A clinicopathological cohort study of liver pathology in 301 patients with HIV/AIDS

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A CLINICOPATHOLOGICAL COHORT STUDY OF LIVER PATHOLOGY IN 301 PATIENTS WITH HIV/AIDS

Introduction: Globally approximately 34 million people are HIV infected with sub-Saharan Africa disproportionately affected. Whilst HIV itself causes no direct liver disease, consequences secondary to HIV are common. Liver pathology data is limited and findings differ between high, middle and low-income countries. In the era of highly active antiretroviral therapy (HAART), morbidity and mortality due to liver disease has emerged as a significant issue. The spectrum of liver pathology and disease in patients with HIV/AIDS in South Africa, the epicentre of the pandemic, is largely unknown.

Aim: To determine the spectrum and clinicopathological correlates of liver pathology in patients with HIV/AIDS in a dedicated liver clinic and referral centre in a middle and low-income country.

Methods: A database of all liver biopsies performed in HIV positive patients since January 2000 was established in 2005. Patients were included retro- and prospectively. Clinical and demographic data including age, gender, WHO clinical stage, CD4 cell count, hepatitis B and C status, complete drug history prior to biopsy, liver chemistry and tissue culture results, amongst others, were recorded. All liver biopsies were assessed by one of two experienced liver pathologists. Statistical comparisons were performed using the Fisher’s exact, Chi-squared and Mann-Whitney U-test and where indicated, odds ratios were calculated. Multivariate logistic regression analysis was performed to identify factors associated with specific patterns of drug induced liver injury.
Results: 301 patients, median age 34 (IQR 29-40) years were evaluated including 158 men, median age 35 years (IQR 31-41) and 143 woman, median age 33 years (IQR 28-37), \( P=0.001 \). Ethnically, 76.1%, 11.6%, 11.6% and <1% were black African, Caucasian, Mixed Ancestry and Asian/Indian, respectively. Median CD4 cell count at time of liver biopsy was 127 (52-260) and not significantly different between men 118 (46-249) and women 132 (55-270), \( P=0.56 \). Drug induced liver injury (DILI) was observed in 127 (42.2%) patients with patterns of drug injury including non-specific hepatitis 51(40.2%), cholestasis 20 (15.7%), mixed hepatitis-cholestatic 25 (19.7%), steatohepatitis 5 (4%), vanishing bile duct syndrome 11 (8.6%), submassive necrosis 13 (10.2%) and granulomatous 2 (1.6%). Of the patients with DILI, 85 (67%) used cotrimoxazole, 78 (61%) HAART and 41 (32.2%) TB drugs either individually or in combination. Other implicated drugs were fluconazole 9 (7%) and herbal toxins 8 (6%). With univariate analysis, cotrimoxazole and HAART conferred risk for a DILI (OR 2.78 (1.72-4.48), \( P=0.001 \), OR 1.69 (1.06-2.68), \( P=0.027 \), respectively), but anti-TB drugs did not (OR 0.68 (0.42-1.09), \( P=0.112 \). With multivariate logistic regression analysis, cotrimoxazole predicted strongly for cholestatic and ductopenic injury, whilst efavirenz was mostly associated with non-specific hepatitis and submassive necrosis. Female gender predicted for cholestatic injury, whilst a CD4 cell count >200 and younger age predicted for submassive necrosis. A total of 86 (29%) patients had granulomatous inflammation, mostly non-necrotizing (92%) and predominantly due to *Mycobacterium tuberculosis*. Hepatitis B was the prevalent viral hepatitis observed in 56 (19%) with PCR confirmed hepatitis C noted in only 10 (3.3%) patients. Steatosis/steatohepatitis was observed in 58 (19.3%). Notably, 16.2% of the cohort had >1 histopathological finding.

Conclusion: In this large cohort of patients with mostly advanced HIV/AIDS, DILI was the dominant pathological process with cotrimoxazole and HAART, mostly efavirenz and nevirapine, conferring a significant risk. Although TB drugs likely also confer risk; significance is influenced by selection bias for liver biopsy in the study. Tuberculosis is a highly prevalent opportunistic infection involving the liver and given its endemicity, hepatitis B is a frequent viral co-infection.
PART A Research Protocol

“A review of liver pathology and a clinicopathological correlation in HIV positive patients in Cape Town”

1. Outline of the study population

A database will be established of all adult HIV positive patients presenting with liver disease who have undergone and will undergo a liver biopsy. The Liver Clinic and Division of Hepatology serves as a referral centre for patients with liver disease. Referrals come from related secondary/district hospital both regionally but also supra-regionally e.g. from adjacent Provinces. Part of the evaluation and management of these patients may include the possibility of a liver biopsy. The study is not designed to automatically biopsy every patient, but rather the indications for a liver biopsy will be varied and entirely clinically guided. Typically there may be several indications but common indications can be summarised as:

i. An abnormal liver profile

ii. Unexplained hepatomegaly (as defined clinically by the treating physician or as determined radiologically)

iii. For pyrexia of uncertain aetiology (no cause determined despite an adequate workup).

Initially all patients biopsied from January 2000 will be retrospectively added, thereafter all new patients biopsied will be included prospectively. Hence, the database was initially populated with patients retrospectively and thereafter patients were prospectively included. All patients included retrospectively will have their biopsies accessed from the archive and reviewed by an experienced liver histopathologist. They will only be included if they fulfil the criteria (below) for inclusion.
2. Patient evaluation

i. The HIV positive status of patients will have been confirmed with ELISA based testing for HIV antibody and p24 antigen.

ii. Liver enzyme profile including total and conjugated bilirubin, alanine and aspartate aminotransferase (ALT, AST), alkaline phosphatase (ALP) and gamma-glutamyl transpeptidase (GGT) recorded at or 1 day before liver biopsy.

iii. The CD\textsubscript{4} positive T-cells (cells/mm\textsuperscript{3}) count obtained within 4 weeks of liver biopsy.

iv. Serological and virological markers (or previous results) for hepatitis A (Hepatitis A IgM antibody), hepatitis B (HBsAg, HB core IgM antibody, HBcore IgG antibody, HBeAg, anti-HBe antibody) and hepatitis C (Hepatitis C IgG antibody) available on all patients. HBV DNA viral levels and Hepatitis C genotype and viral load performed where appropriate.

v. Liver biopsies are performed via the standard technique and use the Quick-Core® Biopsy Needle Set when the biopsies are performed by the Division of Radiology (under ultrasound guidance). Alternatively ward based biopsies are performed using the modified Menghini needle, the Hepafix® 88mm biopsy needle. As is our practice, an approximately 1mm piece of the core of liver tissue from every biopsy is submitted for mycobacterial and fungal culture in sterile 0.9% normal saline and confirmation of culture results are followed up from the microbiology laboratories. Biopsy specimens are placed in formalin for histology processing.

vi. All relevant patient clinical and demographic data will be recorded including age, gender, history of opportunistic infections including tuberculosis and mode of TB diagnosis, WHO clinical HIV/AIDS staging, complete drug or toxin exposure history and alcohol history. The data will be captured on a datasheet. (see Part D)
3. **Histopathological examination**

i. The liver biopsy will be deemed adequate if: at least ≥1.5cm in length and/or 6 portal tracts are present.

ii. Each biopsy specimen will be processed according to a standard protocol with haematoxylin and eosin-staining as well as utilizing Bile sirius red, reticulin, periodic acid-Schiff (PAS), PAS diastase and Perl’s Prussian blue stain, routinely for evaluation. Where necessary, Ziehl-Neelsen stain is utilized to assess for acid-fast bacilli, Grocott’s methenamine silver stain for fungal organisms, and immunohistochemistry stains for hepatitis B surface and core antigen.

iii. All the liver biopsies in this study are assessed by one of either two experienced liver histopathologists.

iv. Clinicopathological assessments are done concurrently with 3 hepatologists.

v. The most likely clinicopathological diagnosis(es) will be captured in the database. Suspected drug induced liver injuries will be based on the clinical presentation, exclusion of other aetiological factors and a suggestive histological pattern of injury. The most likely offending drug, in the opinion of the hepatologists, will be recorded.
PART B: Literature Review

Table of contents

1. Introduction

2. The HIV/AIDS epidemic and burden of disease

3. Epidemiology of HIV associated liver disease

4. The liver and HIV
   4.1 Direct effects on the liver
   4.2 Indirect effects on the liver

5. Pathology of HIV-associated liver disease
   5.1 Liver enzyme abnormalities in patients with HIV/AIDS
   5.2 Liver pathology in HIV/AIDS
   5.3 Specific liver pathology in HIV/AIDS
      5.3.1 Granulomatous inflammation
         5.3.1.1 TB-Immune reconstitution inflammatory syndrome
      5.3.2 Drug induced liver injuries
      5.3.3 Viral hepatitis
         5.3.3.1 Hepatitis B
         5.3.3.2 Hepatitis C
      5.3.4 Steatosis/steatohepatitis
      5.3.5 HIV/AIDS cholangiopathy

6. Conclusion

7. References
1. Introduction

HIV and liver disease covers a vast area of available literature. Even though HIV is a relatively “young” disease, it has generated an almost exponential rise in research. The major focus of this dissertation is to analyse the liver pathology observed in patients with HIV/AIDS in our clinical experience; our setting being a country with the highest prevalence of HIV globally. Having determined this spectrum, a clinic-pathological analysis will endeavour to identify major factors determining pathologies. The foundation of this literature review is the basic immunological processes between HIV and the various cellular components of the liver. Building on this foundation is the specific objectives of this review viz. to evaluate the current body of data pertaining particularly to liver pathology in HIV/AIDS. Having determined the scope, attention will be given to specific common pathologies to expand upon particular aspects of these pathologies and their natural clinical histories.

Pubmed (MEDLINE) was utilized for the literature search. Search terms included “Liver pathology and HIV” in combination with “liver disease”, “viral hepatitis B and C”, “granulomatous inflammation” and “steatosis/steatohepatitis”. Additional publications were identified from the relevant literature. Publications from the last 13 years were identified but older publications were not excluded.
2. The HIV/AIDS epidemic and the burden of disease

At the end of 2011, 34 million people globally were living with HIV. An estimated 0.8% of adults aged 15-49 years worldwide are HIV positive. Sub-Saharan Africa is the most severely affected region accounting for 70% of HIV worldwide with almost 1 in every 20 adults HIV infected (1). South Africa has the highest HIV prevalence rates in the world. The total number of persons living with HIV in South Africa increased from an estimated 4 million in 2002 to 5.26 million by 2013 (2).

Approximately 10.9% of the South African population is HIV positive (3) while in 2011, the overall HIV prevalence amongst antenatal women was 29.5% (4). The HIV pandemic is now demonstrating features of stabilizing in South Africa, but its impact on healthcare delivery and the disease burden is incalculable. Together with tuberculosis, HIV forms part of the now recognized 4 major burdens of disease in South Africa (5). HIV/AIDS has had a significant influence on all aspects and disciplines of medicine. Clinical practice and the understanding of pathophysiological processes have needed to be adapted to the mode of presentation of patients with HIV/AIDS. Liver disease is no exception and over the last decade, liver-related complications have become a significant cause of morbidity and mortality in HIV-infected patients, most notably in the developed world (6). Little is known about the range of liver pathology in the developing world and in particular in a high HIV prevalence area such as sub-Saharan Africa. This study aims to add to the relative paucity of pathology data from the developing world.
3. Epidemiology of HIV and Liver disease

Liver disease, including both liver-related morbidity and mortality, secondary to acute and chronic liver disease in addition to hepatocellular carcinoma, is emerging as a significant management challenge in HIV infected people, mostly reported in high income countries (7). The Data Collection on Adverse Events of Anti-HIV Drugs (D:A:D) study investigating adverse effect of antiretroviral therapy in over 33,000 patients noted liver related mortality as the leading cause of non-AIDS mortality in HIV-positive patients (7). In the study, liver related mortality was strongly associated with viral hepatitis (84% of liver related deaths, 11% of total deaths), however 16% of liver related mortality (2.3% of total deaths) was unrelated to viral hepatitis (7).

Data from the developing world or low and middle income countries regarding liver-related mortality in patients with HIV/AIDS is very limited. A retrospective study of over 6007 patients from Latin America, found 42% of total deaths were attributed to cirrhosis or liver failure (8). This was mostly related to co-infection with hepatitis B or C. In a 2005 autopsy study from India, 3.3% of patients had cirrhosis (9), whilst a more recent autopsy study from India in 236 patients with HIV/AIDS, a similar cirrhosis incidence of 3% was observed (10).

