INDICATIONS FOR AND HISTOLOGICAL DIAGNOSIS OF LIVER BIOPSIES AT HELEN JOSEPH HOSPITAL

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A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of Master of Medicine in Internal Medicine

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DECLARATION

I, M. Nobert Muramira, declare that this research report is my own work. It is being submitted for the degree of Master of Medicine in Internal Medicine at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

……..

M. Nobert Muramira

……...day of ……...2017

DEDICATION

I thank the Almighty God for without Him, I'm nothing. He has brought me this far through thick and thin.

To my dear Wife Sharon and lovely Daughter Gianna, you are an inspiration to me.

To my family, Dad and Mum, my Uncle, my Sisters and Brothers; you are a constant source of encouragement, love and support. You have inspired me to attain great heights. I love you and you mean the world to me.

ABSTRACT

Introduction: Liver pathology is an important contributor to the global burden of disease and is the eighth leading cause of death among South Africans.(1-3) Liver disease is particularly frequent in HIV-infected South Africans.(4, 5) The ongoing evolution of the HIV/AIDS epidemic of Southern Africa requires that the spectrum of liver disease in this region be well described and meticulously monitored.**Objectives:** The primary aim of this study was to describe the histological result of liver biopsies of patients attending the Helen Joseph Hospital (HJH). **Methods:** This was a cross-sectional, retrospective study. A review of medical records of adult patients admitted to the HJH, who had liver biopsies done from 1st January 2008 to 30th June, 2013 was performed. **Results:** 107 liver biopsies fulfilled the entry criteria of the study. Patients' mean age was within ±13.5 years of 41.9 years of age. Two-thirds (65.4%) were HIV-infected. Of this group, 70% were severely immune compromised viz. CD4≤200 cells/mm^3. Two-thirds (62.9%) of the HIV-infected were on ART. A definite and/or probable diagnosis was achieved in 72% of the entire cohort, where the single most frequent infectious pathogen was *Mycobacterium tuberculosis* (n=18/33, 54.5%).Of the group with confirmed TB of the liver, a pre-biopsy diagnosis of hepatic TB had been made in only nine patients (50%). Eighty percent of those diagnosed with drug-induced liver injury (DILI) were HIV-infected. Exposure to either or both TB and antiretroviral drugs was frequent. HIV-infected women were three times more likely to experience DILI than infected men. Liver biopsy confirmed the diagnosis of all patients categorised as having malignancy, alcoholic liver disease (ALD) and non-Alcoholic Fatty Liver Disease (NAFLD), p=0.000. In addition, a high level of certainty was achieved with regard to the categories of Infection and DILI, p=0.000. The causes of liver disease in the HIV-infected and the uninfected *differed* with regard to all the diagnostic categories studied. Specific and coherent antiviral management of those with hepatitis

B and C infections was weak or non-existent. **Conclusions:** In the era of a changing HIV epidemic, liver biopsy continues to provide diagnostic value to the management of both the HIV-infected and uninfected.

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NOMENCLATURE

ADPKD	Autosomal Dominant Polycystic Kidney Disease
AFB	Acid-Fast Bacilli
AIDS	Acquired Immunodeficiency Syndrome
ALD	Alcohol-related Liver Disease
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
ART	Antiretroviral Therapy
ARV	Antiretroviral
AST	Aspartate Aminotransferase
CPS	Child-Pugh Score
CTX	Cotrimoxazole
DILI	Drug Induced Liver Injury
FBC	Full (complete) Blood Count
GFR (eGFR)	Glomerular Filtration Rate (e=estimated)
GGT	Gamma Glutamyl Transferase
GXP	GeneXpert
GBD	Global Burden of Disease (Study)
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HCC	Hepatocellular Carcinoma
HIV	Human Immunodeficiency Virus
HJH	Helen Joseph Hospital
HREC	Human Research Ethics Committee

INR	International Normalised Ratio
IRIS	Immune Reconstitution Inflammatory Syndrome
IQR	Interquartile Range
LFT	Liver Function Tests
NAFLD	Non Alcoholic Fatty Liver Disease
NASH	Non-Alcoholic Steatohepatitis
NICD	National Institute of Communicable Disease
NNRTI	Non-Nucleoside Reverse Transcriptase Inhibitors
NRTI	Nucleoside Reverse Transcriptase Inhibitors
SD	Standard Deviation
TB	Tuberculosis
ULN	Upper Limit Normal
IREP	Isoniazid, Rifampicin, Ethambutol and Pyrazinamide
IR	Isoniazid and Rifampicin.

CHAPTER 1

1.0 INTRODUCTION

The liver is particularly vulnerable to metabolic, toxic, microbial, circulatory and neoplastic injury.(6) Liver pathology is therefore an important contributor to the global burden of disease. (1, 2), In the 2010 Global Burden of Disease (GBD) Study, liver cirrhosis accounted for 2.0% (n=1,030 800) of deaths worldwide.(1) Although this condition is only the eighth 'leading' cause of death among South Africans, the incidence of liver disease in this population appears to be rising. (3, 7) In the United Kingdom (UK) as a cause of death, liver disease is now ranked fifth, after cardiovascular, stroke, chest diseases, and cancer. (7, 8)

Two percent of deaths in the United States of America (USA) are attributed to liver disease. (9) Infection with the hepatitis B and C viruses (HBV, HCV), alcohol-related liver disease (ALD) and non-alcoholic fatty-liver disease (NAFLD) are the leading causes of these deaths. (10) NAFLD has become the most common liver disorder in industrialised countries, affecting 20-40% of North Americans and Europeans.(11-14) A subset of NAFLD, non-alcoholic steatohepatitis (NASH), is particularly aggressive, and affects 6-17% of the group. NASH carries the risk of progression to cirrhosis and to hepatocellular carcinoma (HCC). The incidence of NASH reflects the prevalence of overweight and obesity in the human community. In South Africa, more than 29% of South African men and 56% of women are classified as overweight or obese.(15)

Drug-Induced Liver Injury (DILI) is difficult to manage, is associated with significant morbidity and mortality, and is an important cause of liver disease in South Africa.(16, 17)The dual

epidemics of Human Immunodeficiency Virus (HIV) and tuberculosis (TB) in Southern Africa has resulted in the exposure of large numbers of people to drugs that are hepatotoxins.(4, 16, 18) Nonetheless, liver disease itself is frequent in HIV-infected South Africans, whether or not exposed to ART or TB drugs, (4, 5) and liver biopsy is occasionally required to resolve diagnostic uncertainty in the care of these patients.(19)

By the end of 2011, thirty-four million people (range, 31.4-35.9) worldwide were living with HIV, 6.4 million of which were South Africans. (17) Globally and in Africa, greater numbers now access ART and live longer, yet remain susceptible to TB, opportunistic disease, comorbid conditions, and the diseases of aging. The prevalence of liver disease among these is expected to increase. (20) Chronic infection with the HBV and HCV is common in various parts of Africa. In Southern Africa, the background prevalence of infection with HBV is high, 5-17 percent. While the prevalence of HCV in this region is low (<1%), effective oral treatment has recently become available to some in Southern Africa.(21-23) Dual infection with HIV and either HBV or HCV renders patients at greater risk of progressive liver disease. (18, 24) The expansion of therapeutic options and the growing pressure on manufacturers to provide affordable drugs provides hope that all in need will eventually access appropriate treatment.

TB is the most frequent cause of death of HIV-infected Africans (25), where its presence is easily missed in those with advanced HIV. Undiagnosed TB was found in 50% of HIV-infected Kenyans at the time of autopsy. The TB was disseminated in 80 percent of these cases. Fifty percent had evidence of liver involvement viz. hepatic granulomas.(26) TB treatment predisposes patients especially the HIV infected to elevations of liver enzymes, and the onset of DILI.(27, 28)

Occasionally, abnormal liver tests following the start of ART, signal the onset of an unmasking or paradoxical 'immune reconstitution inflammatory syndrome' (IRIS). Both DILI and TB-IRIS can be life-threatening. Both cause diagnostic uncertainty. Liver biopsy is sometimes useful in resolving the dilemma.(29, 30)

Most clinicians view liver biopsy as the ultimate tool with which to confirm a problem diagnosis. (31) But liver biopsy has its limitations. Firstly, obtaining an adequate tissue-core is operator dependent; then, sampling errors arise when lesions are small or few or where radiographic biopsy-guidance is unavailable; furthermore, the biopsy may not be representative. Histology can be non-specific and non-diagnostic, e.g. drug and toxin-induced injury, overwhelming sepsis; system-defects are frequent and bias defines the patient population; people requiring liver biopsy have failed easier attempts at diagnosis and are often ill with comorbid conditions, and in receipt of multiple, potentially toxic drugs; randomised controlled studies are not done in this study population; and there are no 'controls'. Notwithstanding these impediments, the constant evolution of health care in Africa and in particular, the fluid nature of the HIV/AIDS epidemic, demand the clearest description of disease and its meticulous monitoring. It is in this context that this study has been undertaken.

1.1 AIMS AND OBJECTIVES

1) To describe the histological diagnosis of liver biopsy specimens of patients attending the Helen Joseph Hospital between January 2008 – June 2013.
2) To tabulate the indications for liver biopsy.
3) To ascertain the demographic characteristics of patients who have had a liver biopsy.

4) To describe the results of liver function tests (LFTs) taken at the time of the liver biopsy.

5) To describe the liver biopsy findings of a subgroup of patients with tuberculosis-related drug-induced liver injury (TB-DILI).

6) To compare liver biopsy findings in the HIV-infected and the HIV-uninfected.

