = 0.06)

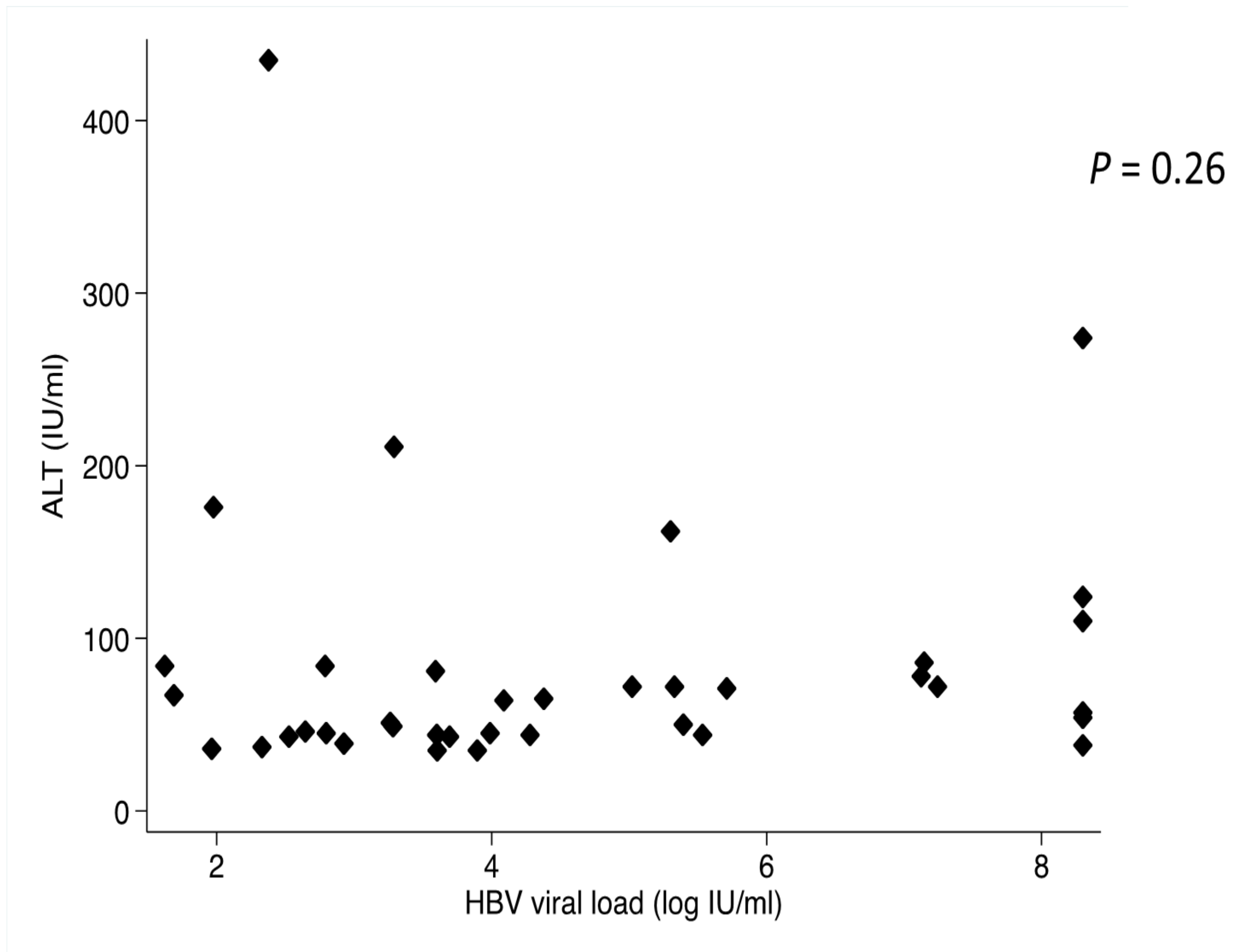
Graph 3: HBsAg levels in HBeAg Positive and HBeAg Negative patients



4.5 Correlation between serum HBV DNA and ALT

There was no significant correlation between serum viral load and ALT. ($\rho=0.26$)

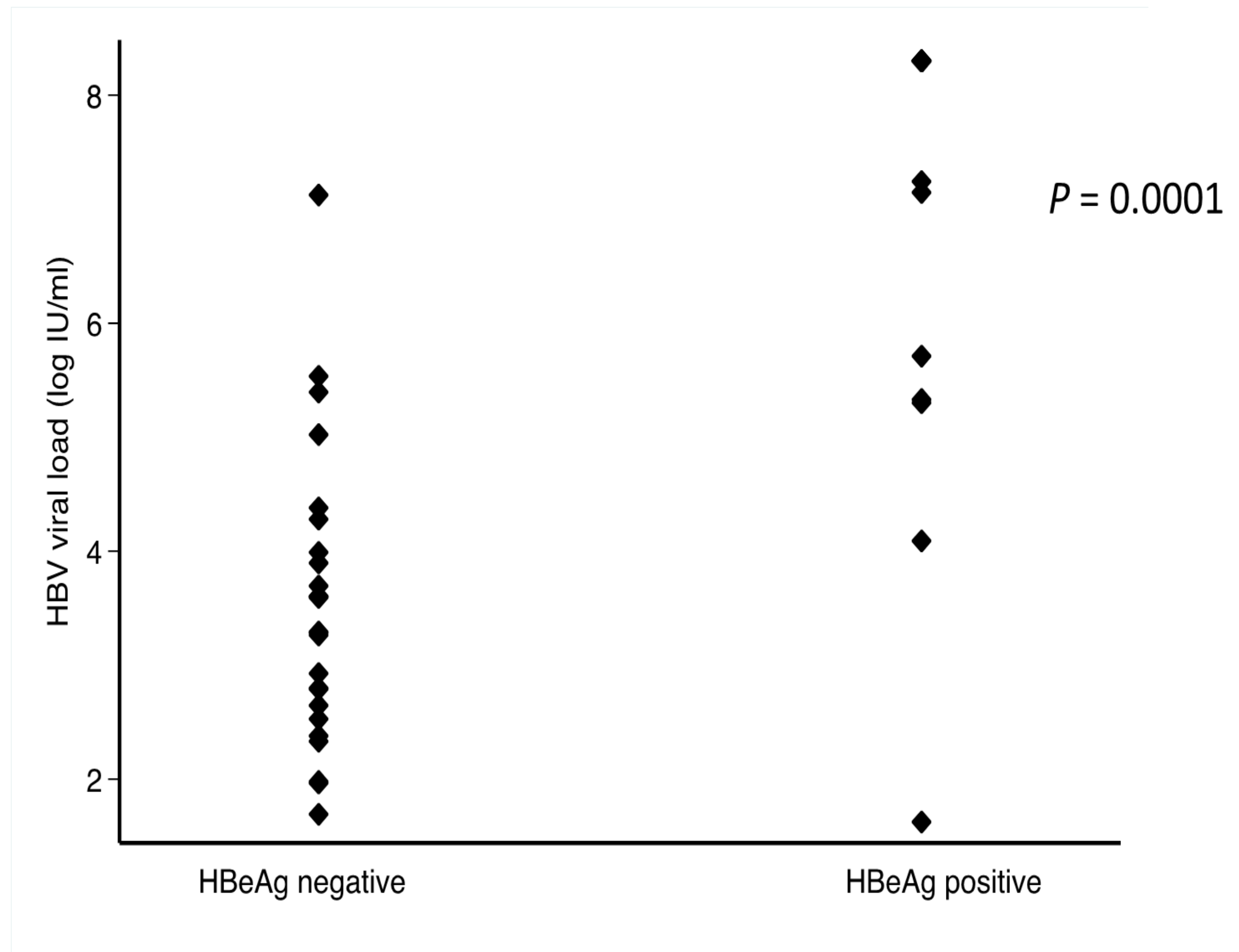
Graph 4: Correlation between Serum Viral Load and ALT