An autopsy study in 86 patients with HIV/AIDS from the Eastern Cape in South Africa noted that 10% had liver disease, although no specific details of the liver disease were provided in this study(11). A more recent autopsy study from Johannesburg looking at causes of death in 39 adults after initiating antiretroviral therapy, found tuberculosis as the leading cause of death (12). Mortality secondary to liver disease accounted for 2 deaths but notably 14 patients (36%) had granulomatous change due to *Mycobacterium tuberculosis* involving the liver.
4. The liver and HIV

4.1 Direct effects on the liver

HIV has a significant effect upon cell mediated immunity producing both immune deficit and dysregulation through predominantly infecting CD$_4^+$ T-cells, macrophages/monocytes and dendritic cells. What is now clear is that HIV also infects a wide range of non-immunological cells, including cells of the liver (13). It is well established that HIV gains entry into most target cells by forming a complex consisting of its outer envelope glycoprotein (gp120), CD$_4$ receptor and members of the chemokine co-receptor family, in particular CCR5 and CXCR4 (14).

A variety of seminal studies has demonstrated the susceptibility of the liver to a wide variety of infectious and neoplastic conditions (15-17) as a result of HIV related immune compromise. However, no primary hepatic process directly related to HIV is observed in clinical practice (18). This is despite Kupffer cells (the resident hepatic macrophages), sinusoidal and biliary epithelium all expressing CD$_4$ receptors (19). In some instances, a non-specific hepatitis very similar to Epstein Barr virus related hepatitis, can be seen in primary HIV infection (20). This is in sharp contrast to the many liver abnormalities observed in the later clinical stages of HIV/AIDS. Of curiosity is that evidence to suggest CD$_4$ receptor expression by hepatocytes is ambiguous. Some studies have demonstrated CD$_4$ expression on hepatocyte cell lines, whilst others are CD$_4$ negative (21, 22). Notably, chemokine co-receptor CXCR-4, CCR3 and CCR5 have been demonstrated on hepatocytes (23). HIV is however capable of infecting several CD$_4$ negative cell types such as fibroblasts, neural cells and renal tubular cells (24) suggesting CD$_4$ independent mechanisms of infection. This may explain the finding of HIV RNA as well as other HIV proteins in
hepatocytes (25). What is now known is that HIV via gp120 protein signalling can induce hepatocyte apoptosis through the CXCR4 chemokine co-receptor in the absence of direct HIV infection of hepatocytes (26). Interestingly, this effect is augmented in the presence of the hepatitis C E2 protein (22). An additional effect of hepatocyte apoptosis is that it can trigger pro-fibrotic activity of hepatic stellate cells, as has been demonstrated in both HIV-HBV as well as HIV-HCV co-infection (27, 28).

Kupffer cells are a target for HIV and immunohistochemical evidence of the presence of HIV proteins confirm this finding (29). Kupffer cells harbour HIV proviral DNA and can support in vitro HIV replication (30). Furthermore Kupffer cell densities correlates with the degree of HIV related immunosuppression and may increase after antiretroviral therapy (31). This decreased density may play a critical role in enhanced microbial translocation with consequent systemic immune activation with advancing HIV/AIDS (31).

Limited data exists of the effect of HIV upon the lipid storing and fibrogenic hepatic stellate cells as well as the hepatic sinusoidal epithelium, suffice to say that both cell types are permissive to HIV entry (13). Hepatic stellate cells express the chemokine co-receptors CCR5 and CXCR4, however this appears to be CD4 independent (32). There is also data to suggest a pro-inflammatory and pro-fibrogenic influence of HIV on stellate cells (33). This may play a role in enhancing hepatic inflammation and fibrosis in patients with acute or chronic liver injury. HIV infected hepatic stellate cells demonstrate increased activation and fibrogenesis as measured by the production of collagen and alpha-smooth muscle actin as well as increased levels of
monocyte chemotactic protein-1 (32). This may well explain the accelerated progression to advanced fibrosis and cirrhosis observed in HIV-HCV co-infected patients (34).

4.2 Indirect effects on the liver
HIV infection of the gastrointestinal associated lymphoid tissue, in particular the CD4+ T-cells, has an effect on gut permeability. It enhances translocation of bacterial endotoxins such as lipopolysaccharide or LPS. The consequence of this is increased circulating levels of LPS that are thought to activate monocytes and contribute to chronic immune activation in HIV-infected patients (35). LPS, via toll like receptor 4 (TLR-4), can activate Kupffer cells through a host of pro-inflammatory cytokines as well as hepatic stellate cell activation to produce monocyte chemotactic proteins such as CCL-2 (36). The net result of this LPS driven cytokine and chemokine activation is an induction of chemotaxis of both T-lymphocytes and monocytes to the liver which can contribute to the progression of liver disease.
5. Pathology of HIV-associated liver disease

5.1 Liver enzyme abnormalities in patients with HIV/AIDS

Liver enzyme abnormalities are commonly encountered in HIV/AIDS and are reported in the literature to occur in 20 – 93% of HIV infected populations (37). An important question is what is “normal” in the context of liver enzymes? By evaluating local “normal” populations laboratories typically derive values for normal ranges and then determine reference ranges. However it has been suggested, for example, that the upper limit of normal (ULN) for alanine transaminase (ALT) levels should be 30 U/L for men and 19 U/L for women (38). This corresponds to the 95th percentile of ALT levels in a cohort of nearly 4000 first-time blood donors at low risk for liver disease. Another issue is that liver enzymes are often mildly abnormal in the HIV population creating another difficulty in defining what is a “normal” baseline (39).

The range of liver enzyme abnormalities is wide and aetiologies vary between populations in the developed and developing worlds in addition to differences in the pre-HAART and HAART era. In a Boston based study, 308 HIV positive patients attending a municipal clinic, one-third with CD₄ counts less than 200, were evaluated over a 2 year period from 1990 – 1991. In this group, 75 % had abnormal liver function tests, 20% regarded as severe (40). In an Indian Public Hospital, 63% of HIV/AIDS patients with CD₄ less than 200, had elevated liver enzymes (41). In a Johannesburg based study at a large Academic hospital, 93% of those evaluated, had abnormal liver function tests (37), whilst 87.6% of patients in a Nigerian tertiary hospital had abnormalities of their liver chemistry profiles (42). In a more recent study in the USA, excluding hepatitis B and C co-infected patients in a cohort of HIV patients, 31.5%, 23.8% and 46.9% of patients had an elevated AST, ALT and ALP,
respectively (43). Most elevations however were mild to moderate. In the large Swiss-HIV cohort study, 16% had a chronic elevation in liver enzymes (44). A Mexican study found abnormal alkaline phosphatase and alanine transferase in 45% of a cohort of 161 patients with HIV/AIDS (45).

5.2 Liver pathology in HIV/AIDS

Several biopsy and autopsy studies are published that describes liver pathology in patients with HIV/AIDS. It is evident from this data that differences in findings are accounted for by the geographical source of the data viz. the developed or developing world as well as the presence or absence of highly active antiretroviral therapy (HAART).

The seminal paper on the pathology observed in liver biopsies performed in patients with HIV/AIDS was published from a New York in 1985 (46). Here 29 biopsies done in 25 patients were reviewed. The most common histological finding was of macrovesicular steatosis in 55% of cases. One third had non-caseating granulomata secondary to *Mycobacterium avium* complex and a single case due to histoplasmosis. The first study from a developing country noting liver pathology was a 1987 Brazilian autopsy series of 12 patients with HIV/AIDS. It revealed mostly opportunistic infections (*Cryptococcus neoformans* and *Mycobacterium tuberculosis*) as well as Kaposi’s sarcoma (47). The same year another biopsy (29 biopsies) and autopsy (59 autopsies) series from the USA was published (15). Steatosis, non-specific portal tract inflammation and granulomata accounted for the majority of pathological findings. Similarly, as with the previous USA based studies,
granulomatous change was due to *Mycobacterium avium* complex. Cytomegalovirus hepatitis as well as lymphoma accounted for other significant findings.

The largest liver biopsy series to date, published in 1996, involved a retrospective review of liver biopsy findings in 501 HIV patients from the New York area (48). Sixty-two percent of the patients had CD$_4$ counts <100/mm$^3$. Granulomatous hepatitis was the most prevalent finding in 37.2% of patients. In 50% of cases, it was accounted for by *Mycobacterium avium* complex and in only 7% was it due to *Mycobacterium tuberculosis*. Viral hepatitis was seen in 18.2% of patients with normal or non-specific findings in almost one-third of patients.

The only purely biopsy based pathological study to emerge from a high HIV prevalence country in the developing world is a 1999 Thailand study that evaluated biopsies from 46 patients (49). Again granulomatous inflammation accounted for the majority finding in 50% of patients. Granulomata were mostly non-caseating and in two-thirds were confirmed due to *Mycobacterium tuberculosis* based on culture. Of the remaining third, 75% had acid fast bacilli found elsewhere and responded to the anti-tuberculosis therapy. Other pathological findings included opportunistic infections such as histoplasmosis and cryptococcosis (13%, each) and penicillinosis (8.7%). Other important findings included steatosis (4.4%), viral hepatitis (4.4%) and importantly drug induced liver injury (2.2%).

A 2004 autopsy (155 patients) and biopsy (16 patients) from India reported non-caseating granulomatous inflammation in 41% of cases, invariably due to tuberculosis (50). Similarly, a 2005 autopsy based series from India of 60 HIV
positive patients again highlighted the common finding of granulomata on liver histology in 31.6% of cases (9). Interestingly in this study most were caseating type epitheloid granulomata, again mostly due to tuberculosis. Steatosis (10%) and viral hepatitis B (2.6%) and C (10%) accounted for other major findings. CD4 cell counts were not reported in these studies.

A Mexican study looked at hepatobiliary diseases in 161 patients with HIV/AIDS treated with HAART. Liver biopsies were performed in 85 (51%) patients. The frequency of findings was granulomatous hepatitis (29%), steatosis plus granulomatous hepatitis (19.5%) and steatosis alone (14.6%). Opportunistic infections were isolated in 27.9%, *Mycobacterium tuberculosis* (26.6%) being the most frequent, *Histoplasma capsulatum* (20%), Cytomegalovirus (13.3%), and *Mycobacterium avium intracellulare* (11%). The HBsAg was positive in 21 of the 69 patients (30.4%) (45).

In Africa, only one liver biopsy based pathology study in HIV/AIDS patients has been reported (51). Several autopsy studies have been reported although none of them concentrate specifically on liver disease. In 2008, a series of 12 liver biopsies was reported from Nairobi, Kenya (51) with 7 of the 12 biopsies demonstrating acid fast bacilli positive granulomatous hepatitis. A Nigerian study in 100 patients with HIV/AIDS who underwent post-mortem evaluation of the liver was reported in 2006 (52). Tuberculous granulomatous hepatitis (34%), chronic hepatitis (20%), non-specific reactive hepatitis (15%) and steatosis (12%) were the most frequent findings. An autopsy study in 86 patients from the Eastern Cape, South Africa found liver disease in 10.1% of cases but did not specifically make reference to the types of
liver pathology observed (11). A 2012 autopsy study of 39 adults from Johannesburg looking at post mortem causes of death after initiating antiretroviral therapy (12) found that granulomatous inflammation secondary to tuberculosis was the most frequent liver pathology in addition to drug induced liver injuries.

5.3 Specific pathology of the liver in HIV/AIDS

5.3.1. Granulomatous inflammation

Granulomas are focal collections of modified macrophages often coalescing to form multinucleate giant cells. Occasionally they may be surrounded by a rim of lymphocytes and fibroblasts (53). Granulomas can occur in the liver as a result of a wide variety of disorders. Occasionally it may be due to a primary liver process but mostly it is secondary to a generalised systemic process. Given the extensive likely aetiologies of granulomata, geographical location as well as a given patient population tends to determine the most likely cause (54). Inter alia, these include infectious agents, immunological diseases (PBC, PSC etc), neoplasms and drugs.

As has been indicated, the histological finding of granulomatous inflammation in liver pathology from patients with HIV/AIDS is the common finding in both autopsy and liver biopsy studies, ranging from 31 – 50% (48, 49). Opportunistic infections account for the vast majority of this finding, however there is a clear difference between the infectious aetiology in the developed and developing world. *Mycobacterium avium* complex (MAC) and fungal infections are the dominant aetiologies in the developed world whilst *Mycobacterium tuberculosis* dominates as the aetiology in the developing world (55). *Mycobacterium avium* complex appears to be significantly less common in developing countries and may be exceedingly rare or
almost non-existent in parts of Africa (56). There is no clear reason to explain this observation in geographical variation. It may well be related to the overwhelming burden and incidence of *Mycobacterium tuberculosis* in the developing world that virtually eclipses MAC as an opportunistic infection.

### 5.3.1.1 TB immune reconstitution inflammatory syndrome (TB IRIS)

HIV results in a progressive depletion of CD$_4^+$ T cells (57). Tuberculosis related granulomas as well as lymph nodes are depleted of T-cells and contain significant neutrophils as well as necrosis (58). Following the introduction of combination antiretroviral therapy (cART), HIV replication is rapidly suppressed. A prompt increase in naïve and memory T-cells is observed (59). Despite T-cell function in HIV-TB co-infected patients not being equal to that of TB non HIV infected individuals, the improvement in cell-mediated immunity is associated with a reduction in the clinical rate of TB (60). Paradoxically, the incidence of TB increases during the first 3 months of cART before declining (61).