CHAPTER 2

2.0 METHODS

2.1 STUDY DESIGN

This is a cross-sectional, retrospective study. We performed a record review of all adult patients admitted to the Helen Joseph Hospital (HJH), who had liver biopsies done from 01.01.2008 to 30.06.2013.

2.2 STUDY SETTING

The HJH, in Johannesburg, South Africa, is a tertiary-level public hospital that forms part of the teaching-hospital complex administered by the state, and supported by the University of the Witwatersrand and its Medical School.

2.3 STUDY POPULATION

Patients who attend the HJH are mostly residents of the cities of Johannesburg and Soweto in the Gauteng Province, South Africa, where a small number are referred from distant clinics and hospitals, and the occasional patient is a visitor from a neighbouring country in the southern African region.

2.3.1 Sample Size

Convenience sampling was applied and included all adult patients who had liver biopsies done at Helen Joseph hospital, and who met the selection criteria.

2.3.2 Selection Criteria

2.3.2.1 Inclusion Criteria

1- Adult patients (age ≥18 years)

2- Patients who had had a liver biopsy during the time period 01.01.2008, 30.06.2013 and for whom medical records and a liver histology result were available for assessment.

2.3.2.2 Exclusion Criteria

1- Patient hospital record/file not retrievable

2-Liver biopsy sample 'inadequate' or unavailable for histological assessment

2.4 STUDY MEASUREMENTS

Demographic data included the subject's gender and age at the time of the liver biopsy. Where available, the following additional information was retrieved from the patient notes, viz. the patient's HIV status, the most recent CD4 cell count i.e. within 6-12 months of the time of the liver biopsy, alcohol use, traditional medication and recreational drugs, the indication for the liver biopsy and the clinically suspected diagnosis prior to the biopsy, the use of prescribed drugs such as co-trimoxazole, antiretroviral therapy (ART), anti-TB treatment; and other antimicrobials. Laboratory parameters included the patient's hepatitis A/B/C status, liver function tests (LFTs) and the international normalised ratio (INR), the full blood count (FBC), renal function tests e.g. the urea and electrolytes and the estimated glomerular filtration rate (eGFR), histology, serology, microscopy, cultures, and tests to diagnose sepsis including tuberculosis (TB). Acute blood tests such as the FBC, and renal and liver function tests, were taken as close as possible to the time of the biopsy, and therefore do not necessarily reflect admission results. Where available or known, the Child-Pugh score (CPS) was recorded. Furthermore, data on the clinical outcome, the presence of co-morbidities, as well as pertinent radiological data, such as the abdominal sonar and/or the

report of an abdominal CT scan, were collected. The CT scans and liver histologies were reviewed and reported on by a senior member of the radiology and histopathology departments of the University of the Witwatersrand medical school and/or the National Health Laboratory Service (NHLS), based at the Helen Joseph hospital or the National Institute for Communicable Diseases (NICD), Johannesburg. The data was collected from the original CT scan and histopathology reports and no further review with the investigator was done as part of this study.

2.5 STUDY IMPLEMENTATION

Each patient's file was reviewed for the required information. Additionally, the patient's clinical course, management, outcome, and, if available, follow-up data, were noted. The liver biopsy and laboratory results were obtained from the NHLS database based on the original histological report without any further clinical interpretation. Once retrieved, all data was captured on a data-collection sheet (Appendix A). All patient-identifying data was removed between initial retrieval and recording in the database. Anonymised information from the data collection sheet was captured onto a Microsoft Excel™ spreadsheet to form the database of the study.

2.6 ETHICS APPROVAL

Ethics approval was obtained from the Human Research Ethics Committee (HREC) of the University of Witwatersrand-clearance certificate number M121197. Approval was sought and obtained from the Chief Executive Officer (CEO) of the HJH to review patient files. Professor M.J. Hale, Head of the Department of Anatomical Pathology at NHLS/University of the Witwatersrand, granted permission to review the liver histology results.

2.7 STATISTICAL ANALYSIS

Data was imported from the MS-Excel™ spreadsheet into a statistical software package, viz. STATA version 12. A descriptive analysis was chosen to summarise the patients' demographic, clinical, laboratory and radiological characteristics. Categorical variables such as gender have been characterised as frequencies and percentages. Normally distributed variables, such as age and lab results, e.g. LFTs, are represented as means and standard deviations. The data is presented in frequency tables, bar graphs and pie charts. Medians and inter-quartile ranges have been determined for abnormally distributed data. The Chi-square test was chosen to describe associations between two categorical variables and significance was set at a value of $p<0.05$.

The liver function tests are grouped into four categories, based on the predominant pattern of liver enzyme derangement, as per the ratio (R) of ALT to ALP levels (expressed as multiples of the upper limit of normal, ULN): (19)

- Cholestatic pattern (ALT/ULN: ALP/ULN, R<2)
- Hepatocellular pattern (ALT/ULN: ALP/ULN, R>5)
- "Mixed" pattern (R= 2-5)
- Normal (ALP and ALT: R<1.3xULN)

The Child-Pugh score (CPS) was calculated as per the accredited system.(32)

CHAPTER 3

3.0 RESULTS

3.1 PATIENT CHARACTERISTICS

During the study period of 2008-2013, 401 liver biopsies were performed at the Helen Joseph Hospital. Two-hundred and ninety-four had to be excluded, because patient records could not be found. Hospital records were available for the remaining 107 patients. Liver biopsies were performed to identify the cause of persistently abnormal liver function tests in patients who were otherwise well (patients who were found to have deranged liver function tests and were not resolving with no cause that could be established) (n=29, 27.1%), or to assist in the diagnosis of ill patients with deranged liver function tests (n=78, 72.9%). Fifty-six (52.3%) patients were female, and 51 (47.7%) were male, a gender ratio of 1.1:1. Ages ranged from 18-84 years, but the mean age was 41.9±13.5 years, and was the same for men and women. A third (30.8%) were aged from 31-40 years and half (51.4%) were ≤40 years. (Table 1, Figure 1)

Two-thirds of the group (n=70/107, 65.4%) was infected with HIV. The HIV status of 11 (10.2%) was unknown. Two-thirds of the HIV-infected (n=44/70, 62.9%) were on ART. Seventy percent of the group of infected subjects had a CD4 count below 200 cells/mm3. The mean CD4 count of the entire group was only 95 cells/mm^3. Most were on a 'first-line' regimen, comprising two nucleoside/tide reverse transcriptase inhibitors (NRTIs) and one non-nucleoside reverse transcriptase inhibitor (NNRTI). Eighty percent (n=31/44) were on efavirenz (EFV). Only two (n=2/44, 5.2%) were on nevirapine (NVP). Six (n=6/44, 15.4%) were on a lopinavir/ritonavir (LVP/r, aluvia) protease inhibitor-based second-line regimen. The nucleoside/tide backbone comprised standard combinations of tenofovir, zidovudine, abacavir and lamivudine, drugs not

usually associated with hepatotoxicity. The antiretroviral regimen of five is unknown. The mean duration on ARTs before liver biopsy was 55.2 weeks (3-240).

Twenty-four (n=24/107, 22.4%) were on anti-tuberculous medication (TB-drugs). All except one, were HIV-infected. Nineteen (n=19/24, 79.2%) were on both anti-tuberculous and ART medication. Seven (7/24, 29.2%) were on Rifinah (Isoniazid and Rifampicin), while seventeen (17/24, 70.8%) were on Rifafour (Isoniazid, Rifampicin, Pyrazinamide and Ethambutol). Concurrent exposure to cotrimoxazole (Bactrim) was confined to the HIV-positive subjects, but did not include all of them, n=14/70, 20 percent. Co-infection with Hepatitis B and C viruses was confirmed in nine and three subjects, respectively. The clinical severity of the liver disease at the time of the biopsy was measured in 99 subjects by means of the Child-Pugh score (CPS). This was normal or near normal, viz. 6.3±1.4 (range: 3-11) in the majority. Concurrent HIV infection was frequent among subjects in each of the three CPS-severity grades viz. A, least severe (n=37, 66%), B, moderate severity, (n=24, 61.5%) and C, most severe (n=4, 100%). HIV positive status did not appear to influence the CPS-score (p=0.28) per se. A small number of patients were known to abuse alcohol (n=5), use recreational drugs (n=2) and to take African traditional medicines (n=3) regularly.

