Drug-related toxicity of HIV patients on Antiretroviral and tuberculosis therapy

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A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, in partial fulfillment of the requirements for the degree of

Master of Medicine in branch of Intern Medicine

Johannesburg, 2019

Declaration

I, Lifa Dhlamini, declare that this research report is my own work. It is being submitted for degree of Master of Medicine in the branch of Internal Medicine. This report is submitted in the publishable format as recognized by the Faculty of Health Science. I further declare that this work has not been submitted for any degree or examination at any other University.

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ACKNOWLEGMENTS

I am deeply grateful to my supervisors.

To Professor Prudence Ive. I am truly thankful for the opportunity to work on this dissertation with you. Your assistance and patience has been deeply appreciated. Thank you for believing in me and for giving me the opportunity to grow. You were motivating and cultivated excellence within me.

To Dr David Spencer. The great knowledge you have in this field established the foundations for this dissertation. It would not have have been possible without your steady and firm leadership. I am deeply grateful for your assistance.

To Ms Vuyo Jezile, who is a senior co-ordinator of research at the Right-to-Care institute at Helen Joseph hospital, and her team. I am deeply grateful for providing me with all the the data I needed as part of the dissertation.

To Michelle, who is lecturer at School of Public Health Biostatistics. I am deeply grateful for your assistance with statistics, and guiding me to be confident with my own ability to implement the statistics needed for the dissertation. You have helped ignite my passion for statistics.

To my family, including my late father, Dr Hezekiel Mangena Dhlamini (6 Oct 1958-2005), my mother , siblings, and partner. I remain grateful for your support throughout the dissertation and my personal life and for remaining as my pillars of strength.

To my God, my Rock and my Shepard. May You forever shine in my life and continue to shelter me under your wing. I do remain grateful forever, that what You have started in me, You have completed. Amen

PUBLICATIONS/ PRESENTATIONS ARISING FROM THIS PROJECT

None

ETHICAL CONSIDERATIONS

Permission for retrospective study was obtained from Prof Ive (Head of Division of Infectious Diseases, Helen Joseph), Dr Kirby (Clinical Manager, Helen Joseph Hospital), and the Human Research Ethics Committee of the University of Witwatersrand(clearance number-M160410)

Abstract

Background: There are large numbers of people on both antiretroviral therapy (ART) and tuberculosis (TBT) therapy in South Africa due to the burden of disease. Both treatments may have overlapping toxicities resulting in drug induced liver injury (DILI) and drug induced renal injury (DIRI) which impacts on both morbidity and mortality in HIV patients.

Objectives: To determine the incidence, expression, severity and risk factors of DILI and DIRI, as well as to determine the effect of DILI and DIRI on the length of hospital stay in a large HIV patient cohort receiving ART alone, TBT alone or concomitant TBT and ART .

Methods: A retrospective descriptive study was conducted on primary data obtained from the Infectious Diseases division database of patients who were admitted at Helen Joseph Hospital (HJH) from 1 January 2013 to 30 June 2015. Data collected included socio-demographic, treatment regimen, and laboratory variables. Descriptive statistics and association of the variables were studied. Ordered logistic regression was used to analyse the predictors for the incidence of DILI and DIRI.

Results: Thirty per cent of patients with liver impairment (102/342) were classified as DILI. ART alone (41.2%) and combination of TBT and ART (42.2%), had a greater contribution to the development of DILI compared to TBT alone (16.7%). The predominant expression and severity grading of DILI were cholestasis (59.5%) and mild injury (58.8%), respectively. Only 10% (32/333) of patients with renal disease, were classified as DIRI. ART (62.5%) and combination of ART and TBT (34.4%) were important contributors to the incidence of DIRI. Pure TB therapy had a little role in this regard (n=1). The significant predictors for DILI were age, female sex and length of hospital stay, while that for DIRI was baseline creatinine.

Conclusion: DILI was more common than DIRI in the study. ART alone and combination of TBT and ART, had a greater contribution to the development of DILI and its severity compared to TBT

alone. Both ART and combination of ART and TBT treatment were important contributors to the incidence of DIRI. Pure TB therapy had little role in this regard. There is an important need for prospective studies to investigate the incidence and outcomes of DILI and DIRI.

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ABBREVIATIONS

3TC	lamivudine
ABC	abacavir
AKI	acute kidney injury
AIN	Acute interstitial nephritis
ALP	alkaline phosphatase
ALT	alanine aminotransferase
ART	antiretroviral therapy
AST	aspartate transaminase
ATN	acute tubular necrosis
AZT	zidovudine
CDC	Centres for Disease and Prevention
CKD	chronic kidney disease
CYP	cytochrome
d4T	stavudine
DIKD	drug induced kidney disease
DILI	drug induced liver injury
DIRI	drug induced renal injury
DOTS	directly observed treatment

DRESS	drug rash with eosinophila syndrome
EFV	efavirenz
EMB	ethambutol
FDC	fixed does combination
FS	fanconi syndrome
HJH	Helen Joseph hospital
HLA	human leukocyte antigen
IL	interleukin
INH	isoniazid
INSTI	intregrase strand transfer inhibitors
IRIS	immune reconstitution inflammatory syndrome
KDIGO	Kidney Disease Improving Global Outcomes
MRP	multidrug resistant protein
NRTI	nucleoside reverse transcriptase inhibitor
NNRTI	non-nucleoside reverse transcriptase inhibitor
NVP	nevirapine
OAT	Organic anion transporter
PI	protease inhibitor
RIF	rifampicin

TBT	tuberculosis therapy
TDF	Tenofovir
TNF	tumour necrosis factor
UK	United Kingdom
WHO	World Health Organisation

Chapter1: Protocol and Extended review of the literature

1.1 Introduction:

The highest proportion of people living with HIV are in South Africa, a country with an HIV-burden of approximately 7.9 million (2017)[1]. According to the Human Sciences Research Council, the national prevalence of HIV infection among South African adults was 12.6% in 2012[2].With the change in the World Health Organization (WHO, 2015) and South African (2017) guidelines, all irrespective of baseline CD4 cell count, are now being initiated on antiretroviral therapy (ART) [3].

South Africa has the 8th highest TB incidence globally [4]. The problem of dual infection is magnified in South Africa, as it has the highest prevalence of HIV associated TB cases[5] . The TB epidemic in South Africa is driven by HIV. ART coverage in South Africa has increased from 24-61% (2010-2018), which has lead to the decline in the incidence of TB[4]. Apart from incidence and prevalence, the societal impact of TB can be measured in TB-related morbidity and mortality.

In the case of both HIV and TB, treatment is frequently complicated by drug toxicity. The signs and symptoms of drug toxicity, the immune reconstitution inflammatory syndrome (IRIS), and other HIV-related diseases overlap and obscure the primary diagnosis. Under these circumstances the definitive diagnosis of a drug-related injury is difficult, nevertheless this diagnosis is important. ART and tuberculosis therapy (TBT) can cause debilitating and/or life-threatening disease e.g. severe skin reactions, hepatitis, renal disease, marrow suppression, and incapacitating neuropathies[6].

For the purpose of this study, only drug-induced liver injury (DILI) and drug-induced renal injury (DIRI) were investigated. DILI accounts for 7% of adverse drug reporting and for 30% of fulminant liver failure[7]. Hepatotoxicity complicates ART in 8.9-10.8% of cases[8]. The incidence of TBT related DILI is 2-28%. Incidence rates for hepatotoxicity are not commonly reported in sub-Saharan

Africa as liver function tests in patients on TBT are not routinely carried out[9]. There are often overlapping toxicities between ART and TBT. DIRI related to ART accounts for 14% of late onset acute kidney injury (AKI)[10]. AKI is a rare complication of TBT that leads to treatment interruption and irreversible kidney injury. The incidence varies from 0.05-7%[11-13]. DILI and DIRI impact both morbidity and mortality in HIV patients [3,11].

The aims of the study were to determine the period prevalence, expression, severity and risk factors of DILI and DIRI, as well as to determine the effect of DILI and DIRI on the length of hospital stay in a large HIV patient cohort receiving ART alone, TBT alone or concomitant TBT and ART.

1.2. The definition of DILI

There are many guidelines available globally for the definition of DILI e.g. American Thoracic Society (ATS),[7] however in South Africa, clinicians use a South African Consensus Guideline. These were established in 2013, in order to facilitate a coordinated approach to the management of DILI[13]. In addition, a standard definition allows comparisons between clinical studies to be made more accurately. The defining criteria in the consensus guidelines includes the following: an alanine aminotransferase (ALT) level > 120 IU/L in symptomatic patients (i.e. nausea, abdominal pain, jaundice); or ALT > 200 IU/L in asymptomatic patients; a total bilirubin > 40 micromoles/L[13].