In most cases this is due to an immune restoration syndrome as a result of an immune response against subclinical *Mycobacterium tuberculosis* infection. TB IRIS is observed in HIV-infected patients as either an unmasking of undiagnosed TB or a ‘paradoxical’ worsening of currently treated TB affecting 20-25% of HIV patients in the first 3 months after cART is commenced (61). Evidence suggests that a high mycobacterial load is a major determinant of a pathological immune response (61). Hence, TB-IRIS tends to occur more frequently in patients with disseminated (extrapulmonary) TB, a shorter latency period of TB therapy before cART is started or drug-resistant TB (62-65). These all have the potential for an elevated
mycobacterial load. TB-IRIS is characterized by a pronounced and atypical inflammatory response and severe CD4+ T-cell deficiency (invariably <50/mm$^3$) is a potent predictor of TB-IRIS (66). The pathological immunological response is not entirely clear but an exaggerated Th1 response seems central to the process. It is not clear why Th1 responses are higher in patients with TB-IRIS and a deficiency of Treg cells (FoxP3+CD4+ cells) in patients with TB-IRIS has not been demonstrated. In summary available data suggests that TB related Th1 responses are increased in all HIV-infected patients with treated TB following cART and that the responses are higher in patients who develop TB-IRIS (67).

Very limited data of TB-IRIS in the liver exists, suffice to say that it poses a significant clinical challenge given the possible likely aetiologies in a given patient who develops liver dysfunction after cART initiation (68). In a previous series of patients with TB-IRIS, 21% had hepatic involvement with hepatomegaly and abnormalities of liver enzymes, predominantly involving the canalicular liver enzymes (69).

### 5.3.2 Drug induced liver injuries

A drug induced liver injury (DILI) or hepatotoxicity is essentially a diagnosis of exclusion when in the clinical context of abnormal liver enzyme elevations, other causes such as viral hepatitis, alcohol or drug abuse, immune reconstitution syndrome or biliary tract disorders are not found. Although ALT is a significant marker of liver injury, it is neither sufficiently sensitive or specific to define or categorize hepatotoxicity given that it is present in tissues other than the liver (70). The Adult AIDS Clinical Trials Group (AACTG) produced a modified scale to
measure the fold-changes from baseline of the transaminases. The score is graded as follows: grade 1 (1.25–2.5 × ULN); grade 2 (2.6–5 × ULN); grade 3 (5.1–10 × ULN) and; grade 4 (>10 × ULN). Hence they defined severe hepatotoxicity as grade 3 or 4 change in aspartate transaminase (AST) or ALT levels following drug exposure (39). It is however important to note that this system is primarily focused on hepatocellular injuries. Cholestatic injuries, including severe injuries such as the vanishing bile duct syndrome, would not be accounted for with this system.

The US Food and Drug Administration (FDA) suggests that the most critical evaluation of potential for severe hepatotoxicity is so-called “Hy’s Law,” derived from Hy Zimmerman’s observation that patients with ALT/AST elevations with concomitant jaundice had a poor prognosis (71). In essence 3 basic criteria are utilized to define hepatotoxicity of a predominantly hepatocellular nature. These include (1) ALT or AST >3× ULN; (2) Total bilirubin >2× ULN and (3) no other cause for the enzyme elevations (72).

The question as to whether drug induced liver injuries occur at a greater frequency in HIV positive patients than they do in HIV negative patients, is unclear. A study in the early 1990’s showed that the frequency of drug allergy or hypersensitivity in patients infected with HIV ranged from 3% to 20% (73). Another study documented that drug-related rashes were 100 times more common in HIV-infected patients than controls (74). Consequently, similar observations have been made with regard to various antiretroviral drugs and other medications e.g. antituberculous drugs, cotrimoxazole; in HIV-infected populations. These adverse drug reactions range from simple limited cutaneous rashes to more complicated systemic symptoms e.g. DRESS (drug reaction, eosinophilia and systemic symptoms) and even death from
Stevens-Johnson syndrome, Toxic Epidermal necrolysis and drug induced liver injuries.

Cotrimoxazole, a commonly used antimicrobial for both prophylaxis and treatment in HIV/AIDS is associated with hypersensitivity reactions in 3% to 5% of HIV-negative patients (75). However the prevalence of cotrimoxazole associated adverse reactions is significantly higher in the HIV-positive population with one report noting that in HIV-positive patients treated with cotrimoxazole who experienced adverse effects; rash occurred in 33% (76-78).

An elegant 2008 study suggested that the impairment of HIV-infected cells to deal with reactive drug metabolites may be a putative mechanism for the increased rates of adverse drug reactions seen in HIV/AIDS. The study looked at the toxicity of sulfamethoxazole (one half of cotrimoxazole) and its reactive hydroxylamine intermediate in lymphocytes transfected with the HIV tat gene. There was a significant concentration-dependent increase in cell death in transfected cell lines expressing Tat protein compared to controls. T cells transfected with a dose dependent inducible tat gene showed increased toxicity in response to sulfamethoxazole and its intermediate as more Tat expression was induced. It is thus suggested that HIV tat protein expression may increase oxidative stress within HIV-infected cells (79). This could explain the increased risk of hypersensitivity reactions seen in cotrimoxazole use in HIV/AIDS.

Given the high rates of co-infection in subsets of HIV positive populations, another important confounder in the frequency of DILI’s in HIV positive patients is the role
that co-infection with hepatitis B or C possibly contributes to increased risk. In a study of 134 consecutive patients receiving antituberculous drugs, the relative risk of developing a TB DILI if the patient had hepatitis C or HIV was elevated 5-fold and 4-fold, respectively. If a patient was co-infected with both, the relative risk was increased 14.4-fold (80). An interesting aspect of this study was that in a few patients treated for hepatitis C with alpha-interferon, anti-TB drugs were successfully re-challenged without DILI recurrence (80). In several studies, predictive factors for cART related hepatotoxicity were evaluated. Rates of toxicity ranged from 2 to 23.6%. Apart from the type of antiretroviral drug used e.g. nevirapine, ritonavir, hepatitis C then B, were the most consistent additional factors predicting for risk (81-84). In a South African based cART study, rates of hepatotoxicity after commencing cART was 7.7 episodes per 100 person years. The presence of HBsAg increased the risk of hepatotoxicity 3 fold (85). In a study of 400 HIV positive patients, of whom 15% were hepatitis C and 8% were hepatitis B co-infected, viral hepatitis remained associated with an increased risk of hepatotoxicity with relative risks of 2.78 (95% CI, 1.50–5.16) and 2.46 (95% CI, 1.43–4.24) for HBV and HCV infection, respectively (86). The risk of a DILI with nevirapine use, particularly in pregnancy, is notably elevated in the presence of hepatitis C co-infection. This was the observation from the ATHENA cohort where 425 pregnant and 1121 non-pregnant women were evaluated. Here, independent risk factors of hepatotoxicity in all women were the presence of detectable HCV RNA (OR 5.48, 95% CI 2.25-13.38, p<0.001) and nevirapine use (OR 2.63, 95% CI 1.54-4.55, p<0.001). However, stratified for pregnancy, the adjusted risk of hepatotoxicity was significantly associated with HCV co-infection only during pregnancy (OR 23.53, 95% CI 4.69-118.01, p<0.001) (87).
HIV increases the risk of tuberculosis 6 - 50 fold. (88). In the treatment of TB, one of the most frequent adverse events in patients is liver toxicity (89, 90). Up to 20% of patients develop asymptomatic elevation of liver enzymes (as a result of adaptation) which is usually self-limiting in most (91). In those who develop jaundice, ascites, encephalopathy or acute liver failure, the outcome is far less favourable (92, 93). Antituberculous DILI encompasses a wide spectrum of liver injury ranging from asymptomatic minimal elevation of liver enzymes to acute liver failure, often leading to death. Unlike paracetamol and other non-TB antimicrobials in high income countries, TB drugs are a leading cause of acute DILI leading to death in low and middle income countries (93, 94).

In a single centre registry of 303 patients in Bangalore in India, TB drugs contributed to 58% cases of DILI whilst in another series looking at acute liver failure in New Delhi in India, TB drugs contributed to 5.7% patients with acute liver failure carrying a 67% mortality (92, 94). An area of limited data is that although the risk factors for liver toxicity during TB treatment have been assessed in HIV-negative patients, there is limited data in HIV/AIDS patients. What is accepted is that HIV-infection increases the risk of hepatotoxicity during standard multidrug treatment of active TB (80, 90, 95). The reasons as to why HIV-TB co-infected patients have an increased risk of TB DILI are unclear. HIV/AIDS patients with acute illnesses have altered activities of oxidative pathways, which may partly explain their increased risk of TB DILI (96). The additional use of cART amplifies potential risks of toxicities and the concomitant use of fluconazole, which is often used in HIV/AIDS patients, is also a risk factor for TB DILI (97).
Liver enzyme elevations following the initiation of highly active antiretroviral therapy has been a frequently observed complication of HIV treatment with grade 3 and 4 hepatotoxicity observed in 8.5 – 23% of patients (39, 44, 98, 99). In longstanding exposure to cART, cholestatic changes in liver enzymes seem to predominate (100). More recently approved antiretroviral drugs demonstrate a better hepatic safety profile with risks of grade 3 to 4 hepatic toxicity less than 3% (101). Severe DILI can present as acute liver failure and in a retrospective analysis of 16 studies in the US AIDS Clinical Trials Group, 8851 patients were studied to assess risk factors for developing severe hepatotoxicity (102). Early (0 – 6 months) and late (7 – 12 months) severe hepatotoxicity was associated with abnormal baseline liver function test results and didanosine containing cART. Elevated creatinine (>1.5 X upper limit of normal) and low platelet count (<75 X 10^9) were also noted as risk factors although these variables might have been indicative of unrecognised chronic liver disease and portal hypertension (103). An additional factor noted was the presence and degree of fibrosis given that in a prospective study of 107 patients co-infected with HIV and HCV, hepatotoxicity events were higher in advanced fibrosis (Metavir F3 or F4) than in mild fibrosis (38% vs 15%) (104). A 12-fold increase in nevirapine hepatotoxicity has been reported in female patients with CD4 cell counts of greater than 250/mm^3 (105, 106). Although mechanisms of hepatotoxicity of protease inhibitors are not clearly understood, 1.9 - 5% of patients on protease inhibitors experience hepatotoxicity (107).

Five patterns of liver injury can occur with cART: hypersensitivity/immunoallergic, idiosyncratic reactions, mitochondrial toxicity, immune reconstitution syndrome and hepatic steatosis/steatohepatitis. Hypersensitivity reactions are immune mediated
and are in essence very similar to DRESS syndrome (drug reaction, eosinophilia, and systemic symptoms). It usually starts within 7 - 14 days of starting offending drug although delayed presentation at 6–12 weeks has been reported (106, 108). Hypersensitivity reactions are reported to occur with nevirapine, abacavir and efavirenz. There is no dose relation and symptoms generally resolve after stopping the drug although systemic steroids may be required. Nucleoside reverse transcriptase inhibitors (NRTIs) cause mitochondrial DNA depletion through their effect on mitochondrial gamma-polymerase. This results in impaired fatty acid oxidation, microvesicular steatosis and occasionally lactic acidosis. The risks of toxicity are greatest with didanosine and stavudine (109). Mitochondrial toxicity usually occurs weeks to months after starting cART and is associated with elevated ALT/AST, lactate, amylase, and lactate dehydrogenase (LDH). Insulin resistance secondary to longstanding cART, notably the NRTIs, contributes to the development of hepatic steatosis and steatohepatitis (110).
5.3.3 Viral hepatitis

5.3.3.1 Hepatitis B

Hepatitis B virus (HBV) is the most common cause of chronic liver disease worldwide, with over 400 million people chronically infected (111). HBV is endemic in sub-Saharan Africa, with prevalence rates for hepatitis B surface antigen (HBsAg) positivity ranging from 5–20% (112). Furthermore, up to 80% of HBsAg negative individuals in a West Africa study have circulating antibodies against hepatitis B core antigen immunoglobulin G (HBc-IgG), which confirms previous exposure to HBV (113).

Approximately 5 – 10% of HIV positive patients worldwide are co-infected with HBV (114). Given the HIV pandemic in sub-Saharan Africa, there are probably a large number of HIV/HBV-co-infected people. The prevalence of HBV infection varies by risk factor and geographic region and is influenced primarily by the age at which infection predominantly occurs (114). The endemicity of infection is high in parts of the world where infections predominantly occur during childhood or in the perinatal period e.g. South-East Asia, Sub-Saharan Africa, whilst it is intermediate in areas of mixed pattern acquisition i.e. infancy, childhood and adulthood e.g. Eastern Europe, Russia, Middle East. It is low in other parts of the world (typically high income countries) and immigration from high endemic areas to low prevalence areas accounts for recent increases (115). Co-infection rates in South Africa have been reported to occur between 5 – 20% (85, 116, 117).