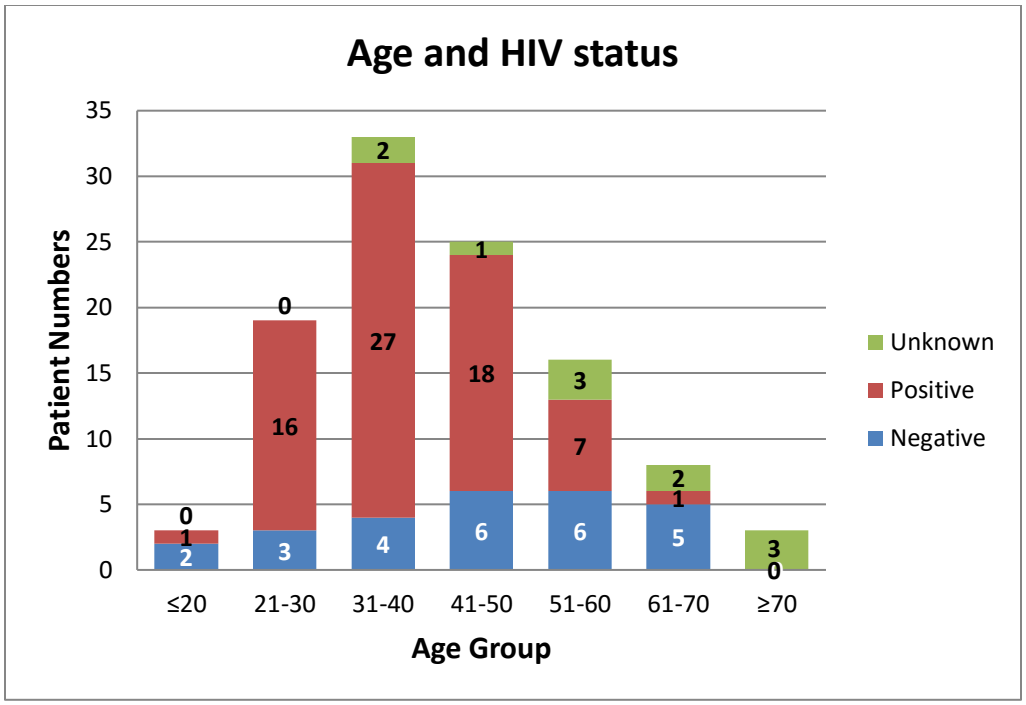

Figure 1 Age and HIV Status of Patients Undergoing Liver Biopsy

Table 1 Age, Gender, HIV Status, CD4 level and Antiretroviral Therapy (ART) Use

Study Parameter	Number, n (%) or Mean (±SD)	HIV Positive: n (%)	HIV Negative: n (%)
Patients, n (%)	107 (100)	70 (65.4)	26 (24.3)
Age (yr., Mean/SD)	41.9±13.5	37.8±9.6	45.7±14.2
Gender: Female n (%) Male n (%) Male : Female ratio	 56(52.3) 51(47.7) 1.1:1	 *41 (73) *29 (57)	 *11 (20) *15 (29)
HBsAg Positive	9 (8.4)	7	2
Anti-HCV Positive	3 (2.8)	1	2
Antiretroviral Therapy (ART)	44/107 (41.1)	44/70 (62.9)	N/A
Cotrimoxazole (CTX, Bactrim®)	14/107 (13.1)	14	0
Anti-Tuberculous Medication	24/107 (22.4)	23 (32.9)	1 (3.8)
Child-Pugh Score	99/107 (92.5%)	6.3± 1.5	6.0±1.2

SD = standard deviation; IQR= inter-quartile range. *Note that for some subjects, viz. four women and seven men, their HIV status is unknown.

3.2 LABORATORY CHARACTERISTICS

All blood test results in this study were the most recent prior to the biopsy: taken at the time of the liver biopsy, or within a two-week prior period (the liver function tests), or within a two month prior waiting period for all other blood tests.

3.2.1 Haemoglobin (Hb)

Moderate and/or severe anaemia i.e. Hb 9.9-8.0g/dl (moderate) and ≤7.9g/dl (severe), was present in 42 of the 106 subjects (39.6%) tested. Most (n=35/42, 83%) were HIV-positive. Five (n=5/42, 12%) were negative. The association of anaemia with HIV-infection was significant, and correlated with decreasing haemoglobin levels (p=0.006). (Figure 2) Patients with severe anaemia, viz. Hb. ≤7.9g/dl, were also more likely to have very low serum albumin levels i.e. albumin <25 g/l (p=0.000). However, a direct association of anaemia with other indices of severe liver dysfunction, such as the INR (p=0.191) or an elevated Child-Pugh Score (p=0.257), was not confirmed.

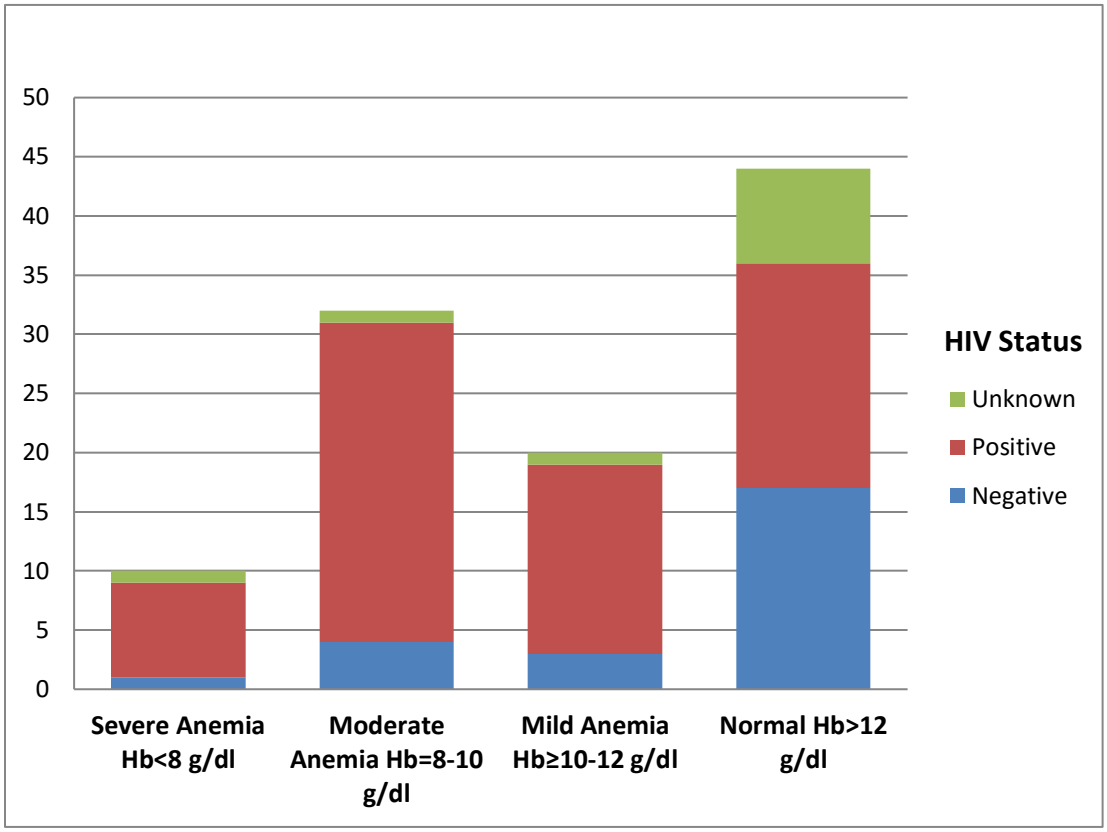

Figure 2 Prevalence and Severity of Anaemia in Patients undergoing Liver Biopsy

3.2.2 Liver Function Tests (LFTs)

Each patient's biochemical/liver enzyme results were grouped into the following patterns: cholestatic/infiltrative, hepatocellular and mixed. Chapter 2.7, p. 8, (Table 2, Figure 3) HIV-infected subjects formed the majority of individuals in each group. No particular pattern was characteristic of the HIV positive cohort, p=0.534. A small number (n=13/107, 12.2%) had normal liver enzyme levels at the time of the liver biopsy. The cholestatic/infiltrative pattern was the most frequent over all viz. n=61/107, 57%. This pattern with the addition of an elevated total bilirubin, characterised those on TB drugs, n=20/24 (83.3%). All were on rifampicin-based TB therapy. No-one on TB drugs had a purely hepatocellular pattern, but three (12.5%) had a 'mixed' pattern and one had normal liver enzymes. Only seventeen (n=17/107, 16%) had a predominantly hepatocellular pattern, seven (n= 7/17, 41%) of these had a DILI. The remaining 13 DILI patients had either a cholestatic (n=8/20) or a mixed pattern (n=5/20). Sixteen patients (n=16/107, 15%) had a 'mixed' enzyme pattern.

Table 2 Baseline Serum albumin, Liver-Enzyme Patterns by HIV status at the Time of Liver Biopsy

Study parameter	Total group n (%)	HIV positive n (%)	HIV negative n (%)	p-value
Patients	107 (100%)	70 (65.4)	26 (24.3)	0.007
Hepatic Biochemistry: Bilirubin-Enzyme Patterns				
Cholestatic/ Infiltrative	61 (57.0)	42 (68.8)	12 (19.6)	0.224
Hepatocellular	17 (15.9)	10 (48.8)	5 (29.4)	0.553
Mixed	16 (14.9)	12 (75.0)	4 (25.0)	0.837
Normal LFTs	13 (12.2)	6 (46.1)	5 (38.5)	0.145
Liver Biochemistry at the Time of the Liver Biopsy				
Abnormal LFTs	94 (87.9)	64 (91.4)	22 (84.6)	0.477
	Mean *(SD)	**Mean *(SD)**	**Mean *(SD)**	
Albumin (g/l)	25±7.6	23.6±7.6	28.2±7.4	0.074

*SD = standard deviation;
Note that this table does not include the biochemical data of subjects whose HIV status is unknown.