4.6 HBV DNA in HBeAg positive and HBeAg negative

There serum viral load was significantly higher in HBeAg positive than in HBeAg negative patients (ρ=0.0001)

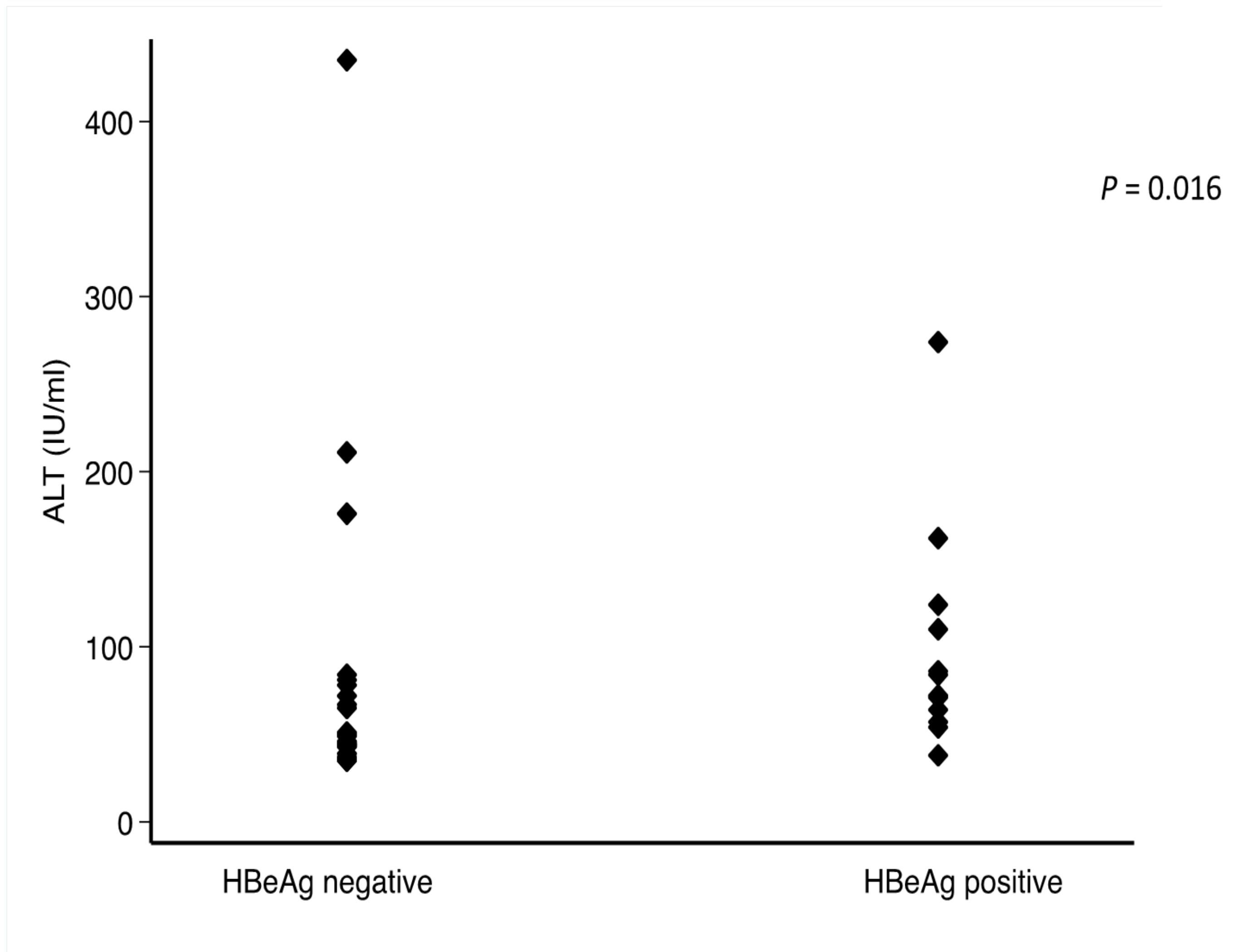
Graph 5: Serum HBV DNA in HBeAg Positive and HBeAg Negative patients



4.7 ALT in HBeAg positive and HBeAg Negative Patients

There was significantly higher ALT in HBeAg positive than in HBeAg negative patients. (p=0.016)

Graph 6: ALT in HBeAg positive and HBeAg Negative Patients



Chapter 5

5.0 Discussion

5.1 Correlation between baseline serum HBsAg and serum HBV DNA

There was a significant inverse correlation between serum HBsAg and serum HBV DNA (r = -0.38, p = 0.02). Chan et al[15] found a correlation between the level of baseline serum HBsAg and that of intrahepatic markers of HBV infection (cccDNA; r = 0.54, p = 0.004, and total intrahepatic HBV DNA; r = 0.43, p = 0.028) but not with serum HBV DNA. This may be explained by the fact that the production of HBsAg by the cccDNA is independent of viral replication. However, the significant inverse correlation that we found in our study population between serum HBsAg and serum HBV DNA requires further evaluation.

5.2 Correlation between serum HBsAg and ALT

There was no significant correlation between serum HBsAg and ALT (p = 0.94). ALT level is a biomarker that reflects host immune response against virus-infected hepatocytes. Our study population falls under a high prevalence category in which HBV infection is mostly perinatal and is characterized by an immune tolerance phase in which patients usually have positive hepatitis B e-antigen (HBeAg), very high HBV DNA and normal ALT levels in the initial 2–3 decades of life.[5] In addition, the median age of the participants in our study was 30 years, hence, they are likely to be in the immune tolerance phase. Several cross-sectional studies have also shown that serum HBsAg levels are generally higher in patients in the immune tolerance phase than in the immune-clearance phase.[7–11] However, of the 49 patients in our study only 14 (28.6%) were HBeAg positive and 35 (71.4%) were HBeAg negative. In addition, it has been observed that in 45–65% of cases, ALT activity can fluctuate with long periods of normal ALT levels, resulting in misclassification. These factors may contribute to the lack of correlation between baseline serum HBsAg and ALT. This would suggest that a low ALT may not be the best indicator of disqualification for treatment in our study population. However, baseline ALT level is known to be the strongest determinant of HBeAg seroconversion during lamivudine treatment.[69]

5.3 Serum HBsAg quantification and HBeAg status

We found no significant difference in HBsAg level between HBeAg positive and HBeAg negative patients in our study population (p= 0.06). However, HBsAg levels have been found

to be higher in HBeAg-positive than in HBeAg-negative patients[8–10]. Several other recent studies on serum HBsAg monitoring show that HBsAg levels change during the natural course of CHB infection and during on-going therapy. In Italian genotype D patients, the HBsAg levels were higher in patients with HBeAg-negative CHB than in 'inactive carriers' and a single point quantification of HBV DNA <2000 IU/ml and HBsAg <1000 IU/ml identified inactive carriers with a PPV of 88%[39]. However, these findings may require validation across all genotypes. Although we did not ascertain the genotypes in our study, we can speculate that the predominant genotype in our study population may have different characteristics which may explain why there was no significant difference in baseline serum HBsAg levels between HBeAg-positive and HBeAg-negative patients.