1.3. Incidence of TBT/ART related DILI

1.3.1 Incidence of TBT related DILI

TBT is frequently complicated by DILI, with an incidence varying between 6-26%[14-18.].The geographic region may have a role in determining the occurrence of DILI. The incidence seems to be higher in developing countries. There are multiple possible reasons for this difference, namely: higher rates of malnutrition; immunosuppression; pre-existing liver disease, alcoholism and the higher use of traditional medication[15]. Studies in India, Ethiopia, and South Africa showed an incidence of 9-

11% [14-17]. This differs from a study conducted in the United Kingdom which showed an incidence of 6.9% [18]. In the United States, DILI is most commonly due to acetaminophen rather than TBT[19].

1.3.2 Incidence of ART related DILI

There are large numbers of patients on both ART and TBT in South Africa due to the burden of disease. To the best of our knowledge, there are no prospective longitudinal studies that determine the incidence of ART related DILI in South Africa. Prospective studies in Tanzania and Ethiopia, showed the incidence of ART related DILI was 5.9-8.8%[17,20] . A retrospective study in South Africa showed the incidence to be 16.9% (n=12/71) in patients receiving ART alone[16]. A higher incidence is probably expected in South Africa, as the country not only has the highest HIV burden, but also the largest ART program globally[1]. Prospective studies are needed to determine the 'true' incidence of ART related DILI.

In terms of ART regimens, DILI is most prevalent with non-nucleoside reverse transcriptase inhibitor (NNRTI) based therapy. A study comparing the NNRTIs nevirapine (NVP) vs efavirenz (EFV) showed that hepatotoxicity was more frequently associated with NVP than EFV (12% vs 4%, p=0.016)[21].Van Leth et al, showed a similar finding, and that also the combination of these two NNRTIs yielded greater toxicity, without greater efficacy[22].

1.3.3. The incidence of DILI in patients on concomitant TBT and ART

There is overlapping toxicity between ART and TBT. Yimer et al, showed that DILI was more common in patients receiving concomitant ART and TBT (35%), compared to patients on ART alone (8.8%) and TBT alone (2.9%)[17]. In comparison, Shultz et al, showed that TBT contributed more to occurrence of DILI than patients on ART alone and concomitant ART and TBT[16]. In South Africa more vigilance is needed when managing patients on ART and/or TBT.

1.4. Risk factors for TBT/ ART related DILI

The identification of risk factors, including genetic ones, may be important for ensuring patient safety with TBT or ART. A study by Petros et al, showed that HLA allele polymorphisms in black Africans determine susceptibility to both immune-mediated TBT and ART related DILI. Human leukocyte antigen (HLA) B*57 alleles were higher in patients with DILI (25%), compared to those without DILI (1.1%). In comparison, lower HLA-B*47 alleles were found in patients with DILI, which may be protective[23]. This highlights a role for genetic testing, in determining the risk of DILI in patient on ART and TBT. Risk stratification with TBT or ART DILI is not well established. It may help to predict which patient is most likely to develop toxicity[24] and perhaps its severity. The determination of biochemical and/or demographic risk factors for TBT/ART related DILI is established in many studies.

1.4.1 Risk factors for TBT related DILI

With regards to TBT, the following have been shown to be risk factors for DILI: increased baseline liver enzymes (i.e. alkaline phosphatase (ALP), aspartate transaminase (AST) and bilirubin); concomitant HIV disease; female gender; older age; malnutrition and alcohol consumption [14,17,25-27].

These studies were retrospective. It is difficult to assess for a true association of the risk factors for DILI without the use of prospective observational studies.

1.4.2. Risk factors for ART related DILI

In terms of ART related DILI, the only shared risk factor with DILI secondary to TBT is female gender. The other risk factors for ART related DILI were the following: chronic HBV and HCV infection, higher baseline alanine transaminase (ALT), recent commencement of NVP, a higher baseline CD4 count, and longer duration of receiving ART [28-33].

Furthermore in terms of protease inhibitor (PI) based therapy, Sulkowski et al, showed that full dose ritonavir (600mg) was associated with increased hepatotoxicity[29]. However, a study by Cooper et al,

disagreed with this finding[30]. In South Africa, only low dose ritonavir is utilised to boost the PI based second-line ART regimen.

1.4.3. Risk factors for DILI in patients on concomitant ART and TBT

In South Africa, patients on rifampicin, receive a double dose of lopinavir/ritonavir[3], which increases the risk of hepatotoxicity. In a study by Rolla et al, a PI based regimen was added to TBT after 30 days, and subsequently 11.8% (4/34) of patients developed hepatitis. The following factors were associated with the development of hepatitis: hepatitis C co-infection (n=1), paracoccidiodomycosis co-infection (n=1) and heavy alcohol use (n=1)[34]. A similar study done by Moreno et al, evaluating safety of concomitant use of ritonavir and rifampicin, showed that mild transaminitis occurred in 27.8% (5/18) of patients, and notably these patients were co-infected with hepatitis C[35]. It is unclear whether this is a true association or mere co-incidence, as a regression analysis was not done.

1.5. Mechanism of DILI

Hepatotoxicity occurs in either a dose dependent, predictable manner[36] or in an idiosyncratic, unpredictable way. There are a few differences between these mechanisms. Firstly, direct toxicity occurs by release of free oxygen radicals causing hepatotoxicity at a site in the liver which has the highest metabolism, but the lowest detoxifying ability[7,37]. In comparison, idiosyncratic drug reactions, which are the more common type, occur via a hypersensitivity reaction or release of drug metabolites, which cause injury that is not dependent on the dose. Secondly, idiosyncratic reactions cause hepatic necrosis throughout the liver, instead of in a zonal fashion, which is seen with direct toxicity. Thirdly, idiosyncratic drug reactions are insidious and may be subacute (1-8 weeks) to chronic (even up to 1 year), compared to a rapid (<1 week) response, observed with direct toxicity. [7,38]. Thus, due to the nature of these reactions, it is difficult to predict the likelihood of toxicity in a patient.

1.5.1 Mechanism of TBT related DILI

First line TBT is administrated as a fixed drug combination. Amongst all the individual TB drugs, rifampicin (RIF), pyrazinamide (PZA) and isoniazid (INH) are the most hepatotoxic. The mechanism of hepatotoxicity of each drug is different. RIF may cause a transient, unconjugated hyperbilirubinemia (asymptomatic) by direct inhibition of the bile salt exporter pump. It is also a potent inducer of the cytochrome (CYP) 450 system causing hepatotoxicity, and furthermore potentiating the effect of other drugs e.g. INH[7,9]. Another mechanism of RIF hepatotoxicity was explored in rat models, and showed that the drug also caused upregulation of glutathione transferase activity and peroxisome proliferator-activated receptor-gamma (PPAR-gamma) signalling pathways. The activity of these enzymes had a direct correlation to the dose administered[39]. Perhaps the activation of the enzymes system directly lead to hepatocyte apoptosis[7].

In terms of INH toxicity, firstly the drug is metabolized in the liver by N-acetyl transferase2 (NAT-2), into mono-acetyl hydrazine (MAH) and diacetyl hydrazine. Secondly, the genetic polymorphism of NAT-2 differentiate acetylation into fast, intermediate and slow[7]. A study by Sistanizad et al, explored the difference in hepatotoxic potential between fast and slow acetylators, and found that the latter were associated with significantly more hepatotoxicity ($p=0.003$)[40]. The reason for this, is that slow acetylation results in accumulation of the toxic metabolite, MAH[7]. This metabolite causes direct release of free oxygen radicals. INH, compared to rifampicin, inhibits the cytochrome P450 enzyme system, also potentiating the toxicity of other drugs e.g. phenytoin[7,41]. It is important for the clinician to be aware of differences in fast vs slow acetylators. Slow acetylators may require closer monitoring, as they are prone to developing hepatotoxicity[40]. Fast acetylators may require higher doses of INH than the norm, in order to reach an adequate treatment response[42]. The development of resistance, due to 'ineffective' therapy, in fast vs slow acetylators may need to explored.

Compared to rifampicin and INH, less is known about the mechanism of PZA DILI. It is derived from nicotinic acid and metabolized into pyrazinoic acid and subsequently into 5-hydroxypyrazinoic acid[7]. A study by Shih et al, investigated the mechanism of PZA hepatotoxicity in rats, and found that serum levels of PZA and its metabolite(5-hydroxypyrazinoic acid) directly correlated with the AST

and ALT levels in rats ($p<0.005$)[43]. The mechanism of this toxicity is thought to be mediated by the inhibition of nicotinamide acetyl dehydrogenase activity[44]. It is important for the clinician to be aware of the drug-drug interactions that occur with TBT and ART. The mechanisms of ART related DILI are highlighted below.