Given that HIV suppresses the immune system, it intuitively seems that HBV would be more severe in co-infected patients. Conversely, the weakened immune response
from HIV may not accelerate liver disease progression since HBV-related liver
disease is primarily immune mediated. However, pathology studies in the literature
are few but conflicting. One of the earliest studies in 1986 looked at liver biopsies in
54 co-infected men. It was noted that liver injury was milder than anticipated despite
high levels of viraemia and thought to be due to patients being less immunologically
responsive to hepatitis B virus (118). A second study comparing 20 HIV positive co-
infected patients with HIV negative controls similarly found lower histological activity
indices in the presence of high levels of HBV replication. Fibrosis scores were also
lower (119). A later study of 132 patients was the first to demonstrate that whilst
necro-inflammatory activity was not necessarily elevated the degree of fibrosis and
risk for cirrhosis was clearly elevated (120). Another study came to similar
conclusions however in this series of 260 patients; necro-inflammatory activity and
fibrosis were enhanced in HIV-HBV co-infected patients (121). Importantly the first
case of fibrosing cholestatic hepatitis in a co-infected patient was described in 1993
(122). Fibrosing cholestatic hepatitis is an intriguing condition occurring in
immunosuppressed individuals with hepatitis B virus infection, unfortunately carrying
a poor prognosis. Exuberant viral replication produces progressive jaundice and
fibrosis in the absence of significant necro-inflammation.

There are several natural history studies that have shed light on the balance
between the opposing forces of HIV and HBV. As a result of the important role
played by the host immune response in control and clearance of HBV, HIV has
significant impact on the course of disease. To consider co-infection, it is necessary
to view the effect of HBV on HIV and vice versa. Firstly, it is generally accepted that
HBV co-infection doesn’t substantially alter the course of HIV. Studies of how HBV
affects the progression of HIV have produced conflicting results. Studies from the pre-HAART era did not demonstrate a significant impact of HBV on HIV progression and data from the large EuroSIDA cohort found that HBsAg positivity did not affect the incidence of new AIDS defining events. Furthermore even with adjusting for confounding variables e.g. use of cART, baseline viral loads, CD$_4$, age and ethnicity, the time taken for patients to reach undetectable HIV viral loads after 6 to 12 months on cART, was not affected (123, 124). There is however one recent study that suggests that HBV infection might indeed have a deleterious effect on HIV suppression. Patients infected with HIV and HBV who started cART had a significant rebound of HBV DNA after interruption of cART, along with accelerated immune deterioration (decrease in CD4+ cell count) (125).

The natural history of HBV is modified by HIV. There is an elevated risk of chronic HBV infection after acute infection in HIV positive adults (23% in HIV+ versus 4% in HIV -) (126, 127). HBV relapse (so-called sero-reversion) and the re-emergence of HBsAg, HBeAg or HBV DNA together with clinically significant disease can occur (114, 126) In those with chronic HBV infection, the risk of liver-related morbidity and mortality substantially increased in persons with HIV infection compared to those with HBV alone. In a long-term cohort study of 5923 men, liver related mortality rate was higher in men with HIV and HBsAg (14.2/1000) than in those with only HIV-1 infection (1.7/1000, P < 0.001) or only HBsAg (0.8/ 1000, P < 0.001) (128). After initiating cART, the effect of antiretroviral-related immune restoration has been associated with spontaneous recovery from chronic HBV infection but, in other studies, with flares of hepatitis B. Immune reconstitution hepatitis seems to occur most commonly in severely immunosuppressed patients (CD$_4$<200/mm3) (129).
Several studies have found that HBsAg-positive patients with a low CD4+ cell count nadir face an increased risk of liver-related death (128, 130). Data derived from HIV-negative persons suggest that higher levels of HBV DNA \((\geq 4 \text{ log}_{10} \text{ copies/mL})\) may be associated with an increased risk of cirrhosis and hepatocellular carcinoma; however, the relation between HBV DNA level and clinical outcomes has not been adequately studied in co-infected persons. (131, 132). Effective anti-HBV containing cART has beneficial effects on the clinical course of HBV. Lamivudine containing ART was associated with decreased risk of liver-related death in >2000 HBV/HIV co-infected persons (133). There are other data to suggest that antiretroviral regimens that contain drugs active against HBV infection (e.g., tenofovir, emtricitabine, and lamivudine) modifies the natural history of HBV disease in HIV-infected persons by slowing disease progression and, in some patients, leading to seroconversion. Use of dual agents active against HBV dramatically reduces the emergence of resistance; however, long-term follow-up is still needed to evaluate the natural history. The earlier use of HAART \((\text{CD}_4 \leq 500)\) with dual activity to ameliorate HBV liver disease progression before severe immunocompromise occurs is recommended (134). Current thinking therefore holds that early use of HAART containing dual-activity agents is generally positive for preventing severe immune dysfunction, controlling HBV replication, slowing liver disease progression, and preventing immune-reconstitution hepatitis.
Approximately 180 million people worldwide are chronically infected with hepatitis C virus (135). With decreasing mortality from AIDS-related opportunistic infections with the advent of HAART, liver disease has emerged as a very important cause for morbidity and mortality in the HIV-HCV co-infected population (136). As HIV related mortality has declined, hepatitis C related liver disease has become a leading cause of hospitalization and death in the co-infected population. Overall, HIV infection has a detrimental effect on the natural history of HCV disease; HIV-infected patients are less likely to clear hepatitis C following acute infection, have higher HCV RNA loads, and experience more rapid progression to end stage liver disease than those without HIV.(137, 138).

Approximately 30% of patients infected with HIV in the USA and Europe are also infected with hepatitis C (139). The high prevalence of HIV-HCV co-infection is not unexpected because both viruses are transmitted by the same routes, although not with the same efficiency. As a result, the prevalence of HIV-HCV co-infection varies across subpopulations of HIV-infected patients. For example, people acquiring HIV through exposure to contaminated blood (such as injecting drug users or haemophiliacs) are more likely to have HCV co-infection than those acquiring HIV through sexual transmission. In populations of injecting drug users in the USA and Europe, up to 75% of HIV positive patients are hepatitis C co-infected (140, 141). The picture appears to be different in Africa where HCV co-infection rates seem to be much lower suggesting that modes of transmission likely differ. Many patients have no identifiable risk factor and although nosocomial spread is important, the predominant mode of transmission is unclear (142, 143).
The presence of both HIV and HCV infection complicates the natural history of both viruses and their treatment (144). HIV influences HCV and co-infected patients have higher HCV viral loads than patients infected with HCV alone. HIV infection and the related immunosuppression produce more rapid progression of liver disease to cirrhosis, end-stage liver disease and death (145). In some studies, HIV/HCV co-infection was associated with more rapid progression to AIDS and death (145). HCV infection may be associated with an increased risk of ART associated hepatotoxicity (146). Since the introduction of HAART, liver-related morbidity and mortality has increased markedly in HIV-infected patients, particularly those co-infected with hepatitis C. The rate of liver-related complications increased from 5.4 to 26.7 admissions per 100 patient-years in HIV/HCV-co-infected patients treated at a large urban hospital between 1995 and 2000 (147). In addition, liver disease was the second leading cause of death (0.23 cases per 100 person-years) after HIV/AIDS (0.59 cases per 100 person-years) and ahead of cardiac disease (0.14 cases per 100 person-years) among 23,441 patients enrolled in the D: A: D study (148).

Hepatitis C may also have a deleterious effect on HIV, however the mechanisms by which HCV potentially affects HIV are unclear. There is speculation that HIV may be accelerated by HCV-related immune activation with consequent impairment in immune recovery following HAART. In a study of 3111 Swiss patients receiving HAART, it was observed that co-infected patients had an increased risk of progression to AIDS and AIDS-related death (149). This may reflect impaired CD4 cell recovery in co-infected patients on HAART however since there are many variables confounding this population, this finding cannot be confirmed.
5.3.4 Steatosis/steatohepatitis

As the life expectancy of individuals who are HIV positive improves, organ dysfunction associated with ageing becomes a more relevant issue. Apart from chronic viral hepatitis and alcoholic liver disease, one of the major causes of hepatic disease in the developed world is non-alcoholic fatty liver disease (NAFLD). NAFLD has been identified in up to 30% of HIV mono-infected Americans, although this study was unable to demonstrate whether the prevalence of NAFLD was different to HIV-negative individuals (150). NAFLD is the primary manifestation of the metabolic syndrome in the liver with lifestyle, in addition to low exercise and poor diet, being the major determinants for the risk of NAFLD. HIV itself, probably in addition to insulin resistance driven by anti-retroviral drugs, in particular NRTIs, contributes to the higher rate and severity of NAFLD in HIV/AIDS (151, 152). Steatohepatitis (simple steatosis with inflammation), may produce progressive fibrosis leading to cirrhosis (153). Nearly half of HIV/AIDS patients undergoing evaluation for unexplained liver test abnormalities have NAFLD (153, 154). In a French liver biopsy study of 30 HIV positive patients with persistently abnormal liver profiles, 18 had steatosis with severe fibrosis in six patients (155). It should however be noted that alcohol abuse remains prevalent in HIV populations and can produce a liver injury pattern indistinguishable from that of non-alcoholic steatohepatitis. Alcohol consumption thus always needs to be accounted for. Few studies of NAFLD have been published from low and middle income countries, especially Africa, however malnutrition may be a factor in promoting steatosis.
5.3.5 AIDS cholangiopathy

In 1986, Margulis et al described biliary disease resembling sclerosing cholangitis with benign strictures in 3 patients with advanced HIV/AIDS (156). Stenosis of the distal common bile duct and irregularity of the smaller intrahepatic and extrahepatic ducts was observed in patients with either cryptosporidium or cytomegalovirus infection of the biliary tree. It was initially assessed as being due to HIV itself. Cholangiopathy was seen exclusively in patients with advanced HIV and CD4 cell counts were invariably <100 cells/mm$^3$ (157). The aetiology is not entirely clear, but several opportunistic infections (Cryptosporidium parvum, Microsporidium, Cytomegalovirus, Cyclospora cayetanesis, Isospora belli) are suspected to cause it. Elegant work in 2007 suggested that the possible pathogenic mechanism through which HIV infection could cause AIDS-related cholangiopathy is through HIV tat protein enhancing Cryptosporidium parvum induced apoptosis of cholangiocytes via a Fas-ligand dependent mechanism (158).

Endoscopic retrograde cholangiopancreatography (ERCP) or Magnetic Resonance cholangiopancreatography (MRCP) is the diagnostic gold standard. ERCP identifies 4 particular patterns of stenosis: (1) Sclerosing cholangitis and papillary stenosis – 50% of cases, (2) Papillary stenosis only – 15% of cases, (3) Intrahepatic sclerosing cholangitis only – 20% of cases and (4) Long extrahepatic bile duct strictures ± intrahepatic sclerosing cholangitis – 15% of cases (159). ERCP offers a therapeutic means to provide symptomatic relief in cases of papillary stenosis (160). Clinically, the presentation of cholangiopathy may be variable, even completely asymptomatic. Right upper quadrant pain and fever accompanied by an elevated serum alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT) are the most common manifestations. Jaundice is unusual given that complete stenosis is uncommon. The
early initiation of HAART is preventive, without HAART the median survival is 9 months, invariably due to HIV related opportunistic infections (157).

6. Conclusion

The body of literature with respect to HIV/AIDS and liver disease is extensive. What emerges is a clear difference in the spectrum of pathology in the pre-HAART and HAART era. However superimposed on this is another layer of difference between the developed and developing world or more precisely high and middle or low income countries. It is the latter countries that carry a disproportionately higher HIV burden and where the data on liver disease is most limited. Data on clinical liver disease abounds from high income countries however here too pathology based studies are limited.

Our study represents the largest of its kind ever performed in the developing world. It is primarily aimed at filling the data gap for countries with a very high burden of HIV and to determine the factors that characterise liver disease in this population. Patterns that emerge from the data will also potentially assist in guiding clinical practice nationally and sub-regionally. This is of value given that access to tertiary investigations such as liver biopsy to guide patient management is not widely available in middle or low income countries.
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PART C

A clinicopathological cohort study of liver pathology in 301 patients with HIV/AIDS

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Globally, approximately 34 million people are HIV infected with 70% of those infected residing in sub-Saharan Africa (1). South Africa is the epicentre of the pandemic with some 5.6 million people living with HIV and 10.6% of the adult population HIV infected (2). The high tuberculosis (TB) prevalence compounds the challenge. TB-HIV co-infection is a significant burden with up to 65% of newly diagnosed sputum positive TB patients being HIV positive (3). In addition, South Africa, in keeping with sub-Saharan Africa, is an endemic area for hepatitis B with 5-15% of the South African population positive for HBsAg. Over the last decade, a wide scale public access programme means that almost 2 million South Africans currently access highly active antiretroviral therapy (HAART) (4).