5.4 Correlation serum HBV DNA and ALT

There was no significant correlation between baseline serum viral load and ALT ($\rho=0.26$). As earlier mentioned, ALT level is a biomarker that reflects host immune response against virus-infected hepatocytes. Brunetto et al,[12] observed that after the immune active phase, most of the HBeAg-negative CHB population enters the inactive or low replicative phase, characterized by wide fluctuations in serum HBV DNA levels and transaminases. As Most of our participants (71.4% HBeAg-negative patients) may have been in this phase, hence, the lack of significant correlation between the serum HBV DNA and ALT. The clinical spectrum of these HBeAg-negative CHB patients ranges from 'inactive carrier' status to aggressive HBeAg (-) CHB that is generally differentiated from 'inactive carriers' by serial serum ALT and HBV DNA levels.[13,14]

5.5 Serum HBV DNA and HBeAg Status

The serum viral load was significantly higher in HBeAg positive than in HBeAg negative patients ($\rho=0.0001$). This was a reflection of what other studies have shown in the past. Chan et al[5] found that Perinatal acquired HBV infection is characterized by an immune tolerance phase in which patients usually HBeAg-positive, have very high HBV DNA and normal ALT levels in the initial 2–3 decades of life. Our study population being a high prevalence category, the most predominant mode of transmission is believed to be mostly perinatal.[2] In addition, the median age of the participants in the study was 30 years, i.e. third decade of life.

5.6 ALT and HBeAg status

There was significantly higher ALT in HBeAg positive than in HBeAg negative patients. (p=0.016). HBeAg is a marker of active viral replication. It also serves as an immune decoy and directly manipulates the immune system. The pathogenesis and clinical manifestations of HBV infection are due to the interaction of the virus and immune system. Therefore, ALT level being a biomarker that reflects host immune response against virus-infected hepatocytes would be expected to be higher in HBeAg-positive patients. This would explain our finding. However, active viral replication is known to continue despite little or no elevation in ALT level and no symptoms of illness.[12]

Chapter 6

6.0 Conclusion

Baseline Serum HBsAg level inversely correlated with serum hepatitis B viral load, but had no significant correlation with ALT nor was it significantly affected by HBeAg status. This may reflect the inadequacy of serum HBV DNA to represent the level of intrahepatic HBV DNA which correlates with serum HBsAg. There was no significant correlation between baseline serum hepatitis B viral load and ALT. There was significantly higher ALT and serum hepatitis B viral load in HBeAg positive than in HBeAg negative patients. . Therefore, baseline serum HBsAg quantification may not be a useful surrogate marker of other serum markers of viral activity in CHB infection, but requires further evaluation.

6.1 Study limitations

The sample size was small to detect very low correlations, i.e. lower than 0.36, therefore, much lower correlations may have been missed. The distribution of the serum HBsAg levels was limited between 11 – 12.5 log IU/ml, this could have also limited the power to detected lower correlation that may be there if the values were more spread out. In addition, without genotyping, which has not yet been done in the Zambian population, the results may not be generalized for all HBV genotypes.

6.2 Recommendations

The inverse correlation between baseline serum HBsAg level and serum hepatitis B viral load in our study population requires further research, as this is not in keeping with some studies done elsewhere. Further research, with a larger sample size, serial measurements of

variables (serum ALT, HBV DNA and HBsAg) for each participant, with the inclusion of HBV DNA genotyping and liver biopsy for histopathology, would elucidate more information concerning HBsAg quantification.

There is also need for a longitudinal study to determine which parameters (serum HBsAg quantification, ALT, serum hepatitis B viral load and HBeAg status) would predict long term poor outcome of patients with CHB infection.

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Appendices

Appendix 1

Step-down affordable treatment for chronic hepatitis B infection in Africa (Step-HEP)

Information Sheet

Your doctor has told you that you are infected with the hepatitis B virus. This means that you are at risk of developing complications from the infection unless you receive appropriate treatment. We know that the best way to treat people who have active hepatitis B infection is to give them powerful drugs that stop the virus replicating. The old drugs that used to be used to treat hepatitis B are very cheap but they are not very powerful and many people develop resistance to them so that they stop working after a period of time. The new drugs that are used to treat hepatitis B are more powerful and most people don't develop resistance. However the new drugs may have more side effects in the long term and they are much more expensive. In many infections we know that starting treatment with a powerful drug and then reducing the treatment to a weaker drug is very effective. In London a pilot study of this approach has been tried in patients with hepatitis B with success in most cases – specifically we took people who had been treated with the expensive new drug tenofovir and who were responding to treatment and we changed them to treatment with the weaker, cheaper, probably safer drug lamivudine. We now want to find out if this way of treating hepatitis B works in Zambia.

What are we asking you to do?

If you agree to take part we will assess the amount of damage to your liver by a liver biopsy (unless this has already been done). This will involve a short stay in hospital whilst a little needle is put through the skin into the liver to take a small sample of tissue. We will also take about 30 ml of blood to test for hepatitis B and other causes of liver disease. We will also test you for infection with the HIV virus. If your blood tests and liver biopsy show that you have infection with the hepatitis B virus that needs treating we will give you a tablet of tenofovir to take every day for 48 weeks. You will need to come back and see the doctor after 4, 8, 12, 24 and 36 weeks and on each visit we will take another 20 ml of blood to check that the drug is working. You will need to come to see the doctor 8 times in the first year. After 48 weeks treatment we will take another blood test and arrange to see you again in 4 weeks. If the blood test shows that the treatment is working we will change your treatment to a new tablet (lamivudine) and you will then be seen every 4 weeks for 3 months and then seen every 8 weeks for 3 months (a total of 6 visits in 6 months). At each visit we will take an extra 20 ml of blood to check that the treatment is working. If any of these blood tests show that the virus has returned we will change your treatment back to tenofovir. If the blood tests after 48 weeks treatment with tenofovir shows that this treatment is not working well enough you will carry on taking the tenofovir tablets and you will be seen every 12 weeks.

In total you will receive treatment for your hepatitis B for one and a half years. At the end of that time we expect that the liver damage will have reduced and we will stop treatment. You may wish to continue with treatment for your hepatitis B and your doctor will talk to you about the benefits and costs of this. Any additional treatment will be at your own expense. In a few patients it is possible that the experimental treatment will stir up the liver and lead to further liver damage when treatment is stopped. If this is the case we will provide you with a further 24 weeks of treatment at our expense to make sure that the liver damage is brought under control.

What is the current standard treatment?

There is no standard treatment. Currently the sort of treatments we are evaluating are only available in UTH or private clinics for people who can afford to pay. Many patients are being given treatment which is not up to date and may lead to resistance, but most people who would benefit are not being given treatment. We need to find more affordable treatments which is why we are evaluating a way of giving these drugs which uses an expensive drug to prepare for a cheaper drug which we hope can then be given safely.

What are the possible benefits to me?

We cannot promise the trial will help you personally, although we believe that by suppressing the hepatitis B virus we will improve your liver damage and make you less likely to develop problems from hepatitis B in the future.