1.5.2 Mechanism of ART related DILI

ART related DILI occurs via 3 main mechanisms: direct toxicity, hypersensitivity reactions and mitochondrial toxicity. Firstly, direct toxicity occurs in susceptible individuals with polymorphisms of cytochrome pathways. Indeed, Kwara et al, demonstrated higher plasma EFV levels in carriers of polymorphisms of cytochrome (CYP2B6)[44,45]. Secondly, delayed hypersensitivity reactions are commonly associated with both abacavir (ABC) and NVP. These drugs are linked to certain HLA alleles, namely: HLA-B*57:01 and HLA-DRB1 for ABC and NVP, respectively. In terms of NVP, risk of hepatotoxicity is increased in females who have a CD4 > 250 cells/mm^3, which typically occurs within the first 6 weeks after commencing therapy[46]. Thirdly, mitochondrial toxicity is observed with nucleoside reverse transcriptase inhibitors (NRTIs). These drugs cause microvesicular steatosis (within the hepatocytes) and depletion of mitochondria, and consequently leading to liver necrosis, fibrosis and destruction of the biliary ducts[44]. Lastly, initiation of ART may result in immune reactivation of opportunistic infections (e.g. TB or viral hepatitis), which may augment the hepatotoxicity of the drugs[47]. There are clinical implications for these mechanisms, especially in terms of EFV. In the South African setting, patients often receive INH as part of preventative therapy, which interacts with EFV, in susceptible individuals. In these patients, there may be room to consider dose reduction of EFV, in order to avoid higher drug plasma levels, resulting in neurological complications[48].

1.6. Expression of DILI

When a drug is metabolized, the liver may naturally respond with a transient increase in the transaminases. This is called hepatic adaptation and is asymptomatic and reversible. Hepatic

adaptation must be distinguished from a persistent rise in hepatic enzymes, which may require cessation of the drug, which occurs with DILI[7]. One of the ways of classifying DILI is by expression/pattern of injury. The expression of DILI is based on the R value which was defined as [35]:

R= (ALT/ULN (upper limit of normal)) / (ALP/ULN)

- Cholestatic pattern: R value <= 2
- Hepatocellular: R value >= 5
- Mixed pattern: R value > 2 and < 5

Patterns are important to recognise for the following reasons: 1. Liver injury patterns may have a prognostic significance. Jaundice with acute hepatocellular injury may be associated with a 10% mortality. This called Hy's (Zimmerman) law[50]. Zimmerman also stated that cholestasis generally has a better prognosis than an hepatocellular pattern,[51] however Chalasani et al, did not find this to be the case in their study[50]; 2. The patterns of liver injury are of diagnostic importance. These may help to limit the number of differential diagnoses in patients with DILI e.g. hepatocellular expression and viral hepatitis, cholestasis and biliary disease[49]. TBT/ART that are likely to present with an hepatocellular pattern are: INH, PZA, EFV and NVP. Those that are more likely to present with cholestasis are RIF and PIs, and mixed pattern are EFV and RIF[52].

Patterns of liver injury are not static but can change over time and also may not correlate with the histological picture[49]. Yimer et al, demonstrated that the predominant pattern for all regimens (i.e. ART alone, TBT alone and combination of TBT and ART) was cholestasis (61%). TBT alone had the highest proportion of hepatocellular pattern (22%), while ART alone had the most patients with cholestasis (70.8%)[17].

1.7 Severity and outcome of DILI

The other way of defining DILI is by its severity. The South African consensus guidelines assist with this definition. The severity of DILI was as defined as follows:[13]

- Mild DILI: ALT > 40 but < 200 IU/L and total bilirubin < 40 micromoles/L without symptoms;
- Moderate DILI: ALT >200 IU/L or ALT <120 IU/L + total bilirubin > 40 micromoles/L without symptoms;
- Severe DILI: presence of symptoms or features of overt liver failure (elevated INR and /or clinical signs of failure).

Presentation of patients with DILI, may range from asymptomatic to acute or chronic liver failure[52]. The severity is important for a number of reasons: 1. It is associated with a high morbidity and mortality, as stipulated by Hy's law. Severe cases may need liver transplantation [49,52]. 2. Severe disease requires treatment to be stopped,[13] and may result in patients receiving inadequate treatment of TB and perhaps no treatment for HIV. This may be a driver for resistance for both TB and HIV. 3. Severe disease, on a larger scale, may be a reason for drug withdrawal from the market, which may be expensive for drug companies[53].

There are different risk factors for severity in ART and TBT related DILI. Severity specifically with ART related DILI was associated with concomitant viral hepatitis (hepatitis B and C), combination of a PI with EFV or NVP, and greater alcohol intake[54]. In contrast, the risk factors for severe TBT related DILI were a higher baseline liver enzyme abnormality and fluconazole use[55].

1.8. Management of DILI

1.8.1 Management of TBT related DILI

The South African consensus guidelines advocates for treatment to be stopped in both moderate and severe DILI. Therapy is then reintroduced once hepatotoxicity has resolved. A backbone consisting

of moxifloxacin, ethambutol (EMB), and kanamycin is added in the interim, while the liver enzymes settle.[13]

There are different approaches to the re-challenging of the treatment regimen. Re-introduction of the entire regimen has been found to be cost effective due to a shorter hospital stay. However, if DILI re-occurs then the offending agent may be difficult to identify. The stepwise re-introduction allows time for the hepatocytes to adapt, reducing risk of recurrence and identifying a possible offender [56,57]. A study conducted by Costinuik et al, evaluating the re-challenging of therapy with TBT related DILI, showed that the overall recurrence rate was 12% (n=5/42) after re-challenge. There was no significant difference observed between stepwise re-introduction vs full re-institution of treatment (p=0.2773) [56]. Sharma et al had similar findings [58]. Thus, it is up to the clinician to decide which method or approach they prefer. In most academic hospitals in Gauteng, South Africa, step-wise re-introduction is probably preferred.

1.8.2 Management of ART related DILI

South African ART guidelines state that ALT should be monitored at initiation of ART and repeated once symptoms of hepatitis develop. ART is stopped when ALT and/or bilirubin is elevated > 5 fold the ULN without symptoms or ALT and/or bilirubin >2.5 fold ULN with symptoms. A liver friendly regimen can be started, and treatment re-challenge can be considered in certain cases. NVP requires more regular monitoring specifically at 2, 4, 8 and 12 weeks after initiation [3].

2.1 Definition of DIRI

Compared to DILI, DIRI is not as well defined. To the best of our knowledge, there is still no consensus definition for DIRI in South Africa. A standard definition is lacking. This has resulted in inconsistent diagnosis and possible under representation of this entity. In studies, it is challenging to to measure and compare the incidence, severity and outcomes of DIRI[59]. An international expert panel established by Metha et al, which convened in 2016, were the first to develop consensus

guidelines for DIRI[60]. These consensus guidelines referred to DIRI as drug induced kidney disease (DIKD). We use this term interchangeably with DIRI. The occurrence of DIRI/DIKD is described as the 'event', in the following text. DIRI was described to have 4 phenotypes or manners of presentation, namely: AKI, glomerular disease, tubular disorders and nephrolithiasis. For each phenotype, the following minimum criteria should be met in order to fulfil the diagnosis of DIRI: 1. Exposure to the drug must precede the event by >24 hours; 2. There must be a known causal effect between the drug and event, and this association may be biological, immunological or metabolic in nature; 3. Other contributors of the phenotype must be excluded using medical data and investigations; 4. The strength of association, between the drug and the phenotype, should be supported by the duration of drug exposure and the extent to which the criteria for the particular phenotype are met. Time course for the event was defined as: acute < 8 days; subacute 8 to 90 days; and chronic > 90days[60].

2.1.1 AKI phenotype

The phenotype with probably the greatest significance in most studies is AKI. Amongst all the phenotypes, it has the greatest impact on morbidity and mortality. In addition, it may be the least difficult to assess in the clinical setting. This phenotype is defined according to the criteria established by the Kidney Disease Improving Global Outcomes (KDIGO) panel, which are: an abrupt decline in kidney function, resulting in an increase in serum creatinine >26.5 micromoles/L from the baseline value, within a period of 2 days; or a 1.5 fold increase in serum creatinine within the preceding week; or urine output of less than 0.5ml/kg/hr for at least 6 hrs[61]. However, the preceding definition, on its own, is insufficient to meet the criteria for the AKI phenotype. The rationale for this, is that there are a multitude of causes that may cause an increase in the serum creatinine, resulting in AKI (e.g. dehydration, hypotension). In order to increase the sensitivity of the diagnosis for this phenotype, increases in serum creatinine must meet criteria for stage 2 AKI (>2 x baseline creatinine)[60]. This staging is based on the KDIGO guidelines[61]. Although the increase in serum creatinine characterises

this phenotype, it is histologically sub-classified into acute tubular necrosis (ATN) or acute interstitial nephritis (AIN). These subtypes are difficult to differentiate without a renal biopsy [60,62].