HIV-related liver disease has emerged as a significant cause of morbidity and mortality, both in the pre-HAART, but particularly in the HAART era of HIV/AIDS (5, 6). Most data in this regard has come from high income countries with the D: A: D study identifying liver-related mortality as the leading cause of non-AIDS mortality in HIV-positive patients (7, 8). Data from studies primarily assessing liver pathology in patients with HIV/AIDS is limited and studies were mostly performed prior to the availability of HAART (9-13). Invariably, a difference exists in findings between high and middle or low income countries. The largest series to date of 501 liver biopsies, done in the pre-HAART era in New York, reported granulomatous inflammation, predominantly due to Mycobacterium avium complex, and viral hepatitis as the most frequent pathological findings (11). Steatosis has also been frequently reported in other liver biopsy series from both high and low income countries (9, 10, 13, 14). Similarly, limited data from low and middle income areas, has reported granulomatous inflammation as a prevalent pathological finding. However, in contrast to high income countries, Mycobacterium tuberculosis (MTB) is the principal
cause of granulomatous inflammation. A series of 46 liver biopsies from Thailand, apart from MTB related granulomatous inflammation, reported viral hepatitis, steatosis and drug-induced liver injuries (DILI) as prevalent findings (12). A recent autopsy study from South Africa in patients after the initiation of antiretroviral therapy reported MTB related granulomatous inflammation and DILI as notable findings (15).

Liver enzyme elevations following the initiation of highly active antiretroviral therapy has been a frequently observed complication of HIV treatment, with grade 3 and 4 hepatotoxicity observed in 8.5 – 23% of patients (16-18). The question as to whether drug-induced liver injuries occur at a greater frequency in HIV positive patients than they do in HIV negative patients is unclear, although hypersensitivity reactions to drugs such as sulfamethoxazole-trimethoprim (cotrimoxazole, Bactrim\textsuperscript{R}) occur at a greater frequency in HIV positive than HIV negative individuals (19).

Data on the histopathological spectrum in patients with HIV/AIDS presenting with liver disease and the clinical correlates in a high HIV prevalence middle or low income country is limited. Given this, our aim was to evaluate our clinicopathological observations and experience in such an environment.
Patients and Methods

Study population

In 2005, a database of all adult HIV positive patients with liver disease referred to our centre for evaluation and who consequently had a liver biopsy, was established. Retrospectively, patients were included from January 2000 to May 2005. Thereafter, patients were prospectively enrolled until June 2013. Indications for liver biopsy varied but mostly included abnormal liver profile, hepatomegaly (as defined clinically by the physician or radiologically) or pyrexia of uncertain aetiology (temperature $>38^\circ C$ for $\geq 1$ week with no cause despite an adequate workup). The ultimate decision to perform a biopsy was clinically based. In patients with suspected drug injuries, biopsy was indicated with rising liver enzymes, incomplete resolution after drug withdrawal or a consideration for specific therapy e.g. corticosteroids. The HIV positive status of patients was confirmed with ELISA based testing for HIV antibody and p24 antigen and patients were staged clinically using the World Health Organization clinical staging system for HIV/AIDS (20). Liver biopsies were performed via the standard technique using either the Quick-Core® Biopsy Needle Set (Cook Medical, Bloomington, IN, USA) or using the modified Menghini Hepafix® 88mm biopsy needle (B. Braun Melsungen AG, Melsungen, Germany). As per our standard practice, approximately 1mm of the core of liver tissue from every biopsy was submitted for mycobacterial and fungal culture and confirmation of culture results obtained from the microbiology laboratories. Autopsy based data was not included. A liver biopsy was deemed adequate if $\geq 1.5$cm in length with at least 6 portal tracts present. Biopsies of patients that were initially added retrospectively (64 in total) were all accessed from the pathology archives of the University of Cape
Town and also reviewed for adequacy of assessment and inclusion in the study. All relevant clinical and demographic patient data was recorded including age, gender, history of opportunistic infections including tuberculosis and mode of TB diagnosis, WHO clinical HIV/AIDS staging, complete drug or toxin exposure history and alcohol history. The study was performed in accordance with the Declaration of Helsinki and approved by the University Of Cape Town Faculty Of Health Sciences Research Ethics Committee (REC 187/2005).

Laboratory tests

Liver enzyme profiles including total and conjugated bilirubin, alanine and aspartate aminotransferase (ALT, AST), alkaline phosphatase (ALP) and gamma-glutamyl transpeptidase (GGT) were recorded at or 1 day before liver biopsy. Grading of hepatotoxicity for suspected drug injuries was done utilizing the Adult AIDS Clinical Trials Group definitions of hepatotoxicity (21). The CD4 positive T-cells (cells/mm$^3$) obtained within 4 weeks of liver biopsy was recorded. HIV RNA quantification was not routinely recorded given that the antiretroviral treatment protocol only allows for testing 6 months after initiating therapy. Serological and virological markers for hepatitis A (Hepatitis A IgM antibody), hepatitis B (HBsAg, HBcore IgM antibody) and hepatitis C (Hepatitis C IgG antibody) were obtained on all patients. Patients who were HBsAg positive had additional testing for HBeAg and anti-HBe antibody. In some patients negative for HBsAg, total anti-HB core antibody was tested to determine previous hepatitis B exposure status. Anti-HDV was not tested in HBsAg positive patients given that South Africa is an HDV non-endemic area. All serological testing was done using the ARCHITECT I or II system (Abbott Diagnostics Division, Wiesbaden, Germany). HBV DNA viral levels were assessed using the Artus RealArt$^R$ HBV PCR assay (Artus-Biotech, Qiagen, Hamburg,
Germany) up to 2009, when a change was made to the Cobas Ampliprep/Cobas Taqman HBV test, version 2.0 (Roche Diagnostics GmbH, Mannheim, Germany). Those positive for hepatitis C IgG antibody had subsequent hepatitis C PCR confirmed by means of an in-house PCR technique after amplifying the 5’NCR region of the virus. Hepatitis C genotype was done using the Versant HCV Genotype 2.0 Assay Line Probe Assay (Siemens AG Healthcare, Munich, Germany). Hepatitis C viral load assessment was done by a reference laboratory utilizing the COBAS Ampliprep/Cobas TaqMan v2.0 (Roche Diagnostics GmbH, Mannheim, Germany).

**Histopathological examination**

Biopsy material was routinely haematoxylin and eosin-stained in addition to Bile sirius red, reticulin, periodic *acid-Schiff (PAS)*, PAS diastase and Perl’s Prussian blue stain. Where necessary, Ziehl-Neelsen stain was utilized to assess for acid-fast bacilli, Grocott’s methenamine silver stain for fungal organisms, immunohistochemistry stains for hepatitis B surface and core antigen and CK7 stain for bile duct epithelium. All the liver biopsies in this study were assessed by one of either two experienced liver histopathologists. Clinicopathological assessments were done concurrently with hepatologists. The diagnosis of a drug-induced liver injury was determined by the exclusion of viral hepatitis, the temporal relationship between drug or toxin exposure and the injury, signature biochemical patterns of given drugs, effect of drug de-challenge and compatible histological patterns. Drug induced liver injuries were categorised into one of the following histological patterns of injury. Firstly, non-specific hepatitis in which there was portal and/or lobular inflammation particularly in zone 3 with/without cholate stasis in zone 1, with inflammatory cells including lymphocytes & eosinophils. Secondly, cholestatic in
which there was marked bilirubinostasis involving predominantly zone 3 and minimal interface or portal tract inflammation. Thirdly, mixed hepatitic-cholestatic in which there was a combination of portal tract inflammation/interface hepatitis with inflammatory cells, including lymphocytes and eosinophils together with marked zone 3 bilirubinostasis and a ductular reaction. Fourthly, sub-massive necrosis in which zonal or panzonal necrosis was present. Fifthly ductopenia with a “vanishing bile duct syndrome” pattern with cholate stasis, bile duct targeting and absence of bile ducts on CK7 stain. The sixth pattern was microvesicular steatohepatitis with steatosis, ballooned hepatocytes and necroinflammatory foci with or without Mallory’s hyaline occurring in the setting of known drug exposure and other aetiological factors excluded. The seventh pattern of non-necrotizing granulomatous inflammation was ascribed to drugs only after an exhaustive exclusion of mycobacterial infection and in a few instances lack of response to a trial of anti-TB drug therapy. Findings of hepatocyte “pseudo-ground glass change” (in HBV negative patients) thought to be drug-related, but in the absence of any significant inflammatory changes, cholestasis or any typical histological pattern of drug injury, was termed drug adaptive or related changes and not regarded as a DILI. Granulomas were classified as necrotizing (caseous) or non-necrotizing (non-caseous). By local convention and practice, histological grading and staging of hepatitis B utilized the criteria as proposed by Ishak et al (26), whilst hepatitis C was assessed utilizing the METAVIR system (27).
Statistical analysis

Values are expressed as the median and interquartile range for continuous variables. Clinical characteristics were summarized in the total cohort using standard descriptive characteristics. Differences between different qualitative parameters were explored using the chi-square, Fishers exact or Mann-Whitney U test, where appropriate. Odds ratios of a DILI relating to specific drugs were calculated with a 95% confidence interval. Multiple logistic regression analysis utilizing the following factors: age, female gender, CD4 cell count<200, alcohol use, presence of HBsAg and the likely offending drugs was utilized to explore baseline demographic, clinical characteristics and likely offending drug associated with the risk of a specific histological pattern of DILI. A $P$-value of <0.05 was set as the significance level. Statistical analysis was performed using Stata$^R$ v.12.0.
Results

Patient characteristics. Table 1 demonstrates the demographics and characteristics of a total of 301 HIV positive patients who underwent percutaneous liver biopsy and were enrolled in the cohort. There were marginally more men than women, 52% vs. 48%, and patients were young with a median age of 34 years. Woman were significantly younger than men ($P<0.001$) and ethnically, the majority of patients were Black. Patients had advanced HIV/AIDS with a median CD4 cell count of 127 (IQR 52-260). A total of 66.7% had a CD4 cell count $<200$ cells/mm$^3$ with 23.3% $<50$ cells/mm$^3$. Correspondingly almost half of patients were clinically WHO stage 4. Almost a quarter of patients consumed alcohol on a regular basis, males more so than females ($P<0.0001$). Fifty-six patients (18.6%) were hepatitis B co-infected with a far smaller number of patients co-infected with confirmed hepatitis C (3.3%). Medication drug exposure by patients within 4 weeks of liver biopsy is as listed in Table 1. Polypharmacy was prevalent with 83 (27.5%), 67 (22.2%), 62 (20.5%), 35 (11.6%) of patients using HAART/cotrimoxazole, anti-TB drugs/cotrimoxazole, HAART/anti-TB drugs and HAART/cotrimoxazole/anti-TB drugs, respectively. HAART regimens were predominantly dual NRTI plus NNRTI based (Table 2), the predominant NNRTI being efavirenz.

Clinicopathological findings. Histological findings and/or clinicopathological correlates are as listed in Table 3. DILI was the predominant finding (42.2%), followed by granulomatous inflammation (29%), steatosis/steatohepatitis (19.3%) and hepatitis B (19%). Hepatitis C was confirmed in 10 (3.3%) from 11 HCV antibody positive patients. Lymphoma was diagnosed in 7 (2.3%) patients, the majority (86%) being high grade B-cell lymphomas. Siderosis in excess of grade 1
was observed 24 (8%) of patients with the majority, 19 (79%), of black ethnicity. The finding of siderosis was invariably observed in conjunction with other pathology viz. steatohepatitis 8 (33%), steatosis 7 (29%), granulomatous inflammation 4 (17%), respectively. AIDS cholangiopathy was suspected in 7 (2.3%) patients and was confirmed by radiological imaging (5 by MRCP and 2 with ERCP). Drug adaptive or related changes occurred in 7 (2.3%) patients. A total of 49 (16.2%) patients had more than 1 pathological finding on biopsy. The majority of granulomatous inflammation in 79 (92%) patients was non-necrotizing. *Mycobacterium tuberculosis* accounted for the majority of granulomatous inflammation observed and was cultured in 61 (71%) patients – 12 (19.6%) from liver tissue, 32 (52.4%) from sputum, 3 (4.9%) from urine and 14 (23%) from miscellaneous tissues/fluids (lymph node aspirates, pleural fluid, bone marrow). In 3 (3.5%) patients, *Mycobacterium avium* complex was cultured from liver tissue and 1 (1.1%) patient demonstrated a positive Grocott’s methenamine silver stain for fungal elements and subsequently cultured *Cryptococcus neoformans*.