What are the possible disadvantages to me?

i.) If you take part in the trial you will have to have a liver biopsy. This has a very small risk (1 in 3000) of causing bleeding from the liver which may need an operation to stop the bleeding.
ii.) If you take part in the trial you might have an increase in the liver damage when the treatment is stopped (a liver flare). We believe that we will be able to control this by treatment with drugs for hepatitis B.
iii.) If you take part in the trial you may receive therapy with lamivudine which can cause viral resistance and make this drug less effective in the future.
iv.) All participants will need to have an HIV test, but this will help you safeguard your health, and we will offer full pre-and post-test counselling. There is also the discomfort of having blood taken.

If something did go wrong, which has not happened so far, compensation will be available from the insurance company which has provided insurance for medical accidents.

What do we do with the samples we take?

Plasma (the liquid part of blood) will be used for HIV test and for assessment of the severity of the hepatitis infection. You need to know that we intend to store part of every sample in our laboratory for future analysis, and to allow quality control checks with a lab in the UK.

What will happen to the results of the research trial?

The results of the trial may take approximately 2 years to be reported, and a copy of the report will be sent to the Ministry of Health. The results will be published in a medical journal and presented at appropriate clinical conferences here in Zambia and internationally. You will not be identified in any report or publication. It is not intended that we shall provide you with a copy of the scientific trial report, but if you would like to receive a copy please let your doctor know.

Confidentiality

Your details will be recorded on a paper form which will be locked away in our offices in UTH. Your details will be entered on a computer but only in code form with only your ID number and no name. Samples will be stored in the laboratory using a unique number which only the local principal investigator will have the key to. Any information and results will remain absolutely confidential, and family members or work colleagues will not be granted access to this information.

The trial will be monitored by an agency from the UK (Queen Mary University of London Clinical Trials Unit) to ensure that everything is being done properly. This means that we may have to share your information, but it will be coded to remain confidential, and it will not be possible for any monitor to see who the information belongs to.

The study is voluntary

You do not have to participate in this study if you do not want and even if you refuse, we will still provide the best care we can. If you do agree, you are also free to change your mind at a later date. This research study has been approved by the Biomedical Research Ethics Committee of the University of Zambia, and their contact details are:

Principal Investigator: Dr Paul Kelly, Department of Medicine, UTH Lusaka (phone 0211 252269)

Research Ethics Committee: The Chairperson, REC office, department of Anatomy, Ridgeway campus Lusaka (phone 0211 256067).

TO BE KEPT BY PARTICIPANT

Supplementary information for patients being recalled

We have asked you to return to UTH for further evaluation. First of all, we apologise for the inconvenience of asking you to come back after we had told you everything was in order, and for any worry this may have caused you. It is important to emphasise that this is not because there is anything wrong, but we would like to ask for your assistance again.

The problem we have is this. The guidelines for treatment of hepatitis B in Europe and North America are centred on a particular blood test, the ALT test, which helps decide if there is inflammation in the liver and so if the patient requires treatment. The results we have

obtained from the first part of the StepHEP study, which you have participated in, together with results from a similar study in The Gambia, suggest that this test does not work so well in Africa. This may be because there are lots of other infections, such as malaria, which might increase the ALT result, or it might mean that we have more to learn about the way that viral hepatitis progresses in Africa.

So we are asking you to undergo some further evaluation. This means that we need to take another 20ml of blood (about two syringes) to carry out a further evaluation. We also need to arrange a Fibroscan test, which is just like the ultrasound you have already had but involves a shock wave which feels like a little nudge. It is not painful at all. If these show that there is any cause for concern, we will arrange a liver biopsy and treatment just as we originally stated in the information sheet you have already been given.

If you have further questions, Mrs Kanunga or Dr Nsokolo or Dr Sinkala or Professor Kelly will be happy to discuss.

Consent record sheet

I confirm that I have understood the information I have been given about the study. I agree to participate in the study. I confirm that I am joining the study of my free will and that I can withdraw at any time without affecting the care available to me. I understand what will be required of me.

Name

Signed (or thumbprint)

Date

Signature (or thumbprint) of witness

Name

Date

I confirm that I have explained the information fully and answered any questions.

Signed for the study team

Name

Date

Appendix 2

Step-down affordable treatment for chronic hepatitis B infection in Africa

Acronym **– Step-HEP**

Study Protocol StepHEP 2.0

Version 2.0

Principal Investigator:
Professor Graham Foster
Queen Mary, University of London
London UK
Email: g.r.foster@qmul.ac.uk
tel: (0044) 20 7882 7242

Chief Investigator (Zambia)
Dr Paul Kelly,
Department of Medicine,
University of Zambia School of Medicine,
Lusaka
email: m.p.kelly@qmul.ac.uk
tel: (00260) 211 252269 / 955 295493

Study sponsor:
Queen Mary University of London,
Barts& The London School of Medicine Joint R&D office,
Rutland House, New Road
London E1 2AX,
UK
email: g.collins@qmul.ac.uk
tel: (0044) 20 7882

Zambian coordinator:
Dr Bright Nsokolo
Department of Medicine
University of Zambia School of Medicine
UTH
Nationalist Road
Lusaka
tel/fax: 0211 252269
email: b_nsokolo@yahoo.com

Clinical Trial registration: This trial will be registered with Current Controlled Trials

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Appendix: SPC data sheet on Tenofovir and lamivudine

1 Summary

Chronic infection with the hepatitis B virus (HBV) is common in Africa where up to 8% of the population may be infected. Without therapy up to 30% of those who are infected will die of the complications of infection (cirrhosis, hepatocellular carcinoma) after an interval of 20-40 years. Effective treatment is available and widely used in the developed world. Therapy for chronic HBV infection centres around long term viral suppression with potent oral antiviral agents. Provided that suppression of viral replication can be maintained for many years complications of infection can be avoided and rates of liver cancer are reduced by long term therapy. The third generation drugs used to treat chronic HBV (tenofovir and entecavir) are highly potent with a very high barrier to resistance (<1% after 6 years) but these drugs are expensive and not widely used in the developing world. Lamivudine is an old drug, currently inexpensive, that is no longer used in the developed world as it has a very high rate of resistance (70% of patients are resistant after 5 years) reducing the value of the drug in preventing morbidity and mortality. In other chronic diseases with resistance prone organisms, long term disease suppression can be achieved by induction-maintenance regimes where suppression of the replication of the pathogen is initially achieved with highly potent drug combinations which are then exchanged for a less potent combination to maintain suppression of viral replication. This approach is widely used in tuberculosis and has been tested with success in HIV but never assessed in chronic HBV. Induction-maintenance therapy for chronic HBV infection has the potential to make high quality, resistance free therapy available at a significant discount and the purpose of this study is to examine this hypothesis.

This is a pilot study to determine whether induction-maintenance therapy with tenofovir for 48 weeks followed by 24 weeks therapy with lamivudine is safe and effective in patients with chronic HBV infection. We will treat 80 volunteer patients with chronic HBV infection and monitor them closely for a total of 18 months. The efficacy of therapy along with drug resistance, patient compliance and cost effectiveness will be monitored to determine whether a large scale trial is warranted.

2 Background

Chronic infection with the hepatitis B virus (HBV) is common around the world, infecting up to 400 million people. The prevalence in many developing areas (e.g. India & Africa is > 5%[1]). Up to 30% of those infected will die from the complications of their disease (chiefly liver cirrhosis and/or hepatocellular carcinoma) and as many patients in the developed world have uncontrolled viraemia transmission continues to occur, either by materno-fetal transmission during childbirth, by sexual transmission or by inadvertent blood to blood transmission in health care settings or during accidents. Effective therapy is available and controls viraemia and reduces mortality [2]. However therapy is costly, requires complex monitoring and is not universally available – in a preliminary study in Zambia of several hundred HBV infected individuals none were receiving treatment (PK – unpublished observations). This study addresses the need for a low cost treatment strategy for the developing world.