Concerning ART, tenofovir (TDF) is the most common drug implicated in AKI. PIs do not usually cause AKI alone, but perhaps in conjunction with TDF[63]. With regards to TBT, RIF is the most frequently associated drug with AKI, demonstrated by several studies. However, INH and EMB have also been implicated, although to a lesser extent [11,64-66].

2.1.2. Tubular disorder

Compared to AKI, the tubular disorder phenotype, is probably less frequently recognized in the clinical setting. Unless otherwise indicated (e.g. nephrotic/nephritic syndrome, AKI), urinalysis may remain unmeasured. The majority of ART and TBT are handled by the kidneys, especially TDF. This drug has been strongly implicated with proximal tubular disorders (tubulopathies), which include: isolated urinary phosphate or amino acid losses; and overt Fanconi's syndrome. The basis of Fanconi's syndrome, is the presence of the following: bicarbonate wasting resulting in non-anion gap metabolic acidosis; tubular proteinuria; urinary phosphate and glucose losses[67]. Furthermore, a study by Zaiden et al, also showed an association of TDF with distal tubulopathies (i.e. diabetes insipidus). In this study, the proportions of each tubulopathy, proximal and distal, were 87.5% and 12.5%, respectively[68]. With respect to TBT, although rare, RIF has also been implicated with Fanconi's syndrome. Particularly, in a case report, the syndrome was complicated with hypokalaemic periodic paralysis. Moreover, the association between RIF and the syndrome was strengthened, when cessation of the drug lead to the resolution of the syndrome[69]. In South Africa, there is no role for regular urinalysis in patients on ART,[3] as well as TBT. Nevertheless, urinalysis may be measured if clinically indicated (muscle weakness with/without low serum potassium or phosphate)[3].

2.1.3 Nephrolithiasis

With regards to nephrolithiasis, the presentation of this phenotype may range from isolated crystalluria (asymptomatic) to obstructive uropathy. PI based ART is frequently the cause[60]. Amongst the non-PI ART, nephrolithiasis has been reported with EFV. However this was only observed in association with concomitant use of ritonavir[70]. In terms of TBT, Antony et al, reported nephrolithiasis in 2 patients on TBT, which was attributed to PZA (n=1) and EMB (n=1)[71]. The diagnosis of this phenotype is reliant on imaging (i.e sonar)[60], which may not always be available.

2.1.4. Glomerular

Lastly, the glomerular phenotype is an uncommon presentation for DIRI. It often requires a renal biopsy, in order to confirm the diagnosis[60]. With regard to TBT, case studies have described rapidly progressive glomerulonephritis (RPGN) and minimal change disease, that occurred after commencement of RIF[72,73]. On the other hand ART related glomerular disease is even rarer. Only enfuvirtide, a fusion inhibitor, was found to be associated with glomerular disease[74]. In the South African setting, glomerular disease in the HIV population is more closely related to the direct effect of HIV itself i.e. HIV associated nephropathy (HIVAN), rather than drugs.

2.2. Incidence of DIRI

2.2.1 Incidence of ART related DIRI

There was difficultly ascertaining the 'true' incidence of ART related DIRI, even in South Africa. Some of the reasons for this are: there was no standard definition for DIRI, and therefore comparisons between studies were difficult; there were a few studies, mostly retrospective, that determined the incidence of DIRI; and there was no histological confirmation of the entity. Evidently, more studies, especially prospective ones are needed in this regard. Seedat et al, in a prospective study conducted in Klerksdorp, South Africa, found that "TDF induced AKI (DIRI)" was responsible for 53% (93/175) of those hospitalized with AKI. The study was limited by missing values and the lack of histological confirmation[75]. A study done in Nigeria, demonstrated an accumulative incidence of 4.6%[78].

Although these two studies are difficult to compare (the former measuring point prevalence and the latter incidence), they do however highlight the need for clinicians and policy makers, to work on improving the safety of ART.

2.2.2. Incidence of TBT related DIRI

As far as we know, there is no study in Africa that has determined the incidence of TBT related DIRI. This is probably because TBT is not a major renal toxin. To date, the largest study, in this regard, was a prospective study done in Taiwan by Chang et al. The incidence of DIRI secondary to TBT was found to be 7.1 % (99/1394). Furthermore, more than half (61%) of the patients developed DIRI within 2 months[11]. In comparison, the incidence in other studies was 1.8%-12% [12,79]. Perhaps the only way, to begin to consider TBT as a renal problem, is to conduct prospective studies comparing the incidence of DIRI in patients on TBT alone, ART alone and combination TBT and ART.

2.3. Risk factors for DIRI

Compared to DILI, risk factor assessment for DIRI is mostly based on case reports and reporting of adverse events from clinical trials. In addition, there is a small number of prospective trials [59]. With respect to ART, from the few retrospective studies, the risk factors for DIRI were determined to be: an elevated baseline serum creatinine; the dose and duration of TDF administration; the use of other nephrotoxic drugs; advanced age; reduced body weight; a lower CD4 cell count; female sex and PI based ART[80-82]. There is even less data regarding the risk factors for TBT related DIRI. Perhaps the only risk factor reported for RIF was re-exposure to the drug[83]. Emerging biomarkers of kidney damage (e.g. kidney injury molecule (KIM)), may in future not only further classify DIRI phenotypes[60], but perhaps aid in the risk stratification of patients.

2.4. Mechanism of DIRI

The mechanisms of DIRI are similar to those for DILI. They are classified into type A and B reactions. Type A reactions are predictable, dose dependent and occur acutely vs type B, which are

idiosyncratic, unpredictable and more subacute to chronic[60]. With respect to ART and TBT, TDF and RIF are most commonly associated with DIRI, respectively.

2.4.1 Mechanism of TDF induced DIRI

TDF manifests as a type A reaction. It is filtered at the glomerulus and then actively secreted by the tubule. The drug enters the basolateral surface of the proximal tubule using the organic anion transporter (OAT) channel[84]. It subsequently exits the luminal surface, using the multi drug resistance proteins (MRP), to gain entry into tubular lumen[85]. Furthermore, polymorphisms of the ABCC2 gene that encodes for MRP protein, may increase the risk of TDF induced AKI and tubular disorders[86]. Specifically, the mechanism of TDF related toxicity is directly linked to the interaction of the drug with mitochondria in the proximal tubule[87].

2.4.2 Mechanism of RIF induced DIRI

Compared to TDF, the mechanisms of RIF induced DIRI are still unclear. Nevertheless, some studies suggest that the mechanism involves an immune mediated reaction (type B). Specifically, the deposition of RIF antibodies in the interstitium and the glomeruli, may result in interstitial nephritis and RPGN, respectively[12,88]

2.5 Severity and outcomes of DIRI

The severity grading of DIRI is defined according to the KDIGO guidelines[61]:

- Stage 1: Increase in serum creatinine > 26.5 micromoles/L; or less than 2 fold increase from baseline; or urine output (U/O) < 0.5ml/kg/hr for 6-12 hours;
- Stage 2: Between 2 and 3 fold increase in serum creatinine from baseline; or U/O < 0.5mL/kg/hr for >12 hours;

- Stage 3: More than 3 fold increase in serum creatinine from baseline; or U/O <0.3 mL/kg/hr for >12 hours.

There are few a studies to date, that have investigated the risk factors and outcomes for severe TBT or ART related DIRI. More prospective studies are needed in this regard. With regards to ART, Seedat et al, demonstrated that patients with TDF vs non TDF induced AKI developed more severe AKI and had a slower renal recovery at 3 months[75]. Therefore, this means that more patients who received TDF, were more likely to develop CKD, compared to those who did not. In terms of TBT, Chang et al, showed that the majority of study participants presented in stage 1 (84%). Additionally, renal recovery was higher in patients with stage 1 AKI and the least in those with stage 3 disease. Furthermore, the overall predictors of renal recovery in the study, were thrombocytopenia, fever and gastro-intestinal disturbance. Unfortunately, the mortality difference between the DIRI stages was not assessed [11].

2.7. Management of DIRI

There are no differences in management between DIRI vs other forms of AKI. Firstly, the most important step to the stop the offending agent once identified. Notably, according to the South African guidelines, TDF should be stopped when eGFR is < 50[3]. Additionally dose adjustment of the NRTIs and TBT must be done. Secondly, the decision for renal replacement therapy is based on the indications stipulated in the KDIGO guidelines[60]. Thirdly, if renal function remains persistently elevated, even after stopping the drug, then a search for other causes should be done. This may involve the use of non-invasive investigations (ie. sonar) and urinalysis[89]. Follow up appointment should made at 3 months after discharge to assess for development of chronic kidney disease (CKD) [60]. The management of DIRI after discharge, will therefore depend on an efficient primary health care and referral system.