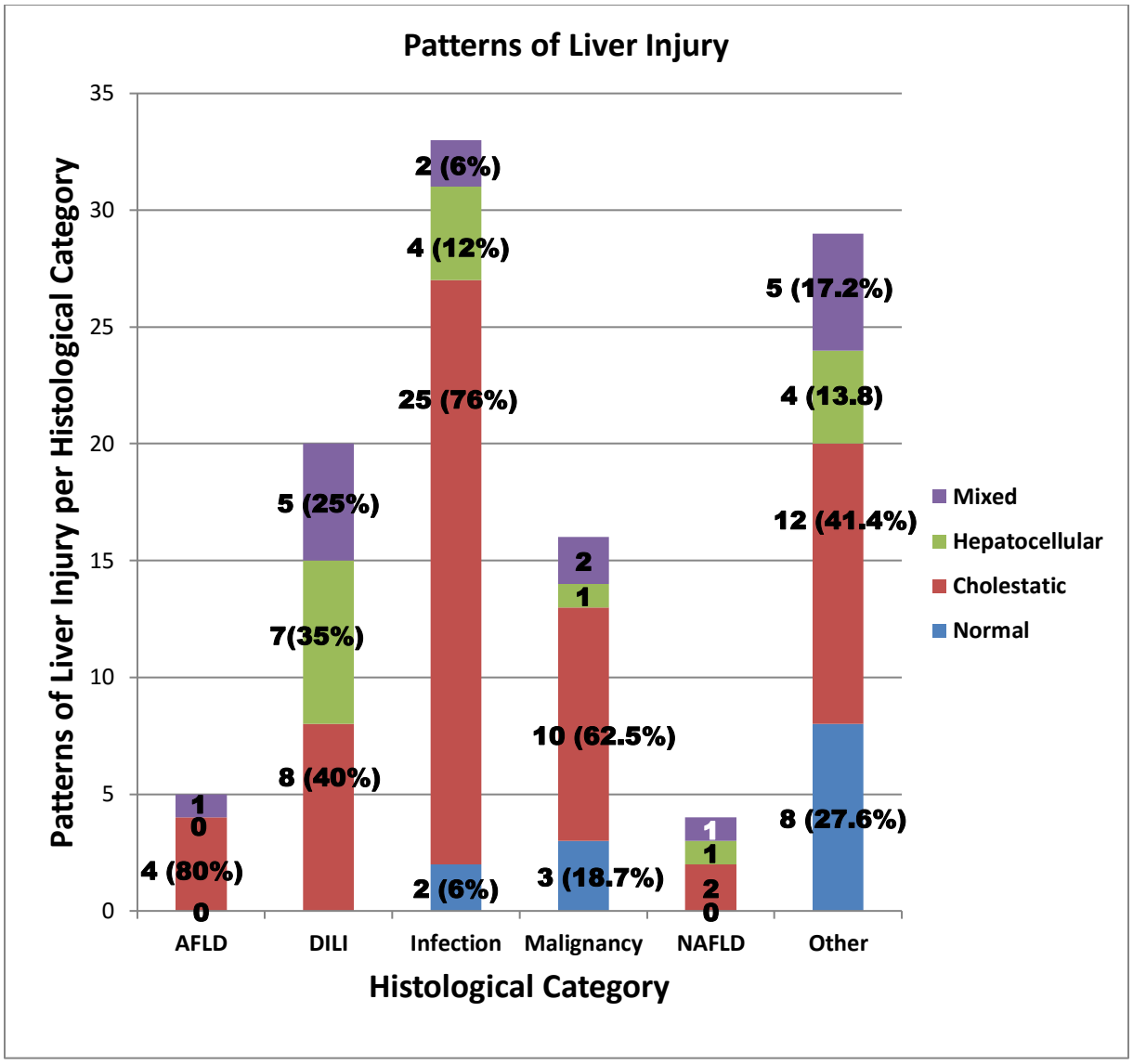

Figure 3 Liver Enzyme Patterns in the Cohort of 107 Patients Undergoing Liver Biopsy

Table 3 Statistical Likelihood that the Cholestatic Liver Enzyme Pattern Identified those Subjects with an Infective Cause of Liver Injury

CHOLESTATIC PATTERN		
	Infection Present	**Infection Absent**
Cholestasis Positive	25	36
Cholestasis Negative	8	29
Sensitivity for Cholestasis	25/(25+8)	75.76%
Specificity for Cholestasis	29/(29+36)	44.62%
PPV	25/(25+36)	40.98%
NPV	29/(29+8)	78.38%

Legend: PPV = positive predictive value; NPV = negative predictive value.

3.2.3 Albumin and the International Normalised Ratio (INR)

The mean serum albumin and INR levels for the entire group were 25±7.6 (g/l) and1.22 ±0.34, respectively. (Figure 4) Most of the HIV-infected cohort had severely depressed albumin levels i.e. serum albumin <25g/l, (n=37/67, 55%) compared with the HIV-negative group, (n=8/24, 33%, p=0.066). Three quarters (75%) of the HIV-infected, who had severely depressed albumins, had low current CD4 counts viz. <200 cells/mm3. No correlation was found between the serum albumin and the INR (*p*=0.222). A severely prolonged INR would have ruled out a liver biopsy. HIV-negative subjects were more likely to have normal serum albumin levels, viz. serum albumin >35g/l: n=3/24, 12.5%, than the HIV-positives, n=3/67, 4.5 percent.

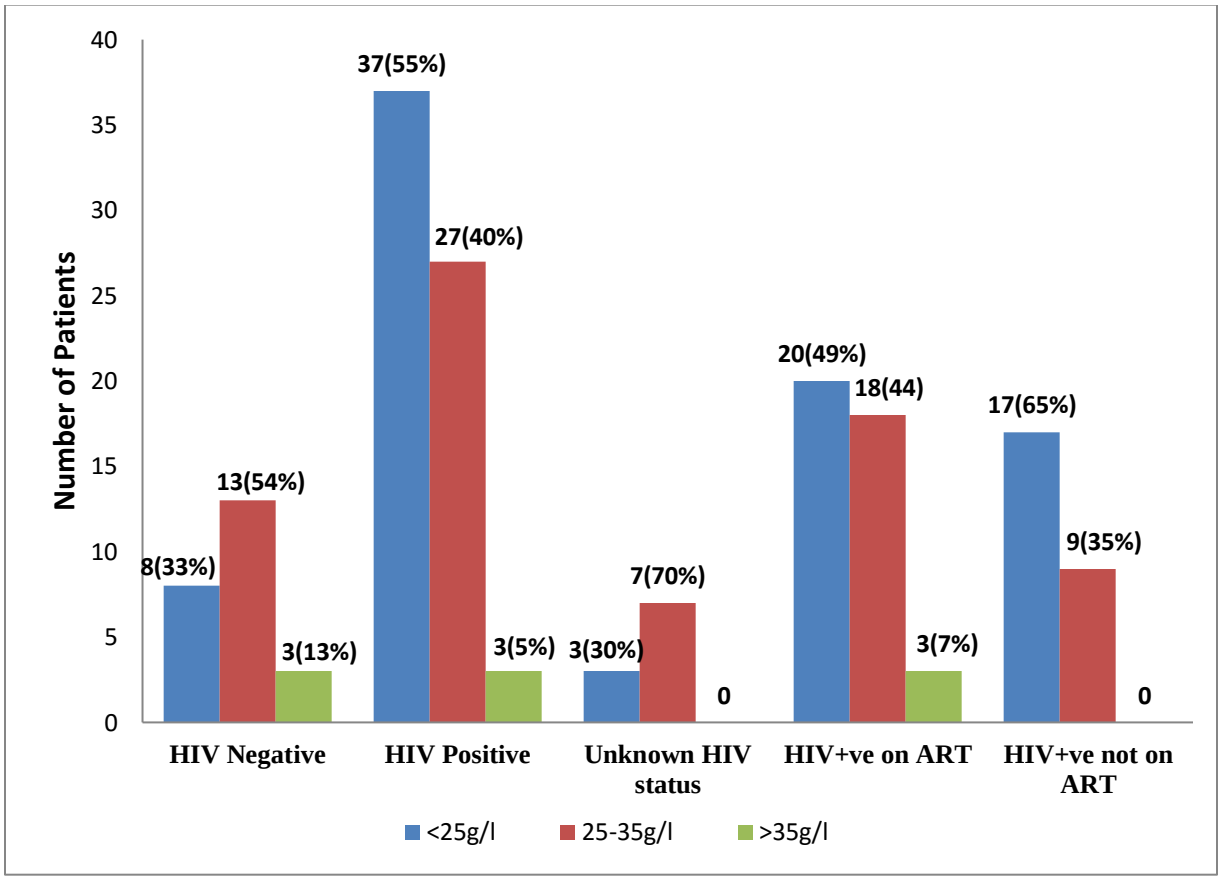

Figure 4 Serum Albumin Levels According to HIV and Antiretroviral Status

3.2.4 Hepatitis B and C virus

Serological tests of hepatitis B and C status were available for 97 (90.7%) patients. Twelve (12.4%) had been exposed to either HBV or HCV. Thirty-three percent (4/12) were also on TB medication. Three (3.1%) had antibodies to hepatitis C, one of whom had a dual infection with both HCV/HIV. Active hepatitis B was diagnosed in the remaining nine (9.3%), seven of whom were also infected with HIV. None of the seven had CD4 counts above 200 cells/mm3, despite concurrent use of lamivudine and tenofovir in four. Neither of the two HBV-infected, but HIV-negative was on tenofovir and/or lamivudine. Of the seven co-infected with HIV, only four 7.1%) were on full ART therapy. No subject was simultaneously infected with both HBV and HCV. No subject had a HBV-DNA (viral load) level measured. Of the three with antibodies to HCV only two had had levels of HCV-RNA (viral load) checked. Detectable HCV was confirmed in only one. No subject with HCV infection had been placed on anti-HCV medication.