Chronic HBV infection moves through different phases [2]. Most people contract chronic HBV in childhood (usually by materno-fetal transfer) and develop viraemia with trivial liver inflammation and a circulating core protein cleavage product (HBeAg). This 'tolerant' HBeAg+ve phase matures into an active phase when an antiviral immune response develops and this activation usually occurs in teenage years. Disease activation leads to liver inflammation, which may progress to cause severe disease, including cirrhosis and liver cancer. In many patients the immune response responds to the infection and controls viraemia leading to a quiescent phase with low-level viral replication, minimal liver inflammation, undetectable HBeAg and HBe antibodies (HBeAb). This HBeAg -ve/HBeAb +ve, inactive phase may be succeeded by viral reactivation and liver damage in an HBeAg –ve/HBeAb+ve active phase which normally develops after the third decade of life.

Therapy for chronic HBV is reserved for phases when liver function tests are abnormal i.e. during the early HBeAg positive 'immuno-active' phase or later during the HBeAg negative disease phase[2]. The immunomodulator, interferon may be used for 48 weeks and induces quiescent disease in ~30% of patients (with better responses in HBeAg +ve patients). Interferon has many side effects, requires monitoring and drug refrigeration, rendering it unsatisfactory in developing countries. An alternative approach is to suppress viral replication with oral drugs. This rarely (<10%), leads to an immune response and HBV surface antigen seroconversion allowing drug withdrawal, so most patients require long term, expensive, carefully monitored, lifelong therapy.

Treatment with oral antivirals often leads to viral resistance, treatment failure and liver damage. L-nucleoside analogues (Lamivudine is the prototype) are associated with resistance in 70% of patients after 5 years [2]. Entecavir is a potent agent with a high barrier to resistance in untreated patients (minimal resistance after 4 years [3]) but in patients with

lamivudine resistance entecavir resistance is seen in 40% after 2 years. The other main class of drugs is the nucleotides (adefovir and tenofovir) which have no cross-resistance with L-nucleosides. Tenofovir is preferred as it is readily available (widely used to treat HIV).Trials in HBV show potency and no resistance after 5 years [4]. Prolonged therapy with tenofovir for HIV rarely leads to renal and bone toxicity. The incidence of these effects in long term therapy for HBV is unknown but regular renal assessments are required and the very long term impact of tenofovir on renal and bone health in patients with chronic HBV remains unclear.

In the developed world entecavir and tenofovir are used to treat HBV. The costs and monitoring needs make these drugs unattractive in resource poor settings – in Africa tenofovir costs £160 per year and needs viral load and other testing costing >£300 annually. By contrast cheap and safe lamivudine (£30 per year and likely to decrease as the patent expires over the next 2 years) has unacceptable rates of resistance. Its availability, ease of use (little safety monitoring is required) make it an attractive agent that continues to be used in resource poor settings, potentially generating resistance and, because of overlap between the polymerase protein and the vaccine epitope of the surface protein, could lead to vaccine escape mutations[5]. Vaccination with the highly successful HBV subunit vaccine has now been incorporated into Zambia's Expanded Programme of Immunisation, and remains the best prospect for long-term control of HBV. However, the benefits will only be apparent in 20-30 years time, and the emergence of vaccine escape mutants would destroy realistic prospects for long-term control or elimination.

Given that a large number of people are infected in Africa and given that the current drugs are expensive it is clear that effective therapy for chronic HBV is unlikely to be widely available unless high quality, cost effective treatment regimes are introduced. A strategy that allowed lamivudine to be administered long term with minimal monitoring and resistance is highly desirable. If viraemia is fully suppressed by potent tenofovir it may be possible to convert to long-term lamivudine because during full suppression of replication the emergence of resistance mutations is much less likely. We have shown that this approach is viable in the UK (see below) and we will now examine this in parts of the world where prevalence is highest.

In the developing world tenofovir and entecavir are widely used to control HBV replication and the effects of therapy are monitored using liver function tests and the level of viraemia (measured as HBV DNA). In general the two tests correlate – the majority of patients with low level HBV DNA have normal liver function tests and vice versa. However monitoring the effects of therapy in the developing world are more challenging, as HBV DNA testing is not widely available. We speculate that the use of liver function tests will allow therapy for HBV to be monitored effectively and in this study we will examine this hypothesis.

3 Justification for the proposed study

Chronic HBV infection is common in the developing world and there are no discounted treatments. The success of current, expensive life long treatments (tenofovir or entecavir) in the developed world has reduced incentives for new drug development. Patients in resource poor settings remain untreated or receive lamivudine – which induces viral resistance in the majority. Discounted strategies to achieve virological control in resource poor areas are needed to reduce mortality from cirrhosis and cancer and prevent the nightmare scenario of drug/vaccine resistant HBV. In developed countries therapy for HBV is monitored using molecular (HBV DNA) assays [2], which are not widely available. However, in patients with active liver inflammation who need therapy, liver function tests (LFTs) correlate closely with HBV DNA measurements and antiviral therapy could be monitored using LFT assessments alone. The success of 'minimal monitoring' regimes in HIV infection shows such approaches are feasible [6] and it is now reasonable to attempt such approaches in HBV. This trial is appropriate for many developing countries. We will base the study in Africa as services are under-developed and if this strategy can be successful here it will inform HBV policies in this region.

Preliminary studies

1. To evaluate the feasibility of induction-maintenance regimes in patients with chronic HBV 25 patients with active hepatitis B who had been treated with tenofovir containing regimes for at least one year were switched to treatment with lamivudine monotherapy. Subjects were reviewed after 1 year. Over 75% of patients who had been changed to lamivudine monotherapy were well controlled on this medication with undetectable HBV DNA and normal liver function tests. In the patients who relapsed on lamivudine monotherapy the return of viraemia was normally relatively rapid (with 3 months) and not associated with significant liver disease. Control of viral replication was rapidly established by re-introduction of tenofovir. These data indicate that induction-maintenance therapy for chronic HBV is feasible, allows a substantial majority of patients to reduce exposure to tenofovir and does not pose risks to patients.

2. We have previously obtained data from blood donors whose blood is rejected by the Zambian National Blood Transfusion Service (Dr Gabriel Muyinda) on grounds of HBsAg positivity. The prevalence of HBV in donated blood samples is 10%, consistent with published estimates (Kasolo et al). We analysed 80 HBsAg-positive blood samples in 2009, and found that ALT was abnormal in 29 (37%) samples. Of these 29, 1 was over 1000 i.u./l, 5 samples were above 100 i.u./l, and 13 were above 50 i.u./l. These data suggest that perhaps 10% of HBV-infected adults in Zambia would fulfil American or European criteria for antiviral treatment, but currently for HIV-uninfected patients no treatment is available.

4 Objectives

The overall objective of this study is to determine whether a large scale clinical trial of induction-maintenance therapy for chronic HBV infection is feasible in Africa. Specifically we will determine:-

1) Whether a treatment regime of tenofovir for 48 weeks followed by lamivudine for 24 weeks effectively suppresses HBV replication in more than 50% of patients

2) Whether re-introduction of tenofovir in patients who have relapsed following introduction of lamivudine successfully suppresses viral replication within 12 weeks

3) Whether viral load testing (HBV DNA testing) can be replaced by cheaper liver function testing without loss of clinically important information

4) Whether induction-maintenance regime for patients with chronic HBV infection in Africa is feasible and acceptable to patients and physicians

5) Whether induction-maintenance regimes are likely to be cost effective.