In conclusion, both ART and TBT are frequently complicated by drug toxicity. Furthermore there are overlapping toxicities between ART and TBT. There is therefore a need to study the incidence, risk factors and outcomes of patients who develop DILI and/ or DIRI.

3.0 Study aims and objective

3.1 Aims

The aims of the study are to determine the incidence, clinical expression, severity and risk factors of hepatic (DILI) and renal drug toxicities(DIRI) in a large HIV patient cohort receiving ART alone, TBT alone or concomitant TBT and ART at Helen Joseph Hospital. In addition, we aim to determine the effect of DILI and DIRI on the length of hospital stay. To date there has been no such study evaluating both drug toxicities in our population.

3.2. Objectives

1. To determine the frequency, clinical expression and severity of drug-induced liver injury (DILI) in HIV positive patients who are on 1st line(NRTI based) / 2nd line (PI based regimen) /3rd line(second line NNRTI, PIs and raltegravir) ART alone vs combination of TB therapy and ART vs TB treatment alone(ARV naïve).

2. To determine the frequency, clinical expression and severity drug-induced renal injury (DIRI) in HIV positive patients who are on 1st line/ 2nd line/ 3rd line ART alone vs combination of TB therapy and ART vs TB treatment alone (ARV naïve).

3. To compare length of hospital stay in HIV positive patients on 1st line/ 2nd line/ 3rd line ART alone vs combination of TB therapy and ART vs TB treatment alone (ARV naive) in drug toxicity (DILI and/or DIRI) compared to remaining cohort.

3.3 Methods

3.3.1 Study Design

This is a retrospective review of patients admitted to Helen Joseph Hospital and interviewed by the Infectious Disease (ID) team during the period January 2013 till June 2015.

3.3.2 Study population

3.3.2.1 Site of study

Helen Joseph Hospital, Infectious disease department.

3.3.2.2. Size

All patients who were admitted and referred to the Infectious disease team between January 2013 to June 2015 were included. The patients were clerked at the bedside by the ID registrar. A medical history, examination, laboratory findings and a provisional diagnosis were recorded on a standardized consultation form. Prior to discharge or death, the provisional diagnosis was reviewed with the ID consultant and a final diagnosis was allocated to the patient. Data from the paper template were then entered by data capturers into a computer database program. The data was subsequently checked and cleaned by a senior member of the ID consultant team. At the time of preparing this report 1127 patient entries had been cleaned and were available for review. The original paper templates were stored in locked cupboards in the ID department.

3.3.2.3 Observations and measurements

- Patient demographics

  a. Age

  b. Race

c. Sex

d. CD4

- Treatment regimen

a. ART regimen

b. TBT regimen

c. Combination of ART/ TBT

d. Date of ART and/or TBT initiation

- Whether DILI and/or DIRI diagnosed or not
- Lab investigations (at baseline and discharge), recording of

a. Renal biochemistry including urea, creatinine, eGFR, sodium, potassium, bicarbonate

b. Hepatic biochemistry including total bilirubin, alkaline phosphatase (ALP),

alanine transaminase(ALT), aspartate transaminase (AST), International normalised ratio(INR)

c. Other: Ferritin, C-reactive protein (CRP), haemoglobin, albumin

d. Sonar (renal and /or hepatic)

e. Biopsy (renal and/or hepatic)

- Whether patient was taking other concomitant drugs?
- Outcomes

a. Whether patient died or not?

b. The length of hospital stay determined by date of admission and discharge

A comprehensive data collection sheet of all the variables is attached.

(Appendix 3.1)

3.3.2.4 Limitations and/or confounders

The limitations of the study are:

- It is a retrospective study hence not all the relevant information may be available from the database. A prospective study is needed.
- An inherent bias is acknowledged in that the patients in the study do not necessarily represent the HIV-positive population at large. The study cohort is drawn from sick and hospitalized patients who are likely to have received multiple medications prior to their referral to the ID unit and to be persons with or exposed to a variety of diseases that can duplicate or mask the signs of drug toxicity.

There are confounding variables in the study:

- The confounding factors for DILI include hepatitis status, chronic liver disease and hepatotoxic drugs
- The confounding factors for DIRI include chronic kidney disease, nephrotoxic agents, and other conditions which may cause renal impairment e.g. dehydration or sepsis

3.4 Statistical analysis

The data from the electronic database will be reorganised and captured into an Excel spreadsheet. It will then be processed using Stata version 13.0 statistical software. Continuous data will be tested for normality and summarized as means and standard deviation where appropriate. Non-normal data will be transformed or analyzed using non-parameter tests. Categorical data will be presented as frequencies and percentages. Associations between categorical variables will be analyzed using Chi-

squared test or Fishers or Kruskall-Wallis test. All tests will be performed at the 95% confidence interval. Statistical significance will be determined when the p-value is <0.05.

3.5 Ethics

An application had been made to Wits Human Resource Ethics Committee. Consent to use patient records will be obtained from the CEO/Superintendent from Helen Joseph Hospital. To assure anonymity of the data and protect confidentiality, the names and personal details of patients entered into the original electronic database will not be transferred to the study database (Excel spreadsheet). As this is an anonymised retrospective analysis of data previously collected, individualized informed consent from the study subjects will not be required.

3.6 Timing

The expected duration of the study is shown below:

	Jan 2016	August	Jan 2017	June	Jan 2018	May 2019
Literature Review						
Protocol preparation						
Protocol Assessment						
Ethics approval						
Data collection						
Data analysis						
Writing up thesis						

3.7 Funding

No costs expected to arise other than necessary for photocopying, stationary, printing and binding, which will be self-funded.

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CHAPTER 2: SUBMISSIBLE ARTICLE

Title: A retrospective review of drug-related toxicity of HIV positive patients on Antiretroviral and Tuberculosis therapy at Helen Joseph Hospital, Gauteng, South Africa

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Short title: The burden of drug-induced liver and renal injury in HIV positive patients at Helen Joseph, South Africa

Conflict of interest: Nil

Keywords: Drug induced liver injury, drug induced renal injury, HIV, South Africa

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Word count: 3456

Abstract word count: 363

Abstract

Background: There are large numbers of people on both antiretroviral therapy (ART) and tuberculosis (TBT) therapy in South Africa due to the burden of disease. Both treatments may have overlapping toxicities resulting in drug induced liver injury (DILI) and drug induced renal injury (DIRI) which impacts on both morbidity and mortality in HIV patients.

Objectives: To determine the incidence, expression, severity and risk factors of DILI and DIRI, as well as to determine the effect of DILI and DIRI on the length of hospital stay in a large HIV patient cohort receiving ART alone, TBT alone or concomitant TBT and ART .

Methods: A retrospective descriptive study was conducted on primary data obtained from the Infectious Diseases division database of HIV positive patients who were admitted at Helen Joseph Hospital (HJH) from 1 January 2013 to 30 June 2015. Data collected included socio-demographic, treatment regimen, and laboratory variables. Descriptive statistics and association of the variables were studied. Ordered logistic regression was used to analyse the predictors for the incidence of DILI and DIRI.

Results: Thirty per cent of patients with liver impairment (102/342) were classified as DILI. ART alone (41.2%) and combination of TBT and ART (42.2%), had a greater contribution to the development of DILI compared to TBT alone (16.7%). The predominant expression and severity grading of DILI were cholestasis (59.5%) and mild injury (58.8%), respectively. Only 10% (32/333) of patients with renal disease, were classified as DIRI. ART (62.5%) and combination of ART and TBT (34.4%) were important contributors to the incidence of DIRI. Pure TB therapy had a little role in this regard (n=1). The significant predictors for DILI were age, female sex and length of hospital stay, while that for DIRI was baseline creatinine.

Conclusion: DILI was more common than DIRI in the study. ART alone and combination of TBT and ART, was associated more frequently with DILI and its severity compared to TBT alone. Both ART and combination of ART and TBT were important contributors to the development of DIRI. Anti TB therapy alone seldom caused DIRI. There is an important need for prospective studies to investigate the incidence and outcomes of DILI and DIRI.

Introduction:

The highest proportion of people living with HIV are in South Africa, a country with an HIV-burden of approximately 7.9 million (2017)[1]. According to the Human Sciences Research Council, the national prevalence of HIV infection among South African adults was 12.6% in 2012[2].With the change in the World Health Organization (WHO, 2015) and South African (2017) guidelines, all irrespective of baseline CD4 cell count are now being initiated on antiretroviral therapy (ART) [3].