3.3 LIVER BIOPSY CHARACTERISTICS

To facilitate analysis, the data from the liver biopsy (the histological result) was organised into the following six aetiological categories. (Tables 4 and 5)

- **Infection**
- **Drug-Induced Liver Injury(DILI)**
- **Malignancy**
- **Alcoholic Liver Disease(ALD)**
- **Non-Alcoholic Fatty Liver Disease (NAFLD)**
- **Other: a group of miscellaneous conditions**

The histology did not always provide a definitive diagnosis. Where additional data supported a specific aetiology, the following terms were used to characterise the final diagnosis: definite, probable or possible. See **Appendix C** for clarification of definitions. A definite and/or probable

diagnosis was achieved in almost three-quarters (72%) of the group: definite (n=30, 28.0%) and probable (n=48, 44.9%). (Table 4) The Infection category was the largest (n=33, 30.8%), followed by 'other' (n=29, 27%) and 'DILI' (n=20, 18.7%). The highest Child-Pugh scores i.e. those with the most severe liver disease, were found in 'infection' and 'DILI' categories. The single most frequent infectious pathogen identified was *Mycobacterium tuberculosis* (n=18/33, 54.5%). Active TB was exclusive to the HIV-infected group. Higher numbers of HIV-positive subjects (n=26/33, 79%) occupied the infection category versus the HIV-negative, n=7/33, 21%.The cause of infection differed between these groups. Infections of the HIV-infected group reflected a background presence of HBV and HCV, as well as advanced immune compromise, viz. disseminated TB and HIV-cholangiopathy. HBV and HCV were present in the HIV-negative group too, but the dominant infections were pyogenic and parasitic. Similarly in the 'other' category, histologically-confirmed causes differed between HIV-positive and negative. Where HIV-infection (Table 5) advanced immune suppression, CD4 counts <200 cells/mm3 characterised the HIV-positive subjects in the infection (69%), DILI (69%) and malignancy (71%) categories, but not the HIV-positive in the 'other' (40%) category. Moreover, in this category, liver histology was non-diagnostic* in 62% (n=18/29), i.e. in 17% (n=18/107) of the entire study cohort. Eighty percent (n= 15/18) of this 'unknown' group were HIV-positive.

The categories, ALD and NAFLD, had the fewest subjects and the smallest percentage of HIV-positive members.

Malignancy was diagnosed in 15% (n=16/107) of liver biopsies. Seven were HIV-infected, n=7/16 (44%). Four were uninfected (n=4/16, 25%) and the HIV-status of the rest was unknown, n=5/16, 31 percent. Of the seven who were HIV-infected, five had advanced immune suppression with CD4 counts <200 cells/mm3. Four of the tumours were AIDS-defining viz. Large B-cell non-

Hodgkin's lymphomas (NHL), n=4/16, 25 percent. All were in HIV-infected subjects. Concurrent HBV or HCV infection was present in three (37.5%) of the patients with hepatocellular carcinoma (HCC). Apart from the presence of non-diagnostic biopsies in the 'other' category, this group also contained a variety of unrelated and unusual conditions that could not have been diagnosed without biopsy. Concurrent infection with HIV was frequent in the 'other' category as well.

Table 4 Liver Histology Categories by HIV Status

Histological Categories	**Number (n, %)**	**Age (yr)**	**HIV Status (n=107)**					
			HIV+ve (Total)	**CD4 Level (HIV+ve)**			**HIV-ve**	**HIV Status Unknown**
				<200 (%)	**200-350 (%)**	**>350 (%)**		
ALD	5 (4.7)	54.2±15.6	0	0	0	0	3	2
DILI	20 (18.7)	37.6±10	16*	11	2	2	3	1
Infection	33 (30.8)	37.9±9.6	26*	18	2	3	7	0
Malignancy	16 (14.9)	49.1±15.5	7	5	1	1	4	5
NAFLD	4 (3.7)	44.8±22	1	1	0	0	2	1
Other	29 (27.1)	43.1±14.3	20*	8	6	1	7	2
Total	107 (100)	41.8±22	70*	42	11	7	26	11

ALD=alcoholic liver disease; DILI=Drug-induced liver injury; NAFLD=non-alcoholic liver disease. *NB. Individual CD4 count results were not available for all of these subjects.

3.3.1 Liver Biopsy, Histology and the Diagnosis of Infection

Thirty-three of the 107 patients in this study (30.8%) had an identifiable *infectious* cause of their liver disease. The diagnosis was definite in nine, probable in 18 and possible in six. Although tuberculosis (TB) was the most frequent single infection found in this study, several additional infections are identified in Table 5. The infectious causes tabulated under the HIV-infected and uninfected columns differ. No subject of unknown HIV status had liver histology fitting the category 'infection' or 'other'. Twenty percent (20%, n=5/26) had an HIV-associated cholangiopathy. A small number (n=10, 9.3%) had suspected or documented concurrent non-tuberculous bacterial infections during their admission, i.e. prior to the liver biopsy. All were given antimicrobial therapy. Only four infections were bacteriologically confirmed viz. *Escherichia co*li (x2, blood and urine), *Enterobacter cloacae* (x1, blood), ESBL-producing *Klebsiella pneumonia* (x1, blood). None were believed to be responsible for the histological changes on liver biopsy. Most (80%) responded to antimicrobial therapy.

*ESBL = extended-spectrum beta-lactamase producing organism

3.3.2 Liver Biopsy, Histology and Concurrent Tuberculosis

Histological examination suggested tuberculous infection of the liver in 18 (16.6%) patients. (Table 5) However the acid-fast bacillus (AFB/Ziehl-Neelsen) stain was positive in only four histology samples. The diagnosis of TB was suggested by the presence of hepatic granulomas in the remaining 14. Microbiologically, infection with TB was confirmed on culture or with a Gene-probe assay [GeneExpert (GXP) test] of non-hepatic tissue in 14, but in only nine (64.3%) was TB microbiologically confirmed (culture/GXP) to be in the liver itself. Of the 24 who were taking anti-tuberculous medication (TB drugs) immediately prior to the liver biopsy, only 10 (41.7%) had biopsy evidence supporting direct infection of the liver with TB. Only one on TB medication prior to the liver biopsy was HIV-uninfected, p=0.002. (Table 1) Nine of the 18 (50%) patients with TB in the liver-biopsy sample (above) had not been diagnosed with TB nor had TB drugs been started prior to the biopsy. Of the 24 on TB drugs at the time of biopsy, liver biopsy supported a diagnosis of DILI in 6 (25%). Of those with DILI, the mean duration of TB treatment prior to the liver biopsy had been 3.3 months ±1.5 SD. The frequency of DILI was unaffected by the TB regimen, i.e. rifafour or rifinah. Nonetheless, the concurrent use of TB drugs did confer a risk of DILI, p=0.001. The majority of those on TB drugs and those with confirmed TB infection of the liver, displayed a cholestatic/infiltrative liver enzyme pattern, n=20/24, 80%, p=0.015 derived by chi-square, and n= 15/18, 88.2%, *p-0.012* derived by fishers exact, respectively.

3.3.3 Liver Biopsy, Histology and Drug-Induced Liver Injury, DILI

Twenty patients were diagnosed with drug-induced liver injury (DILI). A clinical diagnosis of DILI had been made in only 13 prior to biopsy. The histology of 13 showed features of active TB (n=9), HIV-cholangiopathy (n=3) and viral hepatitis (n=1) in addition to DILI. Notwithstanding this and the fact that the histology of DILI is non-specific and that the diagnosis is usually one of exclusion, liver histology compatible with a diagnosis of DILI was determined to be 'probable' in 90%, n=18/20. (Table 5) Eighty percent (n=16/20, 80%) of the DILI subjects were HIV-positive. Women, many of whom were HIV-infected and had CD4 counts ≤200 cells/mm^3 (69%), were three-times more likely than men to be diagnosed with DILI. Of the twenty with DILI on histology, eight (40%) were only taking ARVs, five (25%) were on both ARVs and TB medication, and three (15%) were only on TB drugs. Two (10%) were on traditional (African herbal) medications. Drug data was missing for two patients. Of the 13 subjects with DILI who were on ART, most had been exposed to efavirenz viz. 10 (77%) EFV, two (15%) to NVP and one (7.7%) to LPV/r. No particular regimen appeared to confer a greater risk of DILI (p=0.354). The nucleoside/nucleotide 'backbone' of the ART regimen did not appear to influence the risk of DILI. Additional data concerning DILI and the use of TB drugs is provided in Chapter 3.3.2 page 24 above.

3.3.4 Liver Biopsy, Histology and Other Medical Categories

With regard to the categories of malignancy, alcoholic liver disease and non-alcoholic fatty-liver disease, the liver biopsy enabled diagnostic certainty viz. definite and/or probable, to be reached in 100% viz. p=0.000. A high level of diagnostic certainty was also achieved with regard to the categories of infection and DILI, p=0.000. This certainty however was not possible with regard to

the category of ‘other’. Most were HIV-infected and many (40%) were significantly immune compromised viz. CD4 counts, <200 cells/mm^3. Almost half of the patients had diagnoses that required liver biopsy, i.e. the diagnosis could not have been made without access to tissue microscopy. But for more than half, the liver biopsy was unable to establish a final tissue diagnosis, e.g. ‘liver disease of undetermined origin’ and ‘granulomatous disease of uncertain cause’. (Table 5, page 27 below).

Fifteen patients developed or were admitted with renal dysfunction. Ten were HIV-positive, but none were on ARVs. A small number were hypertensive (n=17/107, 16%), ten had type II diabetes (n=10/107, 9%) and three were obese and had dyslipidemia. A review of the medication taken by these patients failed to suggest a cause for their liver disease.