6) To assess the potential usefulness of quantitative HBsAg testing as a clinical guide to virological control

5 Plan of investigation

Induction/maintenance therapy for HBV is viable if >50% of patients are eligible to convert to lamivudine. Different strategies may be needed for patients with HBeAg +ve and –ve disease but as HBeAg -ve disease is common, a strategy only fit for HBeAg –ve disease will still be of great value. This study will exclude the probability that <50% of patients with HBeAg +ve or HBeAg-ve/HBeAb+ve disease will not benefit from induction-maintenance therapy.

Patients with HBV infection will be recruited from the Blood Bank and clinics of the University Teaching Hospital. These patients will have clinical examination, blood drawn, stools examined for ova of *Schistosoma mansoni*, ultrasound scan performed and will undergo liver biopsy and transient elastography (Fibroscan) testing. 80 patients will then be selected who fulfil the trial inclusion criteria (See below). Of these 80 patients, 40 will be selected who are e antigen (HBeAg) positive and 40 HBeAg negative.

5.1 Inclusion/exclusion criteria

Inclusion criteria:

i. HBV viral load >2000/ml
ii. ALT >36IU/l
iii. Evidence of inflammation on liver biopsy

Exclusion criteria:

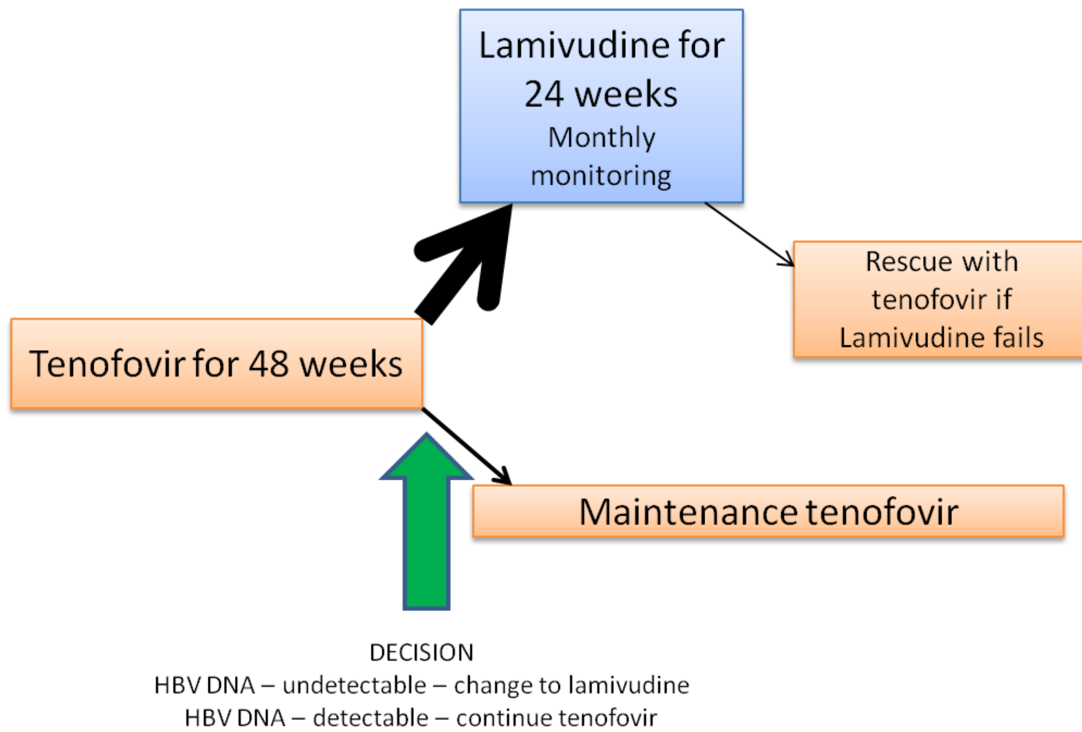
i. Histological or radiological evidence of cirrhosis

ii. HIV infection
iii. History of alcohol abuse or histological evidence of alcoholic liver disease
iv. History of any long-term drug ingestion
v. Histological evidence of metabolic liver disease (haemochromatosis, Wilson's disease, a1-antitrypsin deficiency) or autoimmune liver disease (antibodies to M2, p-ANCA, nuclear antigens, microsomes or smooth muscle)
vi. Histological or radiological evidence of schistosomiasis
vii. Histological evidence of HDV infection
viii. Virological evidence of active HCV or HEV infection

5.2 Study procedures

Eligible patients will sign a consent form and the consent process will be based upon a standardised pro-forma which will be retained with the site file. Patients will be given tenofovir for 48 weeks, with a clinical evaluation after 1, 2 and 3 months and then three monthly. This is standard practice for patients initiating tenofovir therapy. Blood will be tested for ALT, AST and viral load. Compliance with medication will be evaluated by tablet counting. After 48 weeks of therapy HBV DNA will be tested and patients will continue on tenofovir for a further 4 weeks before re-attending. At this week 52 visit patients who have completely suppressed viral replication (i.e. patients in whom HBV DNA is undetectable by sensitive PCR testing) will have their medication changed to lamivudine monotherapy for 24 weeks. They will then undergo monthly monitoring and investigations as above for 3 months followed by 6 weekly assessments for a further 3 months. Any patient who achieves HBeAg seroconversion, will continue treatment regardless of serostatus. Patients who do not fulfil the criteria for conversion to lamivudine will continue on tenofovir with 3 monthly monitoring. Patients who convert to lamivudine and have detectable HBV DNA on follow up blood testing will be re-tested in 2 weeks time and if the increase in HBV DNA is confirmed they will discontinue lamivudine and re-commence tenofovir. Monthly follow up and testing will recommence until HBV DNA is undetectable when 3 monthly monitoring will resume.

A schematic of the flow through the trial is shown below:



A summary of trial related procedures is shown below:

	Screen	Wk 0	Wk 4	Wk 8	Wk 12	Wk 24	Wk 36	Wk 48	Wk 52	Wk 56	Wk 60	Wk 64	Wk 70	Wk 76
Tenofovir dispensed		x	x	x	x	x	x	x						
Lamivudine dispensed (If HBV DNA = 0)									x	x	x	x	x	x
Tenofovir dispensed (If HBV DNA > 100 IU/ml)									x			x		x
Physical examination	x							x						x
Stool exam	x													
Liver ultrasound	x													
Liver biopsy	x													
HCV HEV testing	x													
Screen for other causes of liver disease	x													
HIV testing	x													
Fibroscan	x													
Compliance assessment			x	x	x	x	x	x	x	x	x	x	x	x
FBC	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Renal profile	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Liver function tests	x	x	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	x	x	x
HBV Profile	x	x			x	x	x	x			x			x
Serum sample saved	x	x	x	x	x	x	x	x	x	x	x	x	x	x

5.3 Virological assessments

At recruitment, HCV IgG and HEV IgM will be detected serologically, and positive IgG (HCV) or IgM (HEV) used as exclusion criteria. At every follow-up, HBV viral load (copies/ml) will be quantified by commercially available real time PCR. At a limited number of time points a full HBV serological profile will be carried out: this includes HBeAg and HBeAb.