**Drug induced liver injuries.** Table 4 demonstrates the liver enzyme profiles and grading of the various histological patterns of suspected DILI. Drug related/adaptive changes are included in Table 4, but were not regarded as a DILI. Jaundice was not a significant features in those with non-specific hepatitis. The ALT and AST levels differed only between the non-specific hepatitis and submassive necrosis groups, \( P<0.001 \), whilst ALP and GGT levels differed between non-specific hepatitis, cholestatic, mixed cholestatic-hepatitic and ductopenic patterns of DILI, \( P<0.001 \). The grades of hepatotoxicity based on median ALT are as listed in table 4. Ductopenic injuries and submassive necrosis were grade 3 and 4, respectively. All others were grade 2.
Univariate analysis (Table 5) suggested that age may play a factor for a DILI, $P=0.079$, however gender and CD4 cell count did not play a role. Cotrimoxazole, HAART and herbal medication were most likely to be associated with a DILI. Table 6 demonstrates the clinical and demographic factors as well as the drugs associated with a specific histological DILI pattern using multivariate logistic regression analysis. Non-specific hepatitis was associated with cotrimoxazole (OR, 3.82; 95% confidence interval, (1.82-8.0); $P=0.001$), efavirenz (OR, 4.3; 95% confidence interval, 1.92-9.83; $P<0.001$), nevirapine (OR, 9.6 95% confidence interval, 2.25-40.86; $P=0.002$) and anti-TB drug (OR, 8.1; 95% confidence interval, 2.4-27.81; $P<0.001$) use with no other factors demonstrating an association. Cholestatic injury was associated with female gender (OR, 3.25; 95% confidence interval, 1.08-9.72); $P=0.03$) and cotrimoxazole use (OR, 7.05; 95% confidence interval, 2.5-19.89; $P<0.001$). Mixed cholestatic-hepatitic injury was primarily associated with cotrimoxazole (OR, 3.99; 95% confidence interval, 1.57-10.17; $P=0.003$), whilst efavirenz demonstrated a trend to significance (OR, 2.69; 95% confidence interval, 0.86-11.5; $P=0.07$). Submassive necrosis was associated with younger age (OR, 0.88; 95% confidence interval, 0.81-0.96; $P=0.003$), higher CD4 cell count (OR, 0.21 95% confidence interval, 0.06-0.78; $P=0.012$) and efavirenz (OR, 10.46; 95% confidence interval, 2.7-40.5; $P<0.001$). The vanishing bile duct pattern was only associated with cotrimoxazole (OR, 17.6; 95% confidence interval, 3.26-95.3; $P<0.0001$).

**Viral hepatitis.** Table 7 highlights the 56 patients with hepatitis B, 32 (57%) HBeAg positive and 24 (43%) HBeAg negative. Median age did not differ between the 2 groups ($P=0.49$) and neither did the alanine (ALT) and aspartate (AST) aminotransferase levels ($P=0.8$, 0.5; respectively). Despite the median CD4 cell count and HBV DNA level being significantly lower in the HBeAg negative group
(P=0.03, 0.008; respectively), median histological staging and grading, did not differ (P=0.2, 0.8; respectively). Of note, 99 patients who were negative for HBsAg were screened for anti-HB core antibody and 66 (66.6%) were positive. In the 10 patients with confirmed hepatitis C, genotype 1a was the predominant genotype, 8 (80%), with the remaining 2 patients being genotype 2 and 3a, respectively. Median (range) METAVIR fibrosis score was 2 (1-4), with 4 patients (40%) being ≥F3. Median hepatitis C viral load was 5.95 (3.4-6.4) log_{10} IU/ml.
Discussion

This study, the largest of its kind from a middle or low income country with a high HIV burden, demonstrates the extensive range of liver pathology observed in this particular setting. The demographics of this 301 patient cohort is representative of the broader HIV population and epidemiology in South Africa where patients often present with advanced HIV/AIDS and females are invariably younger (22). The CD4 cell counts at the time of biopsy were low. In keeping with a previously reported pattern, females had a higher CD4 count than men (23). Fortunately, with large scale HIV counselling and testing campaigns, coupled with a comprehensive management strategy for those with HIV since 2007, the epidemic is slowly changing in South Africa (24). Hence, more than half of patients in this study were on antiretroviral therapy prior to liver biopsy. Indirectly, this may also be a factor accounting for the major finding in this study of a high frequency of DILI.

A total of 42.2% of patients in this cohort were clinicopathologically determined to have a DILI. A clinical correlation was noted between the liver enzyme profiles and the histological patterns of DILI we observed. Drug-related steatohepatitis and granulomatous inflammation was only observed in a small number of patients. Patients with grade 1 hepatotoxicity were invariably noted on biopsy to have what was described as drug related or adaptive changes.

Univariate analysis suggested cotrimoxazole, HAART and herbal medications were most frequently associated with DILI, although the number of patients with herbal medications was decidedly small. At first it appears anomalous that anti-TB drugs, well known for their hepatotoxic potential, were not overtly associated with DILI. However, this can be explained by our clinical practice and bias against performing a
liver biopsy in patients with a suspected anti-TB drug DILI. In such patients, prompt withdrawal of anti-TB drugs, the exclusion of other causative factors and a subsequent resolution of an abnormal liver enzyme profile, is a diagnosis by inference of an anti-TB drug DILI and management would not generally include a liver biopsy.

DILI is a well-recognized complication in HIV positive patients, given their potential use of HAART, anti-TB therapy and other antimicrobials such as cotrimoxazole, both for prophylactic and therapeutic purposes. Hepatotoxicity related to HAART is reported to range from 2 – 37% depending on the regimen used, as well as the definition of hepatotoxicity utilized in that assessment (25). In South Africa, HAART hepatotoxicity has also been noted as a frequent observation in clinical HIV practice, particularly in the setting of TB-HIV co-infection and conferred a 35% 3-month mortality risk (26, 27).

In our study, the majority of patients with steatohepatitis related DILI were using stavudine and/or didanosine as part of their HAART regimen. This is understandable given their potential mitochondrial toxicity (28). Owing to this and other toxicity, the use of didanosine has declined and is used less frequently. Furthermore, in 2010, tenofovir replaced as a first line NRTI in the public HAART programme in South Africa.

The most frequent histological pattern of drug injury observed was a non-specific hepatitis and this corresponded with grade 2 hepatotoxicity. In the multivariate analysis cotrimoxazole, efavirenz, nevirapine and anti-TB drugs were all associated
with this pattern of injury. A hepatocellular pattern of drug injury with these drugs is well described (29, 30). Although not specifically assessed in our study, recent data from Tanzania, another middle or low income country, noted an increased risk of efavirenz related DILI when used concomitantly with anti-TB drugs, particularly rifampicin. Another possible factor was polymorphisms of CYP2B6 (31).

Cholestatic and mixed cholestatic-hepatitic injuries were strongly associated with cotrimoxazole, as was female gender. Female gender is generally thought to be associated with an increased risk of DILI, although this has recently been questioned (32). Cotrimoxazole has been associated with a variety of histological patterns of DILI although its commonest association is with cholestatic and ductopenic injuries. Cotrimoxazole hepatotoxicity in patients with HIV/AIDS was recognized early on in the HIV pandemic as a potential problem (33). In this study, it was the drug almost exclusively associated with patients who had a ductopenic or vanishing bile duct syndrome DILI. In these patients, the injury developed after high dose cotrimoxazole use in the treatment of either Pneumocystis jirovecii pneumonia or cerebral toxoplasmosis, rather than those using it for purposes of prophylaxis. This suggests that the risk of a ductopenic injury in this clinical setting may be a dose-related phenomenon.

The question as to whether drug-induced liver injuries occur at a greater frequency in HIV positive patients than they do in HIV negative patients, is unclear although previous studies suggested that the frequency of drug allergy or hypersensitivity in patients with HIV ranges from 3% to 20%, much higher than the general population (34). Cotrimoxazole, a commonly used antimicrobial for both prophylaxis and
treatment in HIV/AIDS is associated with hypersensitivity reactions in 3% to 5% of HIV-negative patients (19).

Co-infection with hepatitis B or C is known to increase the risk of HAART hepatotoxicity (35). The low background prevalence of hepatitis C in South Africa and in our study would limit the influence of this effect. However, a surprising finding was the lack of association between drug injury and HBsAg in our study. It likely relates to this not being a broad population based study looking at all HIV/AIDS patients with hepatotoxicity but rather only those who had a liver biopsy. Furthermore, a local study previously noted an association with HAART hepatotoxicity and hepatitis B, although at a risk lower than populations in high income countries (27). Another factor could be that for a period prior to the widespread availability of tenofovir in the public program, patients were uniformly tested for HBsAg. Those co-infected were defaulted to tenofovir/lamivudine based HAART automatically and at a higher CD4 cell count level. This may have ameliorated the hepatotoxicity risk of hepatitis B in our population (36). Equally a lack of association with regular alcohol use and all patterns of DILI was observed, possibly due to the relatively low frequency of alcohol use in this cohort. In addition, more men than woman used alcohol and a trend towards female gender with some DILI histological subtypes was noted in multivariate analysis.

Efavirenz was strongly associated with submassive necrosis as was younger age and a CD4 cell count >200 cells/mm$^3$, which mirrors the reported risks for nevirapine associated DILI (37, 38). Although nevirapine was associated with a non-specific hepatitis pattern of injury in our study, its lack of association with submassive
necrosis is explained by the small number of patients using nevirapine as the NNRTI in their HAART regimen (21 nevirapine vs. 120 efavirenz). This is due, in part, to a reduction in the use of nevirapine since 2010 in the public HAART program in South Africa, given newer data of the low teratogenic risk of efavirenz in pregnancy. As with anti-TB drugs, bias in not biopsying patients with suspected nevirapine hepatotoxicity given its characteristic pattern, would be an additional explanation for this observation.

The 29% frequency of granulomatous inflammation predominantly due to *Mycobacterium tuberculosis* in this study is not surprising and is similar to other studies (12, 39). *Mycobacterium avium* complex, seen in only 3 patients, is surprisingly uncommon in our setting, although the reasons for this are not entirely clear (40). Granulomas were largely non-necrotizing, a feature well recognized in patients with advanced HIV/AIDS and is due to a marked HIV associated immune compromise (41-43). More than half of the patients fulfilled criteria for a TB immune reconstitution inflammatory syndrome (IRIS) affecting the liver (44). This hepatic predominant type IRIS has not yet been described in the literature and poses a significant clinical challenge after initiating HAART, given that the differential diagnoses in this setting are wide.

Little data on the histological features of hepatitis B in HIV positive patients exists (45). In our study the age, gender and median ALT and AST levels did not differ between HBeAg positive and negative patients. HBeAg negative patients had a significantly lower median CD4 cell count and HBV DNA viral load than the HBeAg positive patients. Despite this, median histological necroinflammatory activity and fibrosis assessment did not differ in the 2 groups. Overall, median ALT and AST levels were elevated >3 times the upper limit of normal, which did not reflect in the
relatively moderate necroinflammatory activity observed. This may in part reflect the degree of HIV related immunosuppression. One patient had fibrosing cholestatic hepatitis related to hepatitis B, a pattern described initially in the setting of liver transplantation and rarely in the setting of HIV-HBV co-infection (46). The presence of anti-HB core in two-thirds of those who were HBsAg negative highlights the endemic nature of hepatitis B in South Africa.

Hepatitis C has low background prevalence in South Africa and this is reflected in the modest number of patients with hepatitis C co-infection, a factor that promotes hepatitis C related liver fibrosis. Genotype 1a predominated (80%) and 40% of patients had advanced fibrosis/cirrhosis (≥F3), a feature frequently observed in co-infected populations (6).

Non alcohol related steatosis and steatohepatitis was observed in 19.3% of patients. Several other studies have made a similar observation (9, 12, 13). NAFLD is a finding in up to a half of HIV patients with unexplained liver enzyme abnormalities (47, 48). The reasons are likely multifactorial and include nutritional factors, HIV itself or insulin resistance related to antiretroviral therapy (47).

In conclusion, we have demonstrated the wide range of liver pathology and disease encountered in a middle or low income country with a high HIV prevalence. Our findings provide insight into the actual pathology that accounts for liver disease in this patient population in the clinical context of a low and middle income country. DILI, notably due to cotrimoxazole and HAART, are frequently encountered and complicate HIV management. Hepatitis B co-infection, although frequent, does not pose a significant clinical problem. However, the phenomenon of TB-IRIS involving the liver potentially complicates the initiation of HAART in a high HIV-TB burden.
area. Given the need for an accurate and early diagnosis in patients with advanced HIV/AIDS presenting with liver disease, we would suggest liver biopsy is considered as early as is clinically warranted. Liver biopsy together with careful clinico-pathological assessment provides a useful tool in guiding management in these clinically complex patients. However it should be recognized that given the burden of disease in South Africa it is unrealistic that liver biopsy will be widely available to all patients.