Table 5. The Histological Diagnosis and Assessment of Probability of 107 Liver Biopsies from 2008-2013 at the Helen Joseph Hospital, Johannesburg, South Africa

Diagnostic category	Diagnosis			Number (n)	HIV profile*			% of total
					+	-	un	
Infection	Tuberculosis, TB (histological features and microbiology)			18	18	0	0	16.6
	HIV-associated Cholangiopathy			5	5	0	0	4.6
	Hepatitis B Virus Infection (active)			3	2	1	0	2.8
	Schistosomiasis of the Liver			3	0	3	0	2.8
	Hepatitis C Virus Infection (active)			2	1	1	0	1.9
	Pyogenic Liver Abscess			1	0	1	0	0.93
	Ascending Cholangitis			1	0	1	0	0.93
Infection Total	**Definite**	**Probable**	**Possible**	**33**				30.8
	9/33 (27.2%)	18/33 (54.6%)	6/33 (18.1%)		26	7	0	
DILI: Total	**Definite**	**Probable**	**Possible**	**20**				18
	0/20 (0%)	18/20 (90%)	2/20 (10%)		16	3	1	
Malignancy	Hepatocellular Carcinoma			8	1	3	4	7.4
	Non-Hodgkin's Lymphoma			4	4	0	0	3.7
	Metastatic Adenocarcinoma			3	1	1	1	2.8
	Neuroendocrine Carcinoma			1	1	0	0	0.93
Malign: Total	**Definite**	**Probable**	**Possible**	**16**				14.9
	15/16 (93.7%)	1/16 (6.25%)	0/16 (0%)		7	4	5	
NAFLD/ NASH: Total	**Definite**	**Probable**	**Possible**	**4**				3.7
	0/4 (0%)	4/4 (100%)	0/4 (0%)		1	2	1	
Alcoholic Liver Disease	Alcoholic Fatty-Liver Disease			3	0	2	1	2.79
	Alcoholic Liver Cirrhosis			2	1	0	1	1.86
ALD: Total	**Definite**	**Probable**	**Possible**	**5**				4.7
	2/5 (40%)	3/5 (60%)	0/5 (0%)		1	2	2	
Other	Autoimmune Hepatitis			2	1	1	0	1.86
	Cavernous Hemangioma			2	2	0	0	1.86
	Liver Congestion			2	2	0	0	1.86
	Granulomatous Hepatitis of Unknown Aetiology			3	3	0	0	1.86
	Iron Overload (Siderosis), Wilson's Disease, Primary Biliary Cirrhosis, Hepatic Fibrocystic Disease e.g. *ADPKD, Cirrhosis of Unknown Aetiology			1each=5				0.93 each
	Liver Disease of Undetermined Cause			15	12	3	0	14.0
Other: Total	**Definite**	**Probable**	**Possible**	**29**				27.1
	4/29 (13.8%)	4/29 (13.8%)	21/29 (72.4%)					
	Definite	**Probable**	**Possible**					
Total (All)	30 (28%)	48 (44.8%)	29 (27.1%)	**107**				100

*ADPKD= Autosomal Dominant Polycystic Kidney Disease; DILI = Drug-Induced Liver Injury; NAFLD = Non-Alcoholic Fatty-Liver Disease; NASH = Non-Alcoholic Steatohepatitis. Un= Unknown.

3.4 CLINICAL OUTCOME

One hundred and one (94.3%) patients were alive at the time of discharge. Two died during admission, both succumbing to nosocomial sepsis. Another subject died of an unknown cause in the six-month period following discharge. Seventy-nine (74.5%) returned for their month follow-up visit. The follow-up records of 22 (20.7%) were unavailable for review.

CHAPTER 4

4.0 DISCUSSION

Liver disease is a major cause of morbidity and mortality among HIV-infected people worldwide. (33, 34) Despite the fact that clinicians have a variety of non-invasive tools to assist with uncovering the cause of liver disease, uncertainty sometimes remains. Liver biopsy is often employed to resolve this uncertainty. Over the past four decades of the HIV epidemic, HIV-related disease has been changing in response to the introduction of ART and the improved control of HIV itself, and its related opportunistic diseases.(35, 36) This evolution of the epidemic has resulted in enhanced survival, the ageing of the infected, and the emergence of comorbid disease in many. The nature of liver disease in this group of patients has changed over the years. (36)

The burden of HIV infection among South Africans is considerable. Approximately 7.1 million (13%) are infected, previously estimated at about 12.5 percent.(37, 38) Data on liver disease in South Africans who are HIV-positive is limited to a small number of descriptive reports (39-42), where there is a need for additional data. It is within this context that this retrospective study was undertaken. The primary goal of the study is to tabulate the histological result of liver biopsies over a period of five-and-a-half years from 01 January 2008, until 30 June 2013. The study subjects were drawn from patients admitted to the Helen Joseph Hospital in Johannesburg, South Africa. The subjects were collected from consecutive liver biopsy records of the hospital and the database of the hospital's pathology service, viz. The National Health Laboratory Service of South Africa (NHLS). Secondary objectives included the construction of a clinical and laboratory profile that describes the characteristics of the study cohort. This incorporates the following: age, gender, HIV status, Child-Pugh Score, hepatitis serology, and at the time of the liver biopsy, the subjects' haemoglobin, serum albumin, and the pattern of the liver enzymes. The biopsy results were broadly

categorised as infective, DILI, alcohol-related, malignancy, non-alcoholic fatty-liver/steatohepatitis, and a miscellaneous group 'other'. Within these categories, patients were sub-divided into a 'final' diagnostic group e.g. tuberculosis, hepatocellular carcinoma etc., based on the histology (biopsy) result. Where HIV status was known, results of the infected and uninfected were compared. The HIV status of a small number in the study was unknown.

The mean age of the cohort was 42 years, where sixty-five percent were HIV-positive. The mean age of the latter was 37.8 years and was lower than that of the uninfected, viz. 45.7 years, p=0.000. This finding is consistent with similar studies in regions where HIV-prevalence is high.(35, 39, 41, 43)The gender ratio was reversed: the HIV-uninfected were mostly male, 57%, while the HIV-infected were mostly female, 58 percent. Women are over-represented in the HIV epidemic of Southern Africa. (37)The HIV-infected women in this study were at a three-fold increased risk of drug-induced liver injury (DILI), when compared with the infected males. Although the reason for this is not apparent from the data, HIV-infected women are at risk of DILI from nevirapine use if their CD4 counts are ≥250 cells/mm^3.(44, 45). Few were exposed to nevirapine in this study, however, many were receiving other hepatotoxic drugs viz. efavirenz, protease inhibitors, ritonavir, TB drugs, trimethoprim-sulfamethoxazole. Growing evidence suggests that South African women may be at increased risk, because of alterations in genes controlling the expression of cytochrome CYP2B6 and the enzyme N-acetyltransferase 2 (NAT2). It is speculated that polymorphisms in these genes result in a 'slow-metaboliser' phenotype i.e. women who have an inability to clear efavirenz rapidly, have unusually high serum efavirenz levels, and have prolonged in-vivo activity of isoniazid and rifampicin when on efavirenz. Many of female subjects were on these agents. Studies in HIV-infected Cambodians on ART and TB drugs have revealed a similar group at increased risk of liver disease. (46-48)

The HIV-infected patients were severely immune compromised. Two-thirds had been taking ART for a year before their biopsy. Nevertheless, most (70%) still had low CD4 counts. The mean CD4 count of the group was only 95 cells/mm3 at the time of the liver biopsy. Almost all (83%) of the HIV-positive cohort were anaemic, and many had low serum albumin levels. Advanced immune suppression is also suggested by the histological categories to which the majority of the HIV-infected belonged, viz. 'infection', 'DILI' and 'malignancy'. Anaemia and low albumin levels occurred with similar frequency in those on ART and those who were HIV-infected, but pre-ART. The low albumin levels were unlikely to have resulted from liver disease. INRs were normal. Few Child-Pugh scores suggested severe liver disease. The anaemia, the low CD4 counts, and the low albumins, most likely reflect ongoing inflammatory activity in the HIV-positive cohort, where many of whom had concurrent TB, other infections and malignancy. Anaemia is a well-recognised sign of advanced HIV disease and of poor survival of the HIV infected.(49, 50)

The HIV-uninfected group differed from the infected. Most (68%) had normal haemoglobins. Albumin levels below 25g/l were infrequent. The histological categories and final diagnoses differed too. TB was absent from the HIV-uninfected group. DILI was an unusual diagnosis in this group. Non-Hodgkin's disease was absent from the HIV-uninfected. Despite the larger absolute numbers of HIV-positives, none had alcoholic liver disease. Although hepatic steatosis/fatty liver has been linked to HIV-positive status and to the use of ARVs, only one of the four with this diagnosis was HIV-infected. (51) Although some South African studies suggest a high frequency of hepatic steatosis in the HIV-infected, the study population was a hospitalised and relatively young cohort, without comorbid disease, and although many were on ART, few had taken protease inhibitors or ART long enough to permit the development of drug-induced steatosis. (39, 41) The

biopsies of 15% (n=16/107) revealed cancer. Though similar numbers were HIV-infected (n=7/16, 44%), uninfected (n=4/16, 25%) or of unknown status (n=5/16, 31%), the cancers differed. Hepatocellular carcinoma predominated among the uninfected or unknown-status groups. Only one of eight with hepatocellular carcinoma was HIV-positive. All four with non-Hodgkin's lymphoma of the liver were HIV-positive.