South Africa has the 8th highest TB incidence globally [4]. The problem of dual infection is magnified in South Africa, as it has the highest prevalence of HIV associated TB cases[5] . The TB epidemic in South Africa is driven by HIV. ART coverage in South Africa has increased from 24-61% (2010-2018), which has lead to the decline in the incidence of TB[4]. Apart from incidence and prevalence, the societal impact of TB can be measured in TB-related morbidity and mortality.

In the case of both HIV and TB, treatment is frequently complicated by drug toxicity. The signs and symptoms of drug toxicity, the immune reconstitution inflammatory syndrome (IRIS), and other HIV-related diseases overlap and obscure the primary diagnosis. Under these circumstances the definitive diagnosis of a drug-related injury is difficult, nevertheless this diagnosis is important. ART and TB therapy (TBT) can cause debilitating and/or life-threatening disease e.g. severe skin reactions, hepatitis, renal disease, marrow suppression, and incapacitating neuropathies[6].

For the purpose of this study, only drug-induced liver injury (DILI) and drug-induced renal injury (DIRI) were investigated. Globally DILI accounts for 7% of adverse drug reactions and for 30% of fulminant liver failure[7]. Hepatotoxicity complicates ART in 8.9-10.8% of cases globally[8]. The incidence of antituberculosis related DILI is 2-28% in Europe, the United States of America and Asia. Incidence rates for hepatotoxicity are not commonly reported in sub-Saharan Africa as liver function tests in patients on TBT are not routinely carried out[9]. There are often overlapping toxicities between ART and TBT. DIRI related to ART accounts for 14% of late onset acute kidney injury (AKI)[10]. AKI is a rare complication of TBT that leads to treatment interruption and

irreversible kidney injury. The incidence varies from 0.05-7% in Romania, Taiwan and South Africa[11-13]. DILI and DIRI impact both morbidity and mortality in HIV patients [3,11].

The aims of the study were to determine the incidence, expression, severity and risk factors of DILI and DIRI, as well as to determine the effect of DILI and DIRI on the length of hospital stay in a large HIV patient cohort receiving ART alone, TBT alone or concomitant TBT and ART.

Methods

1. Study population and data collection

Helen Joseph Hospital (HJH) is a secondary level hospital in Gauteng that serves a large region with a high burden of TB and HIV. A retrospective observational study was conducted from 1 January 2013 to 30 June 2015. All HIV positive patients who were admitted and referred to the Infectious Diseases team were identified. Demographic data including age, gender and race as well as laboratory findings and a provisional diagnosis were recorded on a standardized consultation form (template) by a medical registrar. Prior to discharge or death, the provisional diagnosis was reviewed with the ID consultant and a final diagnosis was allocated to the patient. Data from the paper template was then entered by data capturers into a computer database program. This data had been checked and cleaned by a senior member of the ID consultant team. The original paper templates are stored in locked cupboards in the ID division**.**

All study subjects were HIV positive and aged > 18 years. Patients not meeting the above criteria were excluded from the study, as well as patients referred for drug toxicity secondary to the treatment of an opportunistic infection other than TB, e.g. co-trimoxazole for *Pneumocystis jiroveci* pneumonia (PJP). Where laboratory data was missing from the electronic database, data was sourced directly from the National Health Laboratory Service's database. The study was approved by the University of the Witwatersrand Human Research Council. (HREC ref: M160410)

2. Definitions

Both DILI and its severity were defined according the South African consensus guidelines. DILI was defined if any of the following criteria are met[14] :

I. Alanine aminotransferase (ALT) > 120 IU/L in symptomatic patients

II. ALT > 200 IU/L in asymptomatic patients

III. Total serum bilirubin > 40 micromoles/L.

The severity of DILI was as defined as follows:

- Mild DILI: ALT > 40 but < 200 IU/L and total bilirubin < 40 micromoles/L without symptoms
- Moderate DILI: ALT >200 IU/L or ALT < 120 IU/L + total bilirubin > 40 micromoles/L without symptoms
- Severe DILI: presence of symptoms or features of overt liver failure (elevated INR and /or clinical signs of failure).

The expression of DILI was based on the R value which was defined as [15]:

R= (ALT/ULN (upper limit of normal)) / (ALP/ULN)

- Cholestatic pattern: R value <= 2
- Hepatocellular: R value >= 5
- Mixed pattern: R value > 2 and < 5

Both AKI and its severity were defined according to the Kidney Disease Improving Global Outcomes (KDIGO) guidelines. AKI staging was defined with the following criteria[16]:

- Stage 1: Increase in serum creatinine > 26.5 micromoles/L; or less than 2 fold increase from baseline; or urine output (U/O) < 0.5ml/kg/hr for 6-12 hours;

- Stage 2: Between 2 and 3 fold increase in serum creatinine from baseline; or U/O < 0.5mL/kg/hr for > 12 hours;

- Stage 3: More than 3 fold increase in serum creatinine from baseline; or U/O < 0.3 mL/kg/hr for > 12 hours.

DIRI was defined when AKI developed with drug exposure and in the absence of other nephrotoxic agents, and with biological plausibility for the drug to cause renal injury[17]. Both DILI and DIRI required a compatible drug-exposure history, improvement on withdrawal of the offending drug, and the exclusion of alternative causes of liver and/or renal disease. Diagnostic certainty was not always guaranteed. The categories of 'possible' and 'probable' DILI/DIRI were defined as follows:

- Possible DILI/DIRI: Inability to exclude 'other' contributing factors e.g. infection, hypotension, multisystem disorder etc.

- Probable DILI/DIRI: No contributing/causal factors were identified.

The hepatitis B status was defined as following:

- Acute hepatitis B: presence of surface antigen with a positive core IgM antibody.

- Chronic hepatitis B: presence of surface antigen with absent core IgM antibody, the presence of core IgG antibody, with/ without signs of chronic liver disease.

Statistical Analysis:

All data were expressed as either median (inter-quantile ranges) or mean +/- standard deviation. Inter-group difference was compared using the Kruskal-Wallis test. Unadjusted and adjusted multiple

logistic regression was used to determine factors associated with DILI and DIRI. Parameters with p value < 0.2 in the univariate analysis were included in the multivariate analysis to determine the odds ratios (OR) and 95% confidence intervals (CI). All statistical analyses were conducted using Stata version 13 (StataCorp). P values < 0.05 were considered significant.

Results

The DILI cohort

From January 2013 till June 2015, a total of 1127 HIV positive patients were entered into the database (Figure1). Patients were excluded firstly due to incomplete records (n=344) and secondly due to not meeting criteria for liver impairment (n=441). Of those who met criteria for liver impairment, 29.8% (n=102/342) were classified as having ART and/or TBT related DILI.

The data in table 1 demonstrates the particular characteristics of patients who presented with DILI or DIRI. These two categories were not mutually exclusive as there were 4 patients who developed both drug toxicities. Evidently, characteristics such as age, sex, race, CD4 and length of hospital stay were similar between the categories. Each drug toxicity category was analysed separately. The distribution of ART and TBT regimens in patients who developed DILI are outlined in table 1.

Amongst patients on ART alone, 41.2% (n=42/102) received tenofovir(TDF) based 1st line therapy(i.e. which includes a backbone of lamivudine(3TC)/emtricitibine and efavirenz(EFV)/nevirapine(NVP)). The predominant non-nucleoside reverse transcriptase inhibitor (NNRTI) received in the study was EFV, as only 3/85 patients on ART received NVP. In terms of those on TBT alone, 13.7% (n=14/102) were in the intensive phase of treatment. Patients on both ART and TBT comprised 42.2 % (n=43/102) of patients who developed DILI. The expression and severity of these treatment groups is shown in table 2.

All patients in the various treatment groups presented mostly with cholestasis (59.8%), however those on TBT alone had the largest proportion (70.6%). In contrast patients on ART alone had the highest

proportion of hepatocellular expression (45.2%). In terms of severity, the majority presented with mild disease (58.8%). Figure3 shows that severe toxicity was associated with hepatocellular expression. In contrast mild toxicity was more associated with cholestasis. The risk factors for DILI were determined and shown in table 4.

Characteristics considered in the univariate analysis ($p < 0.2$) were age, female sex, length of hospital stay, and baseline total bilirubin. After adjustment for all these factors, baseline total bilirubin was not found to be significant ($p=0.126$). The outcomes were also assessed.

The mortality rate in those with DILI was 12.75 % (n=13/102). Notably, the mortality rate was highest in patients on TBT alone (23.53%; n=4/17) and lowest in patients on ART alone (7.14%; n=3/42). There was no significant association between DILI severity grading and mortality ($p=0.323$).

The DIRI cohort

Patients were excluded firstly in a similar fashion to DILI (Figure 2). Secondly those not meeting criteria for renal impairment (n=441) were excluded. From the remaining cohort, only 9.6% (32/333) were classified as having ART and/or TBT related DIRI. Table 1 also demonstrates the different treatment regimens for patients with DIRI.