**Abbreviations**

NRTI, nucleoside reverse transcriptase inhibitors

NNRTI, non-nucleoside reverse transcriptase inhibitors

ERCP, Endoscopic retrograde cholangiopancreatography

MRCP, Magnetic Resonance cholangiopancreatography
## Results: Tables

### Table.1 Baseline characteristics of 301 patients at the time of liver biopsy

<table>
<thead>
<tr>
<th>Parameter</th>
<th>N = 301</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>34 (29-40)</td>
<td></td>
</tr>
<tr>
<td>Males - n (%)</td>
<td>158 (52.4)</td>
<td></td>
</tr>
<tr>
<td>Age (years): Male: Female</td>
<td>35 (31-41) : 33 (28-37)</td>
<td>0.001</td>
</tr>
<tr>
<td>CD4 (cells/mm³)</td>
<td>127 (52-260)</td>
<td></td>
</tr>
<tr>
<td>CD4 Male: Female</td>
<td>118 (46-259):132 (55-270)</td>
<td>0.56</td>
</tr>
<tr>
<td>CD4 &lt;200</td>
<td>201 (66.7)</td>
<td></td>
</tr>
<tr>
<td>CD4&lt;50</td>
<td>70 (23.3)</td>
<td></td>
</tr>
<tr>
<td>HIV/AIDS clinical stage, n (%):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1 (0.4)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>53 (17.6)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>97 (32.2)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>150 (49.8)</td>
<td></td>
</tr>
<tr>
<td>Ethnic group# – n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>229 (76.1)</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>35 (11.6)</td>
<td></td>
</tr>
<tr>
<td>Mixed Ancestry</td>
<td>35 (11.6)</td>
<td></td>
</tr>
<tr>
<td>Asian/Indian</td>
<td>2 (0.7)</td>
<td></td>
</tr>
<tr>
<td>Alcohol consumption – n (%)</td>
<td>75 (24.9)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Male: Female</td>
<td>56 (75) / 19 (25)</td>
<td></td>
</tr>
<tr>
<td>HBsAg positive – n (%)</td>
<td>56 (18.6)</td>
<td></td>
</tr>
<tr>
<td>HCV antibody positive – n (%)</td>
<td>11 (3.7)</td>
<td></td>
</tr>
<tr>
<td>HCV PCR positive</td>
<td>10 (3.3)</td>
<td></td>
</tr>
<tr>
<td>Genotype 1a</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Genotype 2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Genotype 3a</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Medication drug use§ – n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cotrimoxazole (Bactrim&lt;sup&gt;R&lt;/sup&gt;)</td>
<td>157 (52.1)</td>
<td></td>
</tr>
<tr>
<td>HAART</td>
<td>162 (53.8)</td>
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</tr>
<tr>
<td>Anti-TB medication</td>
<td>112 (37.2)</td>
<td></td>
</tr>
<tr>
<td>Fluconazole</td>
<td>16 (5.3)</td>
<td></td>
</tr>
<tr>
<td>Herbal</td>
<td>10 (3.3)</td>
<td></td>
</tr>
<tr>
<td>Other¶</td>
<td>18 (5.9)</td>
<td></td>
</tr>
</tbody>
</table>

The data is expressed as medians and interquartile ranges or numbers and percentages

* World Health Organization clinical staging system for HIV/AIDS

# Ethnic group is self-reported

§ Medication use at or within 4 weeks of biopsy

¶ other drugs included amoxicillin-clavulanate, ciprofloxacin, clarithromycin
Table 2. Frequency of various HAART regimens used

<table>
<thead>
<tr>
<th>HAART regimen, n = 162</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nucleoside RT inhibitors</strong></td>
<td></td>
</tr>
<tr>
<td>LAM/D4T</td>
<td>158 (98)</td>
</tr>
<tr>
<td>LAM/TDF</td>
<td>72 (46)</td>
</tr>
<tr>
<td>LAM/AZT</td>
<td>38 (24)</td>
</tr>
<tr>
<td>TDF/FTC</td>
<td>28 (18)</td>
</tr>
<tr>
<td>DDI/D4T</td>
<td>10 (6)</td>
</tr>
<tr>
<td>AZT/LAM/ABC</td>
<td>8 (5)</td>
</tr>
<tr>
<td></td>
<td>2 (1)</td>
</tr>
<tr>
<td><strong>Non-nucleoside RT inhibitors</strong></td>
<td></td>
</tr>
<tr>
<td>Efavirenz</td>
<td>141 (87)</td>
</tr>
<tr>
<td>Nevirapine</td>
<td>120 (85)</td>
</tr>
<tr>
<td></td>
<td>21 (15)</td>
</tr>
<tr>
<td><strong>Protease inhibitors</strong></td>
<td></td>
</tr>
<tr>
<td>Lopinavir/Ritonavir</td>
<td>21 (13)</td>
</tr>
<tr>
<td>Atazanavir/Ritonavir</td>
<td>18 (86)</td>
</tr>
<tr>
<td>Amprenavir</td>
<td>2 (10)</td>
</tr>
<tr>
<td></td>
<td>1 (4)</td>
</tr>
</tbody>
</table>

Abbreviations: RT, reverse transcriptase; LAM, Lamivudine; D4T, Stavudine; AZT, Azidothymidine; TDF, Tenofovir; FTC, Emtricitabine; DDI, Didanosine; ABC, Abacavir
Table 3. Frequency of clinico-pathological findings

<table>
<thead>
<tr>
<th>Finding, n=301</th>
<th>Frequency – n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug induced liver injuries</td>
<td></td>
</tr>
<tr>
<td>Non-specific hepatitis</td>
<td>51 (40.2)</td>
</tr>
<tr>
<td>Cholestasis</td>
<td>20 (15.7)</td>
</tr>
<tr>
<td>Mixed hepatitis-cholestasis</td>
<td>25 (19.7)</td>
</tr>
<tr>
<td>Submassive necrosis</td>
<td>13 (10.2)</td>
</tr>
<tr>
<td>Ductopenia/vanishing bile duct</td>
<td>11 (8.6)</td>
</tr>
<tr>
<td>Steatohepatitis</td>
<td>5 (4.0)</td>
</tr>
<tr>
<td>Granulomatous (drug related)</td>
<td>2 (1.6)</td>
</tr>
<tr>
<td>Granulomatous inflammation</td>
<td></td>
</tr>
<tr>
<td>Necrotizing/Non-necrotizing</td>
<td></td>
</tr>
<tr>
<td><em>Mycobacterium tuberculosis</em>¶</td>
<td>61 (71)</td>
</tr>
<tr>
<td><em>Mycobacterium avium complex</em></td>
<td>3 (3.5)</td>
</tr>
<tr>
<td>Cryptococcosis§</td>
<td>1 (1.1)</td>
</tr>
<tr>
<td>Drug</td>
<td>2 (2.3)</td>
</tr>
<tr>
<td>TB-IRIS related#</td>
<td>45 (52.3)</td>
</tr>
<tr>
<td>Hepatitis B</td>
<td>56 (19)</td>
</tr>
<tr>
<td>Steatosis/Steatohepatitis</td>
<td>58 (19.3)</td>
</tr>
<tr>
<td>Steatosis</td>
<td>42 (72.4)</td>
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<tr>
<td>Steatohepatitis</td>
<td>16 (27.6)</td>
</tr>
<tr>
<td>Alcoholic liver disease</td>
<td>16 (5.3%)</td>
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<tr>
<td>Hepatitis C (PCR positive)</td>
<td>10 (3.3)</td>
</tr>
<tr>
<td>Siderosis &gt; grade 1(49)</td>
<td>24 (8.0)</td>
</tr>
<tr>
<td>Lymphoma</td>
<td></td>
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<tr>
<td>High grade B-cell/Hodgkin’s lymphoma</td>
<td>7 (2.3)</td>
</tr>
<tr>
<td>HIV/AIDS cholangiopathy</td>
<td>7 (2.3)</td>
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<tr>
<td>Drug related/adaptive changes</td>
<td>7 (2.3)</td>
</tr>
<tr>
<td>Indeterminate findings</td>
<td>5 (1.6)</td>
</tr>
<tr>
<td>&gt;1 pathological finding</td>
<td>49 (16.2)</td>
</tr>
</tbody>
</table>

¶ *Mycobacterium tuberculosis* cultured from liver or other fluid/tissue
* *Mycobacterium avium complex* cultured from liver or other fluid/tissue
§ *Cryptococcus neoformans* cultured from liver tissue
# TB-IRIS, TB Immune Reconstitution Inflammatory Syndrome (44)
### Table 4. Liver profiles of the various histological patterns of DILI

<table>
<thead>
<tr>
<th>Pattern (n)</th>
<th>Total Bilirubin 0-21 (µmol/L)</th>
<th>Conjugated Bilirubin 0-6 (µmol/L)</th>
<th>ALT 5-40 (U/L)</th>
<th>AST 5-40 (U/L)</th>
<th>ALP 40-120 (U/L)</th>
<th>GGT 0-60 (U/L)</th>
<th>Grading of hepatotoxicity (\n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-specific hepatitis (51)</td>
<td>8 (6-18)</td>
<td>4 (2-10)</td>
<td>132 (71-296)</td>
<td>127 (68-287)</td>
<td>211 (121-458)</td>
<td>322 (169-586)</td>
<td>Grade 2</td>
</tr>
<tr>
<td>Cholestatic (20)</td>
<td>164 (64-352)</td>
<td>134 (42-257)</td>
<td>102 (63-216)</td>
<td>193 (110-318)</td>
<td>777 (296-1789)</td>
<td>926 (516-1766)</td>
<td>Grade 2</td>
</tr>
<tr>
<td>Mixed hepatitic-cholestatic (25)</td>
<td>84 (18-271)</td>
<td>53 (10-209)</td>
<td>157 (79-611)</td>
<td>185 (136-860)</td>
<td>430 (176-750)</td>
<td>570 (282-1001)</td>
<td>Grade 2</td>
</tr>
<tr>
<td>Submassive necrosis (13)</td>
<td>157 (35-308)</td>
<td>77 (29-255)</td>
<td>660 (244-1288)</td>
<td>611 (253-1804)</td>
<td>181 (145-206)</td>
<td>233 (86-323)</td>
<td>Grade 4</td>
</tr>
<tr>
<td>Ductopenia/vanishing bile duct syndrome (11)</td>
<td>319 (70-494)</td>
<td>255 (48-357)</td>
<td>182 (145-256)</td>
<td>245 (154-426)</td>
<td>1709 (1093-2798)</td>
<td>1370 (472-1697)</td>
<td>Grade 3</td>
</tr>
<tr>
<td>Drug adaptive/related change (7)*</td>
<td>4 (3-11)</td>
<td>1 (1-2)</td>
<td>88 (31-113)</td>
<td>36 (32-94)</td>
<td>201 (148-230)</td>
<td>258 (174-307)</td>
<td>Grade 1</td>
</tr>
<tr>
<td>Steatohepatitis (5)</td>
<td>20 (11-55)</td>
<td>5 (3-17)</td>
<td>101 (77-191)</td>
<td>75 (50-324)</td>
<td>118 (77-189)</td>
<td>97 (34-337)</td>
<td>Grade 2</td>
</tr>
</tbody>
</table>

The data is expressed as medians and interquartile ranges.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transferase

\(\text{¶}\) Grading of hepatotoxicity by ALT level based on median ALT: grade 1, (1.0-2.5 × ULN); grade 2, (2.6–5.0 × ULN); grade 3,(5.1–10 × ULN); grade 4, >10×ULN

# Drug adaptive/related histological changes not regarded as a DILI
Table 5. Demographic and clinical predictors of DILI: univariate analysis

<table>
<thead>
<tr>
<th>Predictors</th>
<th>DILI, n (%) N = 127</th>
<th>Non-DILI, n (%) N = 174</th>
<th>OR (95%CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>34 (29-37)</td>
<td>35 (30-40)</td>
<td></td>
<td>0.079</td>
</tr>
<tr>
<td>Female gender</td>
<td>65 (51)</td>
<td>78 (45)</td>
<td>1.29 (0.82-2.04)</td>
<td>0.276</td>
</tr>
<tr>
<td>CD4 &lt;200</td>
<td>80 (63)</td>
<td>118 (68)</td>
<td>0.81 (0.50-1.33)</td>
<td>0.418</td>
</tr>
<tr>
<td>Cotrimoxazole</td>
<td>85 (67)</td>
<td>72 (41)</td>
<td>2.78 (1.72-4.48)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HAART</td>
<td>78 (61)</td>
<td>84 (48)</td>
<td>1.69 (1.06–2.68)</td>
<td>0.027</td>
</tr>
<tr>
<td>Anti-TB drugs</td>
<td>41 (32.2)</td>
<td>71 (40.8)</td>
<td>0.68 (0.42–1.09)</td>
<td>0.112</td>
</tr>
<tr>
<td>Herbal medication§</td>
<td>8 (6)</td>
<td>2 (1.1)</td>
<td>5.83 (1.22-27.94)</td>
<td>0.027</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>9 (7)</td>
<td>7 (4)</td>
<td>1.79 (0.65-4.96)</td>
<td>0.257</td>
</tr>
</tbody>
</table>