Infection of the liver characterised a third of the study population, and defined the largest category in the study. *Mycobacterium tuberculosis* was the single most frequently identified organism. The relationship between TB and HIV is what the World Health Organisation (WHO) calls a regional and global health emergency, a situation that shapes the daily experience of the health workers in Southern Africa.(43, 52-54) Most subjects (70%) in the 'infection' category were HIV-positive. All who had TB were HIV-infected. Three-quarters of the HIV-infected had TB. The diagnosis of TB had *not* been made prior to biopsy in half (n=9/18): a finding shared with other local studies.(55, 56) Liver disease occurred directly through infection of the liver by *M. tuberculosis*, and indirectly as a consequence of liver damage caused by the TB drugs. TB of the liver is a disseminated form of TB and correlates closely with advanced immunosuppression in the HIV-infected. In a postmortem study of 39 HIV-positive patients on ART, Wong et al. (2012) found active TB in two-thirds (n=26, 66.6%). TB had spread beyond the lungs in all, and many had hepatic TB. The autopsies also uncovered additional causes of death in patients dying from TB, namely viral, bacterial and fungal infections, community acquired pneumonia, malignancy, etc., that had not been diagnosed ante mortem. (56) A larger postmortem review from another South African hospital reported similar findings. (55) Hepatic TB was the "leading opportunistic infection" among 301 consecutive liver biopsies reported from Cape Town in 2015.(39) Although the current study appears to place TB at the head of the causes of serious liver disease in HIV-

positive South Africans undergoing liver biopsy, it ought to be emphasized that this was a retrospective study. Patients who were less sick or whose liver condition was easier to diagnose would not have needed a liver biopsy. These will not have been adequately represented in this sample of patients. Nonetheless, and in stark contrast, hepatic TB was not a diagnosis of our HIV-negative subjects.

Where possible, very effort has been made to confirm the diagnosis of hepatic TB microbiologically. Unfortunately, mycobacterial culture and/or the molecular identification of TB had not been performed on every biopsy sample. Consideration of the histological findings, the concurrent isolation of TB from both liver and/or other body sites, and the clinical response to TB therapy, allowed us to assign a 'definite or probable' outcome to all our cases. The study time period viz. 2008-2013, predated the routine use of molecular TB-identification tests e.g. GeneXpert, (GXP), and the urinary point-of care TB test, viz. the LAM (Lipoarabinomannan) Test. Only 11 subjects had TB confirmed by the GXP method. None had access to the LAM test. These tests facilitate the early diagnosis of TB, and have the potential to reduce the need for liver biopsy in future hospitalised patients in Africa. (57, 58) A number of patients developed hepatic disease and TB shortly after starting ART. Although this suggests an 'unmasking-IRIS', supportive histological features such as multiple granulomas in each high-power field, and a background eosinophilic infiltrate, were not reported.(59) Furthermore the generally 'flat' CD4+ response to ART, together with absent pre and post-viral load data, leaves the diagnosis of IRIS possible, but not definite. The South African National HIV-guidelines discourage obtaining a baseline viral load i.e. before the start of ART. (42) Hence, no baseline viral loads of the 44 HIV-positive subjects on ART were recorded. Few had six or 12-month follow-up HIV-viral load measurements while on ART.

Twenty patients (19%) were diagnosed with DILI. DILI is a recognised cause of increased morbidity and mortality in the HIV-infected. (60) Most of the DILI patients (n=16/20, 80%) were HIV-positive and on ART. In the absence of viral load data the certainty of viral suppression, adherence to therapy and the efficacy of the ART is unconfirmed. Nevertheless, almost all had previous and current CD4 results, and clinical histories that provided information on the start of ART, the timing of the diagnosis of liver disease, and in the case of DILI, the clinical response on withdrawal of the offending drug(s).The HIV-positive women in this study appeared to be particularly at risk. Of those with a diagnosis of DILI, 15(75%) were women and 5(25%) were men.

Many of the drugs to which our patients were exposed, despite being standard-of-care in southern Africa, are hepatotoxins viz. Efavirenz, the drug to which most were exposed, Lopinavir/ritonavir and Nevirapine, Rifampicin and Isoniazid, standard TB drug combination therapy, and Trimethoprim-sulfamethoxazole. TB drugs conferred a definite risk of DILI, P=0.001. Nonetheless, simultaneous exposure to more than one hepatotoxic drug was frequent e.g. ART and TB drugs. Further complicating the allocation of cause was the concurrent presence in several biopsy samples of pathology unrelated to the DILI e.g. active TB, HIV-related cholangitis and hepatitis B virus infection. By comparison DILI was infrequent in the HIV-negative group (n=3/20, 15%), a group not exposed to either the ARVs or cotrimoxazole. Only one HIV-negative patient was on TB drugs at the time of their liver biopsy.

Enzyme patterns did not correlate with specific causes of liver disease in this study. The cholestatic-pattern was frequently encountered among those with TB and HIV in the Infection category. But our analysis of sensitivity, specificity, and positive/negative predictive values failed to confirm a correlation. Clinicians often rely on liver enzymes when determining the likely cause

of a patient's illness. The absence of a correlation is a reminder that a subset of patients will continue to require liver biopsy to clarify the nature of their hepatic disease, and that dependence on the pattern of enzymes for diagnostic purposes is likely to be misleading.

Hepatic steatosis was seldom diagnosed. This contrasts with a number of other biopsy studies. (39-41, 51, 61) There were too few cases and under-representation is likely. The patient base was drawn from an acute-care hospital, rather than a chronic-care, clinic-based facility. Many were young (<40 years) and were HIV-positive. A third were not on ARVs, and of those who were, few had sufficient exposure to the drugs usually associated with HIV-related fatty-liver/hepatic steatosis viz. stavudine and the protease inhibitors. Selection bias will have allowed an early clinical diagnosis with non-invasive tests (liver ultrasound), and the avoidance of biopsy in many patients.

The hepatitis viruses –hepatitis B and C, and, rarely, E – are important causes of liver disease in Africa. (62, 63) Prevalence rates vary across the continent but all are encountered from time to time in South Africa. (64-66) A small number of our subjects were infected with HBV (n=9, 8.4%) and HCV (n=3, 2.8%). Although these numbers are small, they reflect the variable prevalence of HBV and the low prevalence of HCV in this region. (41) Prior selection would have removed the "stable but chronically infected" i.e. not in need of liver biopsy from the cohort. Most of the HBV group were dually infected with HIV (n=7/9, 77.5%). Although the numbers are small, effective therapy is now available: curative in the case of HCV, suppressive in the case of HBV. (67, 68) This group of patients is therefore important to find and manage well.

No patient with HBV had their hepatitis viral load measured. In a recent cross-sectional study of rural Mozambicans and rural Zambians, 7.6% and 11.3% were HBV-positive, respectively.

(69)The authors noted that high HBV viral loads were frequent (49.4%) and correlated with an increased risk of hepatocellular carcinoma and of a poor response to HBV treatment. Recent data from rural South Africa has found a surprisingly large number of pre-ART individuals with "occult" HBV infection (15.1%) i.e. a detectable HBV-DNA and a negative HBsAg. Although this study suggests that infection with HBV or HCV is not of major importance in the context of liver biopsy, the absence of viral load monitoring in this group and limited exposure to any form of treatment implies ignorance with respect to the management of these patients. The current SA Department of Health (DOH) ART guidelines minimises the role of HBV in the context of HIV care. HBsAg tests are only recommended if liver enzymes (ALT) are elevated, or if a tenofovir-based ARV first-line regimen is likely to be discontinued or changed. This is an era where HBV and HCV can be better managed, and where vaccination against HBV is safe, effective and affordable. Tenofovir has a role in the prevention of perinatal transmission of HBV. (70) The pricing of the new HCV-drugs is in flux, and likely to come down. This study is another call to clinicians in Africa to adopt a more supportive approach to the care of the HBV- and HCV-infected.

Sixteen of the 107 patients in this study had some form of liver cancer. Seven (44%) were HIV-positive. The rest were either uninfected (25%) or had not been HIV-tested (31%).Severe immunosuppression (CD4<200 cells/mm^3) again characterised most of the HIV-infected. All with hepatic non-Hodgkin's lymphomas were HIV-positive, and had low CD4 counts. Hepatocellular carcinoma – a diagnosis traditionally associated with chronic HBV or HCV infection – was a disease primarily of the HIV-uninfected or "status-unknown". A large multicenter North American HIV-cancer study has recently reported on 457 cancers in a cohort with 46,318 person-years of follow-up post-starting ART. (71)These investigators found the early occurrence of cancer–within

6 months of starting ART – to be a manifestation of 'unmasking-IRIS' involving *AIDS-related* tumours e.g. NHL of the liver as in our patients. (72) Rates of *non-AIDS-related cancers* in the American study increased continuously after starting ART and the authors suggest that these cancers reflect the accelerated aging of the HIV-infected (on ART) and the persistence of carcinogenic environmental triggers such as alcohol, HBV and HCV. Improved life expectancy and its corollary, aging, is expected to increase the annual cancer incidence in Africa to 1.4 million by 2030. (73-75) With regard to the current study, preventable and treatable infections are likely to have had a role in the development of three-quarters (n=12/16, 75%) of our patient's tumours viz. HBV, HCV (hepatocellular carcinoma, HCC) and Epstein-Barr virus, EBV, (EBV/HIV-related NHL). Currently, age-standardised 5-year net survival with liver cancer is poor, viz. 10-20 percent.(76) If the outlook for liver cancer in Africa is to change, this study suggests that better attention to prevention – including HBV, HCV and HIV (and HIV/EBV), must be prioritised.