Samples will be analysed locally. Samples will be re-tested in the UK by a recognised laboratory to ensure quality of testing. In the event of discordant results the UK result will be deemed correct and appropriate remedial action at the local laboratory will be completed.

5.4 Drug dosage and supply

Tenofovir will be given in a dose of 300mg once daily for 52 weeks. This allows for 48 weeks of treatment followed by a period of 4 weeks for evaluation against criteria for stepping down to lamivudine. Tenofovir will be given as tenofovir disoproxil fumarate (TDF), supplied by Cipla Ltd (Mumbai, India) who make a 300mg tablet rather than the 245mg supplied by Gilead, through Prime Pharmaceuticals in Lusaka. The 300mg dose is the same as used in the national anti-retroviral programme.

Lamivudine will be given in a dose of 100mg daily for 26 weeks, which is the dose appropriate for HBV infection. Patients with HIV receive 300mg daily but co-infected patients will be excluded from the Step-Hep study. This will also be procured from Cipla Ltd.

6 Statistical considerations

We will recruit 80 patients.

We anticipate that ~70-80% of patients treated with tenofovir will successfully convert to lamivudine. If <50% of patients successfully 'step down' to lamivudine the approach is unlikely to be useful. We therefore wish to ensure that our pilot project is large enough to reliably exclude a failure rate of >50%. Assume alpha = 0.0500 (two-sided), power = 0.9000 and alternative p = 0.7000 then a minimum of 62 patients are required.

The pilot study will determine whether ALT measurements are equivalent to HBV DNA testing to determine whether or not patients should be transferred from tenofovir to lamivudine. A misattribution of 10% is acceptable and an error rate of 20% is unacceptable (i.e. 20% of patients with normal ALTs have a high HBV DNA and would be inappropriately transferred to lamivudine). The converse (high ALT but undetectable HBV DNA) is likely to be less common and is not a major concern as these patients would continue to take tenofovir and would not be exposed to clinical risk, albeit they would receive the more expensive therapy. Therefore in the pilot study we wish to exclude a misattribution of >20%. Given a sample size of 80 patients alpha = 0.0500 (two-sided), alternative p =

0.1000 estimated power = 0.6436 (confidence intervals 11% - 30%).

It will be important to confirm that patients who relapse when lamivudine monotherapy is introduced can be successfully re-treated with the re-introduction of tenofovir. Assuming that 80 patients are enrolled and 80% (64 patients) are successfully converted to lamivudine we would anticipate that at least 10% will relapse whilst receiving lamivudine and require the re-introduction of tenofovir. Hence we will be able to assess the impact of re-introduction of tenofovir in 6-7 patients providing sufficient confidence that successful reintroduction of tenofovir is viable to support a large clinical trial using this design.

7 Ethical considerations

7.1 Specific

The chief risk to patients involved in this study is that at the completion of the trial medication may be withdrawn if the patient is unable to purchase further treatment. To prevent any harm from treatment cessation at the completion of the study we will provide, at our expense, a further 24 weeks of free therapy (either lamivudine or tenofovir) and we will ensure that all patients have a minimum of 2 years effective antiviral therapy thereby ensuring that they derive significant benefit from the study and have minimal risk of disease re-activation. Note that previous clinical trials have shown that prolonged viral suppression leads to histological improvement and experience from around the world shows that patients who discontinue therapy do not develop life threatening disease reactivation (‘disease flares’). Since we will not enrol patients with advanced liver disease we are confident that treatment cessation will not be associated with undue risk to participants.

This study will involve testing for HIV. It is probable that a number of patients will be identified with this disease. They will be referred to appropriate specialists for therapy in accordance with Ministry of Health guidelines and procedures.

7.2 General

The study team will follow the tripartite harmonised ICH guideline for good clinical practice E6(R1) 1996, with post-step 4 corrections.

Consent will be obtained from individual participants in a face-to-face interview. Written evidence, verified by a witness, will be obtained in all cases, and a thumbprint obtained from all participants who cannot write. An information sheet will be prepared which will be retained by the participant. This makes clear that participation is voluntary and that withdrawal from the research study will not jeopardise future health care in UTH.

Questionnaires and clinical/laboratory information will be stored in a secure, locked

cupboard, and will be kept fully confidential.

8 Documentation and data

8.1 Data handling

All data will be double-entered by trained personnel in the StepHEP office in the Department of Medicine, UTH. Data will be anonymised and backed up regularly onto secure hard discs. Paper records will be kept securely in locked filing cabinets to which only the PI and data manager will have access.

8.2 Drug Accountability

Drug will be provided free of charge and dispensed by the hospital pharmacy in appropriately labelled containers

8.3 Documentation of Adverse Events

The term "adverse event" is defined for purposes of this study as any unwanted physical, psychological or behavioural change experienced by a patient during the course of the study regardless of its severity or relation to the study. Adverse events may include symptoms, signs, unexpected worsening of pre-existing conditions, clinically significant changes in laboratory values, disease and syndromes, and significant and unexpected failures of appropriate case report forms (CRF) throughout the study, and the severity of each adverse event will be graded on a four point scale: mild, moderate, severe, life threatening. The duration of the adverse event and relationship to the study drug will also be recorded.

8.3.1 Definitions

The following definitions will apply to the reporting of the adverse events:

1. Serious Adverse Experience: Any adverse experience that is fatal of life threatening, permanently disabling, requires in-patient hospitalisation, cancer or overdose.
2. Unexpected Adverse Experience: Any adverse experience that is not identified in nature, severity, or frequency in the investigators brochure/SPC summary.
3. Life-threatening Experience: Subject is, in the view of the investigator, at immediate risk of death from the reaction as it actually occurs. This definition does not include reactions that might be fatal if they were to occur in a more serious form.

8.3.2 Tracing Clinical Adverse Events

At the time of each examination at 0, 7 and 14 days from the start of the study, the subject will be questioned regarding the occurrence and nature of any adverse events. All events will be recorded in the subjects' medical records and in the CRFs. Any subject affected will be examined by the investigator as deemed necessary to ascertain the course of the event and any residual effects. All subjects will be instructed to contact the investigator,

investigator's assistants, or clinical personnel should the subject have any serious adverse experiences. If the adverse event is alarming, it must be reported to the DSMB by telephone within 24 hours of the initial report. Serious adverse events, including death regardless of the cause, must be reported to the DSMB immediately.

The severity of the AE will be classified in the following manner

Score	Severity	Definition
0	None	No symptom
1	Mild	Awareness of sign or symptom, but easily tolerated
2	Moderate	Discomfort caused
3	Severe	Incapacitating with inability to work or perform usual activity

All AEs will be classified by the study team in terms of their likely relationship to the study drugs. The classification is set out below:

Score	Causal Relationship	Definition
1	Definitely	Follows in a reasonable temporal sequence from treatment administration and, in the opinion of the investigator is definitely causally linked to the treatment.
2	Probably	The AE follows a reasonable temporal sequence from treatment administration, and cannot be reasonably explained by the subject's clinical state. The degree of certainty with respect to causality is less than that described above.
3	Possibly	The AE follows a reasonable temporal sequence from treatment administration or could have been produced by the subject's clinical state or by other modes of therapy administered to the subject.
4	Remote	The temporal relationship is such the treatment would not have had any reasonable association with the observed event.
5	Definitely Not	The AE is definitely produced by the subject's clinical state or by other modes of therapy administered to the subject.