* The data is expressed as medians and interquartile ranges
§ Herbal includes African traditional medicine and African potato (*Hypoxis hemerocallidea*)
Table 6. Multivariate Analysis of Clinical, Demographic and Drug Specific Factors associated with a specific histological pattern of DILI

<table>
<thead>
<tr>
<th>Factor</th>
<th>Non-specific hepatitis</th>
<th>Cholestatic</th>
<th>Mixed cholestatic hepatic</th>
<th>Submassive necrosis</th>
<th>VBDS/Ductopenia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR(95%CI) P-value</td>
<td>OR(95%CI) P-value</td>
<td>OR(95%CI) P-value</td>
<td>OR(95%CI) P-value</td>
<td>OR(95%CI) P-value</td>
</tr>
<tr>
<td>Age</td>
<td>0.9 (0.95-1.03) 0.88</td>
<td>1.01 (0.95-1.07) 0.15</td>
<td>0.96 (0.91-1.02) 0.21</td>
<td>0.88 (0.81-0.96) 0.003</td>
<td>0.94 (0.87-1.03) 0.22</td>
</tr>
<tr>
<td>Female Gender</td>
<td>1.1 (0.58-2.22) 0.7</td>
<td>3.25 (1.08-9.72) 0.03</td>
<td>0.95 (0.38-2.4) 0.92</td>
<td>1.36 (0.37-4.88) 0.63</td>
<td>0.37 (0.08-1.62) 0.19</td>
</tr>
<tr>
<td>CD4&lt;200</td>
<td>1.07 (0.54-2.13) 0.8</td>
<td>1.46 (0.47-4.49) 0.50</td>
<td>1.53 (0.56-4.13) 0.39</td>
<td>0.21 (0.06-0.78) 0.012</td>
<td>0.96 (0.22-4.08) 0.95</td>
</tr>
<tr>
<td>Alcohol use</td>
<td>0.6 (0.24-1.45) 0.15</td>
<td>1.28 (0.36-4.58) 0.69</td>
<td>0.8 (0.25-2.67) 0.74</td>
<td>1.22 (0.27-5.36) 0.79</td>
<td>0.36 (0.04-3.13) 0.35</td>
</tr>
<tr>
<td>Hepatitis B</td>
<td>0.6 (0.23-1.51) 0.28</td>
<td>0.29 (0.04-4.58) 0.24</td>
<td>0.17 (0.03-1.31) 0.08</td>
<td>0.36 (0.04-3.04) 0.35</td>
<td>NA</td>
</tr>
<tr>
<td>Cotrimoxazole</td>
<td>3.82 (1.82-8.0) 0.001</td>
<td>7.05 (2.50-19.89) &lt;0.001</td>
<td>3.99 (1.57-10.17) 0.003</td>
<td>1.16 (0.22-5.95) 0.85</td>
<td>17.6 (3.26-95.3) &lt;0.0001</td>
</tr>
<tr>
<td>Efavirenz</td>
<td>4.3 (1.92-9.83) &lt;0.001</td>
<td>0.89 (0.34-2.39) 0.88</td>
<td>2.69 (0.86-11.5) 0.07</td>
<td>10.46 (2.70-40.5) &lt;0.001</td>
<td>0.52 (0.06-4.48) 0.55</td>
</tr>
<tr>
<td>Nevirapine</td>
<td>9.6 (2.25-40.86) 0.002</td>
<td>1.91 (0.21-18.01) 0.56</td>
<td>1.25 (0.14-11.07) 0.50</td>
<td>1.47 (0.13-6.49) 0.75</td>
<td>4.0 (0.36-4.8) 0.25</td>
</tr>
<tr>
<td>Protease Inhibitors</td>
<td>1.9 (0.18-20.51) 0.57</td>
<td>2.93 (0.72-11.84) 0.13</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Anti-TB drugs</td>
<td>8.1 (2.4-27.81) &lt;0.001</td>
<td>NA</td>
<td>3.53 (0.85-14.61) 0.08</td>
<td>4.27 (0.62-29.45) 0.13</td>
<td>NA</td>
</tr>
</tbody>
</table>

# Steatohepatitis/Granulomatous patterns and fluconazole/herbal medication not included given the low frequencies of their occurrence

Abbreviations: VBDS, Vanishing Bile Duct Syndrome; NA, Not Analysed because of insufficient data
### Table.7 Comparison of HBeAg positive and negative patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HBeAg-positive, n - 32</th>
<th>HBeAg-negative, n - 24</th>
<th>P - value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>34 (30-39)</td>
<td>33 (29-39)</td>
<td>0.49</td>
</tr>
<tr>
<td>Male gender n (%)</td>
<td>24 (75)</td>
<td>13 (54)</td>
<td>0.15</td>
</tr>
<tr>
<td>CD4 (cells/mm³)</td>
<td>172 (79-332)</td>
<td>105 (51-163)</td>
<td>0.03</td>
</tr>
<tr>
<td>Total BR</td>
<td>12 (9-21)</td>
<td>22 (10-85)</td>
<td>0.1</td>
</tr>
<tr>
<td>ALT</td>
<td>154 (84-467)</td>
<td>170 (109-417)</td>
<td>0.8</td>
</tr>
<tr>
<td>AST</td>
<td>155 (78-512)</td>
<td>216 (113-387)</td>
<td>0.5</td>
</tr>
<tr>
<td>HBV DNA log copies/ml</td>
<td>7.9 (5.1-8.5)</td>
<td>5.9 (4.4-6.9)</td>
<td>0.008</td>
</tr>
<tr>
<td>Necro-inflammatory score ($)</td>
<td>6 (3-9)</td>
<td>7 (4-11)</td>
<td>0.2</td>
</tr>
<tr>
<td>Fibrosis ($)</td>
<td>2 (1-3)</td>
<td>2 (2-3)</td>
<td>0.8</td>
</tr>
</tbody>
</table>

The data is expressed as medians and interquartile ranges.

Abbreviations (laboratory reference range): Total BR, Total bilirubin (0-21 µmol/L); ALT, alanine aminotransferase (5-40 U/L); AST, aspartate aminotransferase (5-40 U/L)

§ As per Ishak et al (50)
References


21. AIDS Clinical Trials Group, Table of Grading Severity of Adult Adverse Experiences, Division of AIDS, National Institute of Allergy and Infectious Diseases, Rockville, MD, USA, 1996.


Part D: Appendices

1. Author guidelines for HEPATOLOGY
2. Data capture sheet
3. Health Sciences Research Ethics Committee approval letters
1. Author Guidelines

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2. HIV LIVER BIOPSIES DATA CAPTURE SHEET

A. Patient and clinical information

1. Folder number and Hospital: ______________________________________________________
2. Age: _______________________________________________________________________
3. Gender: _____________________________________________________________________
4. Date of biopsy: __________________________________________________________________
5. Name & Surname: __________________________________________________________________
6. History of alcohol use: __________________________________________________________________
7. WHO HIV/AIDS clinical stage: __________________________________________________________________
8. Opportunistic infections: __________________________________________________________________

B. Investigations

9. CD4 count (time of biopsy): __________________________________________________________________
10. HIV viral load: _______________________________________________________________________
11. Hep B status:
   - s-antigen: ____________________________
   - e-antigen: ____________________________
   - core IgM: ____________________________
   - core IgG: ____________________________
   - e-antibody: ____________________________
   - HBV DNA viral load: ____________________________
12. Hep C Antibody:
   - Hep C PCR & genotype: ____________________________
   - Hep C viral load: ____________________________
13. Virology – other:
   - Hep A IgM____________________ IgG____________________
   - CMV IgM____________________ IgG____________________
   - EBV EBna____________________ IgG____________________
   - Herpes simplex IgM____________________ IgG____________________
14. Liver function tests (at biopsy, latest available):
   - TPr______  _____  _____
   - Albumin_____  _____  _____
   - TBr_____   _____    ______
   - CBr____  _____   _____
   - AST______    ______  ______
   - ALT______    ______  ______
   - ALP____   _____    _____
   - GGT______   ______  ______
   - INR_____    ______  _____
   - Hb  _____  _____  ______
   - WCC_____   _____   _____
   - Plts______  ______  _____
15. ERCP/MRCP: _______________________________________________________________________
_________________________________________________________________________________
16. **Other biopsies** e.g. bone marrow, TBBx, lymph node etc.: 

_______________________________________________________________________
_______________________________________________________________________
_______________________________________________________________________
_______________________________________________________________________

17. **Other biopsies – culture** 

_______________________________________________________________________
_______________________________________________________________________
_______________________________________________________________________

C. **Treatment**

a. **HAART**:
   (i) Drugs: (a) NRTI’s: ____________________________
       (b) NNRTI’s: ____________________________
       (c) PI’s: ____________________________
   (ii) Duration: ____________________________
   (iii) Date, if stopped, prior to biopsy: ____________________________

18. **TB drugs**
   (i) Regimen 1 or 2: ____________________________
   (ii) Date started: ____________________________
   (iii) Date, if stopped, prior to biopsy: ____________________________

19. **Additional drugs**
   (a) **Cotrimoxazole** (including dose): ____________________________
       Date, if stopped, prior to biopsy: ____________________________
   (b) **Fluconazole** (including dose): ____________________________
       Date, if stopped, prior to biopsy: ____________________________
   (c) **Antibiotics** (name, dose and duration): ____________________________

20. (iv) **Other drugs**: ____________________________
     ____________________________
     ____________________________
     ____________________________
D. Liver biopsy information

21. Liver biopsy
   (i) Specimen number: ____________________________________________________
   (ii) Pattern of injury:
       ___________________________________________________________________
       ___________________________________________________________________
       ___________________________________________________________________

22. (iii) Final diagnosis: (eg. drug induced hepatitis, drug induced cholestasis, mixed, viral hepatitis, hepatic tuberculosis; steatosis, steatohepatitis, other)
       ___________________________________________________________________
       ___________________________________________________________________
       ___________________________________________________________________
       ___________________________________________________________________
       ___________________________________________________________________
       ___________________________________________________________________

23. Liver biopsy culture:
    ___________________________________________________________________
    ___________________________________________________________________
3.

UNIVERSITY OF CAPE TOWN

Research Ethics Committee
E52 Room 24, Old Main Building Great Schuur Hospital, Observatory, 7925
Queries: Xolile Fuia
Tel: (021) 406-6492 Fax: 406-6411
E-mail: xfuia@curie.uct.ac.za

09 May 2005

REC REF: 187/2005

Dr M. Sonderup
Medicine

Dear Dr Sonderup

A REVIEW OF LIVER PATHOLOGY AND A CLINICO-PATHOLOGICAL CORRELATION IN HIV
POSITIVE PATIENTS IN CAPE TOWN, SOUTH AFRICA

Thank you for submitting your study to the Research Ethics Committee for review.

It is a pleasure to inform you that the Ethics Committee has formally approved the
above-mentioned study on the 08 May 2005.

Please quote the REC. REF in all your correspondence

Yours sincerely

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<table>
<thead>
<tr>
<th>HREC office use only (FWA00001637; IRB00001938)</th>
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<tbody>
<tr>
<td>This serves as notification of annual approval, including any documentation described below.</td>
</tr>
<tr>
<td>☑ Approved Annual progress report Approved until/next renewal date 15/06/2014</td>
</tr>
<tr>
<td>☐ Not approved See attached comments</td>
</tr>
<tr>
<td>Signature Chairperson of the HREC</td>
</tr>
<tr>
<td>Date Signed 3/14/2013</td>
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</tbody>
</table>

Principal Investigator to complete the following:

1. Protocol information

Date form submitted 31 May 2013
HREC REF Number 187/2005
Current Ethics Approval was granted until
Protocol title Liver Pathology and a clinico-pathological correlation in HIV positive patients in Cape Town
Principal Investigator Dr Mark Sonderup
Department / Office Division Hepatology K46 Old Main Building GSH
Internal Mail Address

1.1 Does this protocol receive US Federal funding? ☐ Yes ☑ No

2. Protocol status (tick ✓)

☑ Research-related activities are ongoing
☐ Data collection is complete, data analysis only

3. Protocol summary

Total number of records or specimens collected, reviewed or stored since the original approval 228
Total number of records or specimens collected, reviewed or stored since last progress report
Have any research-related outputs (e.g. publications, abstracts, conference presentations) resulted from this research? If yes, please list and attach with this report. ☑ Yes ☐ No

4. Signature

Signature of PI Date 3/6/17
Signature of Supervisor (if PI is a student) Date

(Not: Please complete the Closure form (FHS019) if the study is completed within the approval period)