CHAPTER 5

5.0 CONCLUSIONS

This retrospective study reports on liver biopsy findings in both HIV-positive and negative subjects admitted to a Johannesburg teaching hospital over a five-and-a-half year period ending in July 2013. Infection with HIV and its consequences dominate these results. The biopsy outcome is sorted into six categories: infection, drug-induced liver injury, malignancy, alcohol-related, non-alcoholic hepatic steatosis and a miscellaneous category, 'other'. The categories with the fewest numbers of patients – the alcohol-related and steatosis groups – were also the only categories where the HIV-uninfected predominated. HIV-infection was associated with anaemia, low serum albumins, and low CD4 counts despite normal/near normal measures of liver function, viz. the INR and the Child Pugh scores. Most of the HIV-infected had liver histology results that suggested ongoing inflammatory activity. Indeed, the largest category of the study was that of infection. *Mycobacterium tuberculosis* was the principle infecting agent and caused liver disease, both directly and indirectly, through TB drug use alone or in combination with ARVs. The comparison of the HIV-infected with those who were uninfected revealed two very different populations: different ages and genders, different infections, different drug exposures and different neoplasms. Biopsy results of the uninfected suggest the era before the HIV epidemic and provide an appreciation of the evolving nature of the epidemic. With regard to DILI, HIV-infected women were at increased risk and as discussed in the text, begging the question of a genetic predisposition. The confirmation of DILI on liver biopsy required ancillary clinical evidence. The retrospective nature of the study meant that we could do nothing with regard to the absence in the patient's

records of post-ART initiation viral load measurements. Furthermore, the presence in DILI biopsies of additional and unrelated liver pathology is a reminder to clinicians to always keep an open mind when managing those suffering from HIV. Many of the patients included in this study belonged to this latter category.

CHAPTER 6

6.0 STUDY LIMITATIONS

This study has certain limitations. It was observational and retrospective in nature. Consequently, the data may have been subject to biases that were not fully appreciated. Incomplete clinical records were frequent and meant the exclusion of many potentially valuable cases that may have influenced the outcome of the findings. Records, when available, were sometimes incomplete, i.e. absence of follow up viral loads, and absence of HIV testing in some cases. Furthermore, the veracity of the data is also subject to observer bias, where the recording of liver enzyme patterns, the absence of outside/independent reporting of liver histology, clinical tests including radiology, and patient variables at times depended on numbers too small for statistical interpretation.

Positive aspects of the study include its systematic collection of patient data and its comparison of two cohorts, the HIV-infected and uninfected, at a time when the HIV epidemic is moving towards its mature phase. Furthermore, findings of the study have potential value with regard to future patient management i.e. the role of molecular diagnostics, follow-up viral load monitoring in those on ART, HBV-viral load measurement in patients with chronic HBV infection. The study confirms the dominance of TB as a major cause of liver disease in the HIV-infected and reminds the clinician to constantly include this diagnosis, even when it appears to have been safely discarded. The study also reaffirms the role of liver biopsy when other avenues of diagnosis have been exhausted. Many conditions diagnosed with liver biopsy would not have been diagnosed in any other way.

APPENDIX

Appendix A: Data Collection Sheet

1. Study number ☐☐☐

2. Date

Demographic data

3. Age: ☐☐☐

4. Gender: Male ☐ Female ☐

Clinical characteristics

5.a) HIV status Positive ☐ Negative ☐ Unknown ☐

b) Most recent CD4 cell count before biopsy ☐☐☐ Date taken:

……..

6. Indication for liver biopsy (pre-biopsy diagnosis) i)…….....

ii) Unknown ☐

7. Histological diagnosis on liver biopsy…….

i) Confirmed.....

ii) Probable.....

iii) Unknown.....

8. a) Use of ARVs at time of diagnosis ☐ Yes ☐ No Regimen: .....

i) NVP ☐

ii) D4T ☐

b) Antibiotic Yes ☐ No ☐ If Yes, ☐cify which antibiotic……

c) Co-trimoxazole Yes ☐ No ☐

9. a) Diagnosis of TB Empiric☐ Confirmed ☐ by.ex culture.....

b) Is the patient on Tuberculosis treatment? Yes ☐ No ☐

If yes, which ones…….and for how long:……..days/weeks/months

10. Sepsis. Blood stream infection ☐ Confirmed ☐ suspected ☐ Not suspected ☐

Organism on blood culture.....

11. Evidence of Renal dysfunction at time of admission:

Normal ☐ mild renal failure (egfr ‹50) ☐ severe RF (‹20) ☐

12. Imaging

Done ☐ Not done ☐

If done, what modality: Abdominal sonar ☐ CT abdomen ☐
Other:…… ☐

Findings on imaging:

a) Ascites: Yes ☐ No ☐

b) Lymphadenopathy Yes ☐ No ☐

c) Features of Fatty Liver: Yes ☐ No ☐

d) Features of Liver Cirrhosis: Yes ☐ No ☐

e) splenic microabscesses Yes ☐ No☐

f) Others: Yes ☐ No ☐ If yes,

specify……

13. Hepatitis studies:

a) Anti-HAV positive ☐ negative ☐

b)HBsAg positive ☐ negative ☐

c) HBeAg positive ☐ negative ☐

d) Anti-HBc positive ☐ negative ☐

e)Anti-HBs positive ☐ negative ☐

f) Anti-HbcIgM positive ☐ negative ☐

g)Anti-HBcIgG positive ☐ negative ☐

h)Anti-HCV positive ☐ negative ☐

i) HBV or HCV viral load.....

14. Most recent (admission) liver function test before liver biopsy:

Test	Result
Total Bilirubin	
Direct Bilirubin	
Alanine Transaminase	
Aspartate Transaminase	
Alkaline Phosphatase	
γ-GlutamylTransferase	
Serum Albumin	
INR	
Prothrombin Time	

15. Child-Pugh score 5-6 ☐ 7-9 ☐ 10-15 ☐

16. The most recent FBC before the biopsy

White cell count	
Haemoglobin	
Platelets	
Eosinophils	

17. History of Alcohol abuse (>4 units/day for men and >3 units/day for women)?

Yes ☐ No ☐ unknown ☐

18. Traditional medication use before biopsy? Yes ☐ No ☐ unknown ☐

19. Clinical outcome at discharge: Alive ☐ Dead ☐ Unknown ☐

20. Follow-up at 3-6/12 post discharge: Alive ☐ Dead ☐ Uknown ☐ no follow-up ☐

21. Co-morbidities

Appendix B: Proposal of a Modified Child-Turcotte-Pugh Scoring System

	1 point	2 points	3 points	4 points
Original CTP*				
Bilirubin (mg/dL)	<2	2–3	>3	
Albumin (g/dL)	>3.5	2.8–3.5	<2.8	
PT prolong (sec) INR	<4	4–6	> 6	
Ascites	None (Absent)	Slight (Easily Controlled)	Moderate (Poorly Controlled)	
Encephalopathy	None	Grade 1–2	Grade 3–4	
Modified CTP†				
Bilirubin (mg/dL) (for cholestatic disease)	<2 (<4)	2–3 (4-10)	3.1–8 (>10)	> 8
Albumin (g/dL)	> 3.5	2.8–3.5	2.3–2.7	<2.3
PT prolong (sec) INR	<4	4–6	6–11	>11
Ascites	None (Absent)	Slight(Easily Controlled)	Moderate(Poorly Controlled)	—
Encephalopathy	None	Grade 1–2	Grade 3–4	—

*Original CTP class: A, 5–6 points; B. 7–9 points; C, 10–15 points.

†Modified CTP class: A, 5–6 points; B: 7–9 points; C, 10–15 points; D, 16–18 points.

Appendix C: Definitions of Definite, Probable and Possible

Definite: The agent (TB/ARV/TB drugs etc.) is confirmed as the cause of the disease or diagnosis (DILI, malignancy, impaired liver function tests etc,). The available evidence establishes/confirms a causal relationship i.e. an association exists between the agent and the event. Furthermore, there are plausible mechanism/features on biopsy that link the two together and rules out other/alternative causes. So, a **definite diagnosis** was recorded if a microbe that could reasonably have caused the liver disease was found in the liver on biopsy e.g. Schistosomiasis, or as in the case of tuberculosis, the hepatic histology revealed compatible changes, such as granulomas, caseation etc. and/or where organisms (acid-fast bacilli) were seen in the specimen while the organism itself was concurrently confirmed with culture, DNA probe, or urinary lipoarabinomannan (LAM), in the same body but at a different site, e.g. lung, blood and/or marrow, urine, lymph node or skin.

Probable: It is likely that the event was caused by the agent (TB). The evidence favours acceptance of a causal relationship. However direct evidence linking one to the other is not available. Nevertheless, the probability exists that the two are related, and that there is a plausible features on the biopsy that supports the proposition. So, a **probable diagnosis** was noted if the liver histology suggested a likely cause and additional clinical, radiographic and laboratory information supported this probability e.g. serology (HBV, HCV), miliary or cavitatory changes on chest x-ray, and/or granulomas in organs other than the liver (TB). The difference between a definite and probable diagnosis was the absence of microbiological confirmation of the microbe in the probable group.

Possible: There is a reasonable possibility that features on biopsy and suggested agent or diagnosis can be linked together, but either the mechanism is unclear or the histological evidence is inadequate to confirm or exclude the diagnosis. An association between the two may be present, but a direct causal link between the two cannot be established. So, a **possible diagnosis** was considered when a microbiological cause could not be confirmed with certainty, but histology was compatible and where clinical, radiographic and laboratory data did not exclude the possibility of the diagnosis, but also did not conclusively support it, and where alternative causes were exhaustively excluded during the patient's pre-biopsy workup. The difference between a probable and a possible diagnosis rests principally on the absence in the possible group of conclusive support from the ancillary investigations/workup.

APPENDIX D: Ethics Clearance Certificate

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Dr Nobert M Muramira

CLEARANCE CERTIFICATE	**M121197**
PROJECT	Indications for the Histological Diagnosis of Liver Biopsies at Helen Joseph Hospital
INVESTIGATORS	Dr Nobert M Muramira.
DEPARTMENT	Department of Internal Medicine
DATE CONSIDERED	30/11/2012
DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 30/11/2012 **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable
cc: Supervisor : Dr E Jong

DECLARATION OF INVESTIGATOR(S)
To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...

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