8.3.3 Reporting Requirement

The Principal Investigator is required to notify the DSMB immediately of any unexpected, fatal, or life-threatening experience and all unusual, alarming, or serious reaction to medication regardless of any opinions as to the cause/effect relationship. A serious event requiring immediate notification by telephone is an event that:

- results in death
- is life threatening
- is permanently disabling
- requires inpatient hospitalization or prolongation of an existing hospitalization
- is a congenital anomaly
- is cancer
- is a drug overdose or results from a drug overdose.

An overdose is defined as any intentional or unintentional consumption of the drug by any route that exceeds the highest dose envisaged in this protocol.

8.3.4 Data Monitoring and the DSMB

A safety and monitoring board (DSMB) of three experienced physicians will be set up to decide if the trial has to be discontinued in the event of life-threatening SAE. No interim analysis will be carried out and it is anticipated that the DSMB may never need to convene unless SAEs are reported according to the above criteria (section 8.3.3). Any SAEs will be reported immediately (within 24 hours) to the Biomedical Research Ethics Committee of the University of Zambia.

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Appendix 3

STEP-HEP CASE RECORD FORM – INITIAL IDENTIFIERS

Name:

Contact person 1:

Contact person 2:

Referred by:

Hospital Number:

What is your house number? _________

Give details of where you live, with nearby landmarks

____

____

____

STEP-HEP CASE RECORD FORM

Screening visit

Inclusion criteria

TICK IF CRITERIA MET

1. AGE ≥ 16 YEARS ☐
2. HBsAg SEROPOSITIVE ☐
3. HBV VIRAL LOAD ≥2000 i.u./ml ☐
4. ALT ≥ 36 i.u./l ☐

Exclusion criteria

TICK IF CRITERIA MET THEN **DO NOT RECRUIT**

1. NOT WILLING TO UNDERGO HIV TEST ☐
2. ETHANOL DEPENDENCY ☐
3. EVIDENCE OF CIRRHOSIS ☐
4. PARTICIPATING IN ANOTHER RESEARCH STUDY ☐

[Evidence of cirrhosis is: ………………………………..]

Initial interview

Age ___ years

Sex 1 male 2 female

Marital status: 1 single 2 married 3 widowed 4 divorced 5 separated 6 co-habiting

Occupation ____ incl. none

Type of work

Level of education attained 0 none 1 primary 2 secondary 3 college 4 university

Do you have any symptoms of illness at the moment? 1 yes 0 no

If so, what are these symptoms?

	Duration (weeks)
1 __________________________________	___________
2 __________________________________	___________
3 __________________________________	___________

Have you ever been told that you have, or been treated for, any of the following?

		Date	Where told
TB	1 yes 0 no		
Hepatitis or jaundice	1 yes 0 no		
Diabetes	1 yes 0 no		
High blood pressure	1 yes 0 no		
Heart disease	1 yes 0 no		
Asthma	1 yes 0 no		

Do you smoke tobacco? 1 yes 0 no

If yes, how many / day? ___

Do you drink alcohol 1 yes 0 no

If yes, is it: 1 every day (beer) 2 every day (kachasu) 3 not every day 4 rare 5 none

Have you had any other serious illnesses and/or operations? List them

1 ____

2 ____

3 ____

4 ____

5 ____

What medication are you currently taking? Also indicate when you started, dose and frequency...

1 ____

2 ____

3 ____

4 ____

5 ____

Examination

General examination: Karnofsky score ___

Is there:	Pallor	0 no 1 yes
	Jaundice	0 no 1 yes
	Cervical lymphadenopathy	0 none 1 left 2 right 3 both
	Goitre	0 none 1 just visible 2 obvious 3 large
	Oral candidiasis	0 no 1 yes
	Oral KS	0 no 1 yes
	Eye problems	0 no 1 yes (what? _____________)
Skin:	BCG scar	0 no 1 yes
	Silky hair	0 no 1 yes
	Pellagra dermatosis	0 no 1 yes
	Other:	

CVS: Pulse /min BP /

Abnormalities on auscultation:

Chest: Breathlessness at rest 0 comfortable 1 just detectable 2 severe

Abnormalities on auscultation:

Abdomen:	Ascites	0 no 1 yes
	Scarring/ markings	0 no 1 yes
	Hepatomegaly	0 no 1 yes
	Splenomegaly	0 no 1 yes
	Kidneys palpable	0 no 1 yes
	Pelvic mass	0 no 1 yes

Weight kg

BMI . kg/m^2

Height m

Record any further evaluations or treatment:

Follow-up visits

Have you had any of these symptoms since your last visit?

Yellowing of eyes	0 no 1 yes
Itching	0 no 1 yes
Abdominal swelling	0 no 1 yes
Nausea	0 no 1 yes
Diarrhoea	0 no 1 yes

Can you estimate how many days you have been unable to take the tablets?

___ out of ___ days since last visit

Examination

General examination: Karnofsky score ___

Is there:	Pallor	0 no 1 yes
	Jaundice	0 no 1 yes
	Cervical lymphadenopathy	0 none 1 left 2 right 3 both
	Goitre	0 none 1 just visible 2 obvious 3 large
	Oral candidiasis	0 no 1 yes
	Oral KS	0 no 1 yes
	Eye problems	0 no 1 yes (what? _____________)
Skin:	BCG scar	0 no 1 yes
	Silky hair	0 no 1 yes
	Pellagra dermatosis	0 no 1 yes
	Other:	
CVS:	Pulse /min	BP /
	Abnormalities on auscultation:	
Chest:	Breathlessness at rest	0 comfortable 1 just detectable 2 severe

Abnormalities on auscultation:

Abdomen:	Ascites	0 no 1 yes
	Scarring/ markings	0 no 1 yes
	Hepatomegaly	0 no 1 yes
	Splenomegaly	0 no 1 yes
	Kidneys palpable	0 no 1 yes
	Pelvic mass	0 no 1 yes

Weight kg

Non-scheduled visits and AEs

What is the current problem? ____

History:

Have you had any of these symptoms since your last visit?

Yellowing of eyes	0 no 1 yes
Itching	0 no 1 yes
Abdominal swelling	0 no 1 yes
Nausea	0 no 1 yes

Diarrhoea 0 no 1 yes

Can you estimate how many days you have been unable to take the tablets?

___ out of ___ days since last visit

Examination

General examination: Karnofsky score ___

Is there:	Pallor	0 no 1 yes
	Jaundice	0 no 1 yes
	Cervical lymphadenopathy	0 none 1 left 2 right 3 both
	Goitre	0 none 1 just visible 2 obvious 3 large
	Oral candidiasis	0 no 1 yes
	Oral KS	0 no 1 yes
	Eye problems	0 no 1 yes (what? ____________)

Skin:	BCG scar	0 no 1 yes
	Silky hair	0 no 1 yes
	Pellagra dermatosis	0 no 1 yes
	Other:	

CVS: Pulse /min BP /

Abnormalities on auscultation:

Chest:	Breathlessness at rest	0 comfortable 1 just detectable 2 severe
	Abnormalities on auscultation:	
Abdomen:	Ascites	0 no 1 yes
	Scarring/ markings	0 no 1 yes
	Hepatomegaly	0 no 1 yes
	Splenomegaly	0 no 1 yes
	Kidneys palpable	0 no 1 yes
	Pelvic mass	0 no 1 yes

Weight kg

Is this a SUSAR? 0 No 1 Yes {IF SO, REPORT IMMEDIATELY TO DR KELLY AND PROF FOSTER]