The comparison of these regimens showed that 62.5% (20/32) of patients were only receiving ART. Most of these patients were receiving a TDF based 1st line therapy (n=16/32; 50%). Patients only receiving TBT had a minimal contribution as only 1/32 developed DIRI. The patient mentioned was on a multi drug resistant (MDR) TBT regimen and receiving an injectable agent (i.e. an aminoglycoside). Further analysis of this patient on TBT was discontinued. Patients on concomitant TBT and ART comprised of 34.4% (n=11/32) of patients who developed DIRI. The severity of these treatment groups in shown in table 3. Analysis in this table excludes the patient with DIRI due to TBT alone.

The majority (54.84%; n=17/31) of patients presented in KDIGO stage1. Comparison of the treatment groups showed that patients on combination of TBT and ART had significantly more severe disease than those on ART alone (p=0.047). The risk factors for DIRI were determined and shown in table 5.

The unadjusted logistic regression ($p < 0.2$) showed that age, duration of hospital stay and baseline creatinine were significant risk factors for DIRI. After adjustment for these factors, age and duration of hospital stay were not found to be significant. The outcomes for patients with DIRI were also determined.

Mortality was 19.35% (n=6/31) in patients with DIRI. The majority (83.3%; n=5/6) of these patients had KDIGO stage 3 severity grading.

Discussion

We conducted a retrospective observational study to determine the incidence, expression, severity and risk factors for DILI and DIRI, as well as their effect on the length of hospital stay in a large HIV patient cohort receiving ART alone, TBT alone or concomitant TBT and ART. The major findings of the study are mentioned below.

Almost a third (30%) of patients with liver impairment (102/342) were classified as DILI. The two treatment categories, ART alone and combination of TBT and ART, had a greater contribution to the development of DILI compared to TBT alone. The predominant expression and severity grading of DILI were cholestasis (59.5%) and mild injury (58.8%), respectively. DIRI compared to DILI, was lower, as only 10% (32/333) of patients with renal disease were classified as having DIRI. ART and combination of ART and TBT treatment were important contributors to the incidence of DIRI, and pure TB therapy had little role in this regard. To the best of our knowledge, this is the first study to describe the incidence, risk factors and outcome of both DILI and DIRI in an African HIV population.

TBT and ART related DILI are important to consider in patients with HIV and liver impairment. Specifically, patients receiving ART or combination of TBT and ART may be at a higher risk of

developing drug toxicity than patients on TBT alone. In comparison, Shultz et al, showed, that TBT contributed more to occurrence of DILI than patients on ART alone and concomitant ART and TBT [18]. In other studies, the incidence of TBT and ART related DILI, ranged from 6-32% [19-23] and 4-20%, [23-25] respectively. In the South African context, the increase in ART rollout, has lead to the decline in the incidence of TB[4]. With this in mind, ART may in time become the largest contributor to DILI in the future. Special emphasis may need to be placed on first line (NNRTI) based therapy, as this was the largest contributor to ART related DILI in the study. Additionally, this study should be considered in developing countries such as South Africa, when considering the development of ART guidelines. The substitution of NNRTI's with intergrase strand transfer inhibitors (INSTI) as 1st line therapy, may dramatically decrease the burden of DILI.

The predominant expression and severity grading of DILI were cholestasis (59.8%) and mild injury (58.8%), respectively. The study showed that ART alone and concomitant ART and TBT increased the risk of DILI severity. In terms of expression, ART alone (45.2%) was more associated with hepatocellular injury than TBT alone and concomitant TBT and ART. These findings correlated with those from Yimer et al, however differed in that hepatocellular injury was more common in patients on TBT alone[23]. The difference in these studies, shows that TBT and ART related DILI may present in different ways. Furthermore expression of liver injury is not static and may change over time[26] . It is important for the clinician to be familiar with these hepatic expressions, as it may help to limit the number of differential diagnoses in patients with DILI e.g. hepatocellular expression and viral hepatitis, cholestasis and biliary disease[27].

Compared to DILI the incidence of DIRI was lower, as only 10% (32/333) of patients with renal disease were classified as having DIRI. This is likely due to the function of the liver, as compared to the kidney, in drug metabolism i.e. the first in line to be affected by toxins, including drugs[7]. On the other hand, diverse conditions influence renal health in the HIV infected[28,29]. The kidneys are nevertheless important to the HIV clinician because where there is renal impairment, our drugs are frequent culprits, such as TDF which is frequently used in first line ART since 2012. The data in our

study indicates that ART and combination of TBT and ART are important in DIRI. Pure TB therapy has little role in this regard. This points in the direction of TDF and its role in renal dysfunction. Again the place of first-line ART in the causation of end-organ disease (renal and liver) is emphasised in the study. This points to the urgent need to better first line ART.

The incidence of DILI and DIRI in patients on TDF based 1st line ART was 31.4% (32/102) and 50% (16/32), respectively. This suggests that TDF may have had a more immediate effect on renal function than efavirenz on the liver. For other regimens including 2nd line (PI-based) therapy, the incidence of DILI and DIRI was only 9.8% (10/102) and 12.5%(4/32), respectively. This demonstrates that these two drug toxicities were less common with second line ART. In terms of TBT alone, there were only 3.1% (1/32) of patients who developed DIRI vs 16.7 % (17/102) who developed DILI. TBT in general is not a major renal toxin[11,13] .

Co-trimoxazole also plays a role in both liver and renal impairment. This can add to the complexity in making the diagnosis of DILI[18] and DIRI. There was a distinction made between those who developed DILI or DIRI and were receiving concomitant co-trimoxazole vs those with drug toxicity secondary to the treatment of an opportunistic infection other than TB, e.g. co-trimoxazole for *Pneumocystis jiroveci* pneumonia (PJP). The latter group of patients were excluded from the analysis. There were similar percentages, 18.6% (19/102) and 15.6% (5/32) for DILI and DIRI, respectively. This suggest that co-trimoxazole was not a major influencer in both drug toxicities. Despite the low CD4 (i.e. on average <100 cells/mm3) in the study, the majority of patients were not on concomitant co-trimoxazole prophylaxis. It was unclear whether this observation was due to technical factors (e.g. unavailability of the drug vs the lack of implementation of guidelines at the local clinic) or personal factors e.g defaulting treatment. A prospective study is needed to evaluate the 'true' role of co-trimoxazole in both DILI and DIRI.

In addition we used multivariate logistic regression models to determine which biochemical and /or socio-demographic factors influenced the incidence of both DILI and DIRI. The significant predictors

for DILI for both TBT and ART were younger age <35years, female sex, and a longer length of hospital stay. In relation to TBT associated DILI, our study differed from Singla et al, who demonstrated that older age >35yrs, together with poor nutritional status were their significant predictors[30]. A prospective study is needed evaluate whether younger patients in this study population are more predisposed to to DILI, owing to other factors such as: increased alcohol use; hepatitis B and C and herbal ingestion. Compared to other studies,[31,32] our study could not determine if chronic viral hepatitis was a major predictor of ART related DILI, as these patients were excluded from the analysis. Furthermore, owing to the retrospective nature of the study, only a few patients had documented HepB or HepC serology. With reference to predictors of DIRI, our study found that a higher baseline creatinine was a significant predictor for DIRI. Similarly, a study by Jafari et al, showed similar findings and in addition the following variables were also predictors: the dose and duration of TDF administration; use of other nephrotoxic drugs; advanced age; reduced body weight, and a lower CD4 cell count[33]. Identifying risk factors may help to predict which patients are likely to develop drug related toxicity[34]. However, this often difficult in clinical practise, as most cases of DILI manifests in an idiosyncratic manner[15]. Nevertheless, it is still important for clinicians, drug production companies and policy makers, to be vigilant of these risk factors, which may motivate for increased surveillance of drug related toxicity and the development of new drugs with less potential for toxicity.

In terms of the mortality in both drug-toxicity groups, the numbers were small and therefore comment is guarded. The mortality in patients with DILI vs DIRI was 12.7% (13/102) and 18.8% (6/32), respectively. This may suggest that DIRI in our context may have been more likely to result in death. Certainly it would be an important area to follow up on with a larger and 'cleaner' study cohort.

Study Limitations

This study has several limitations. It was a retrospective study and hence not all the relevant information was available on the database. Both DILI and DIRI had lower CD4 counts, on average

less 100 cells/mm^3. At the time of the study, the patients were started on ART late i.e. at CD4s <200, then 350 cells/mm^3. This inherent bias is acknowledged, in that the patients in the study did not necessarily represent the HIV-positive population at large. It is also unclear whether the low CD4 baselines contributed to the role of IRIS in the toxicity of drugs given to TB patients.

Conclusion

Up to a third of patients with liver impairment had DILI. ART alone and combination of TBT and ART, had a greater contribution to the development of DILI and its severity compared to TBT alone. DIRI compared to DILI, had far fewer numbers, as only 10% of patients with renal disease were classified as having DIRI. Both ART and combination of ART and TBT treatment were important contributors to the incidence of DIRI. Pure TB therapy had a little role in this regard. There is an important need for prospective studies to investigate the incidence and outcomes of DILI and DIRI.

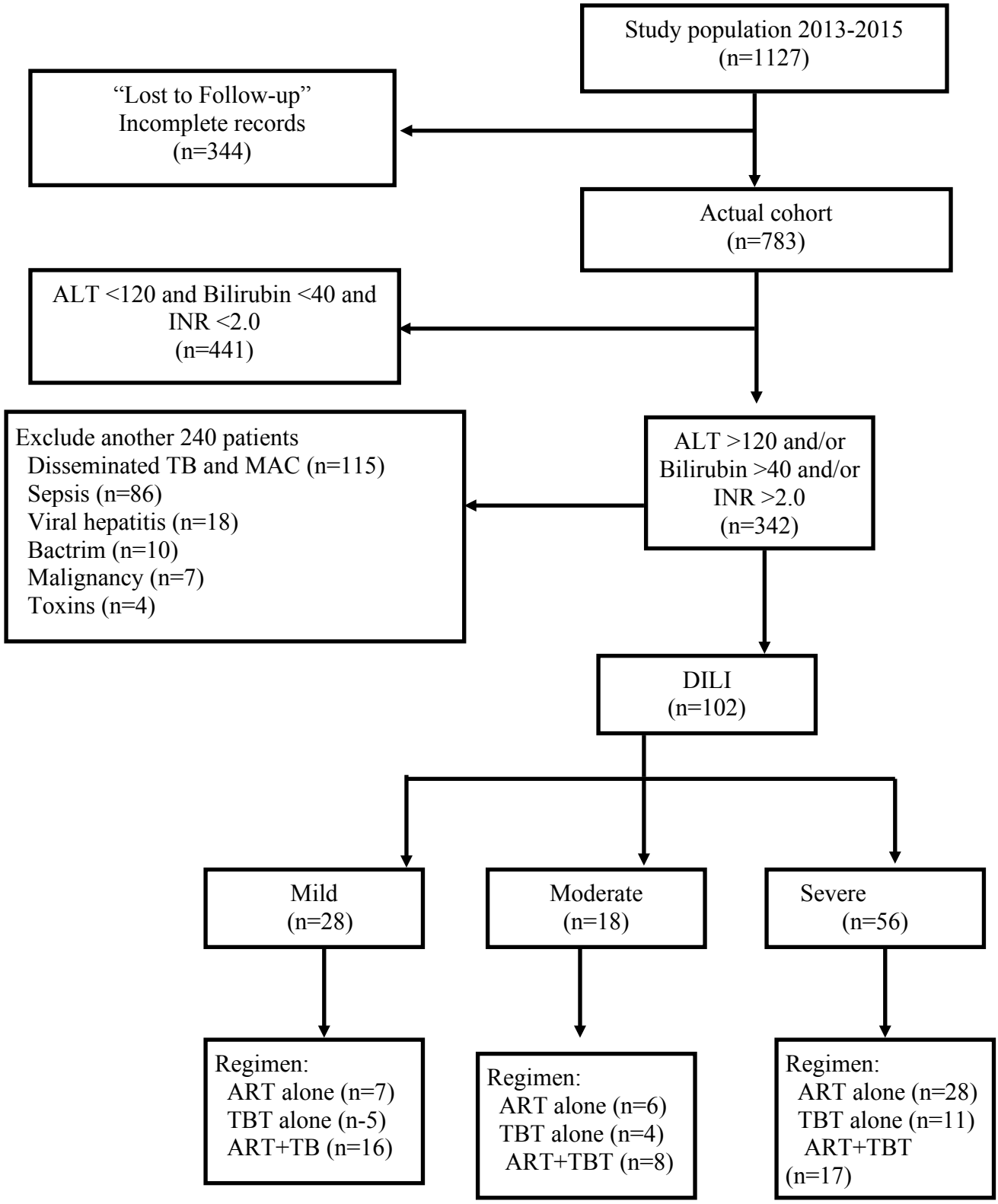

Figure 1 Selection of study subjects for DILI. ALT, alanine aminotransferase; INR-International normalized ratio; MAC, Mycobacterium complex; Sepsis,all other infection other than TB and MAC, Malignancy(primary or secondary liver cancer).

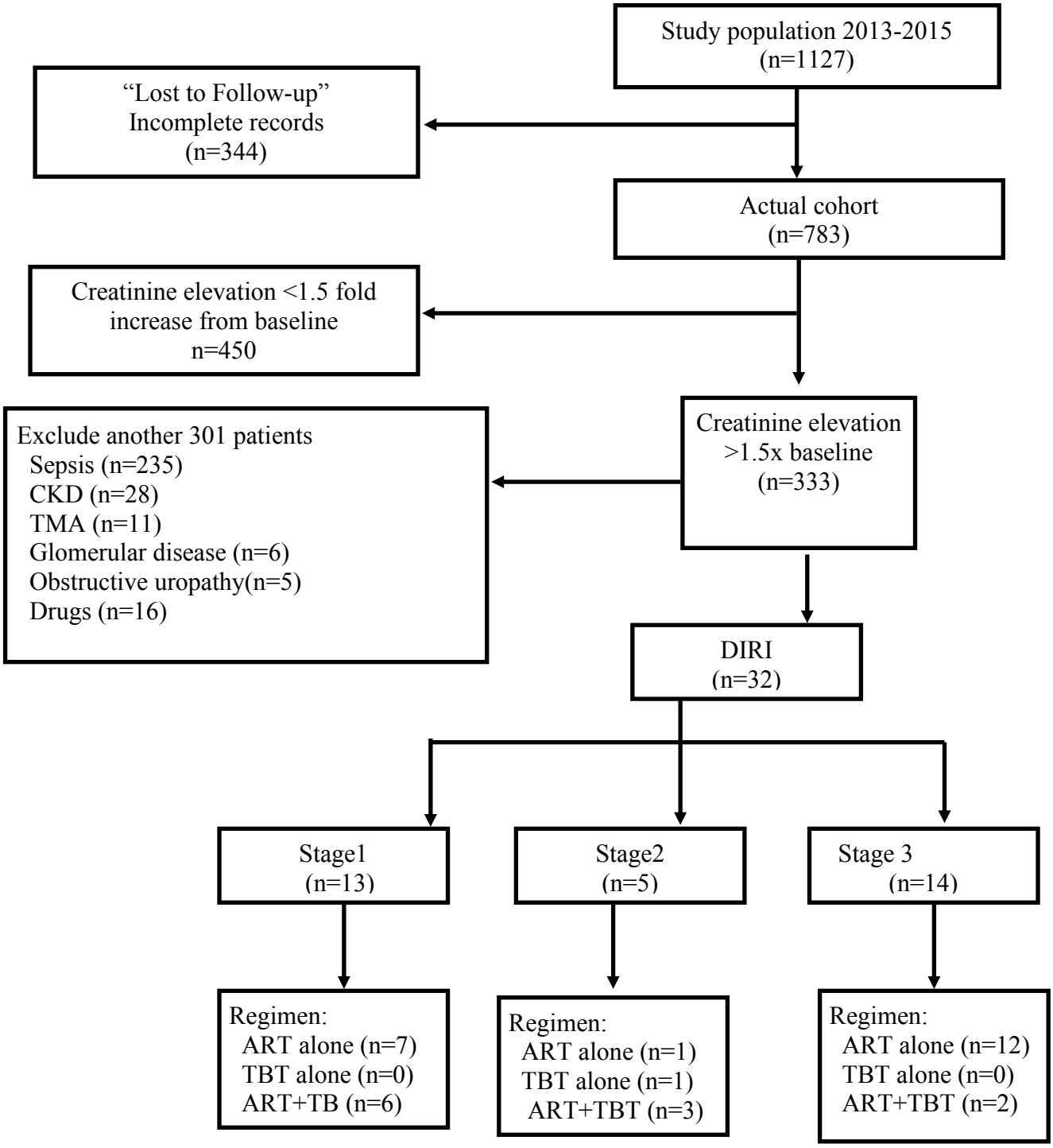

Figure 2 Selection of study subjects for DIRI CKD, chronic kidney disease; TMA, thrombotic microangiopathy including TTP/HUS; Drugs, include amphotericin, aminoglycosides, NSAIDS, Bactrim.

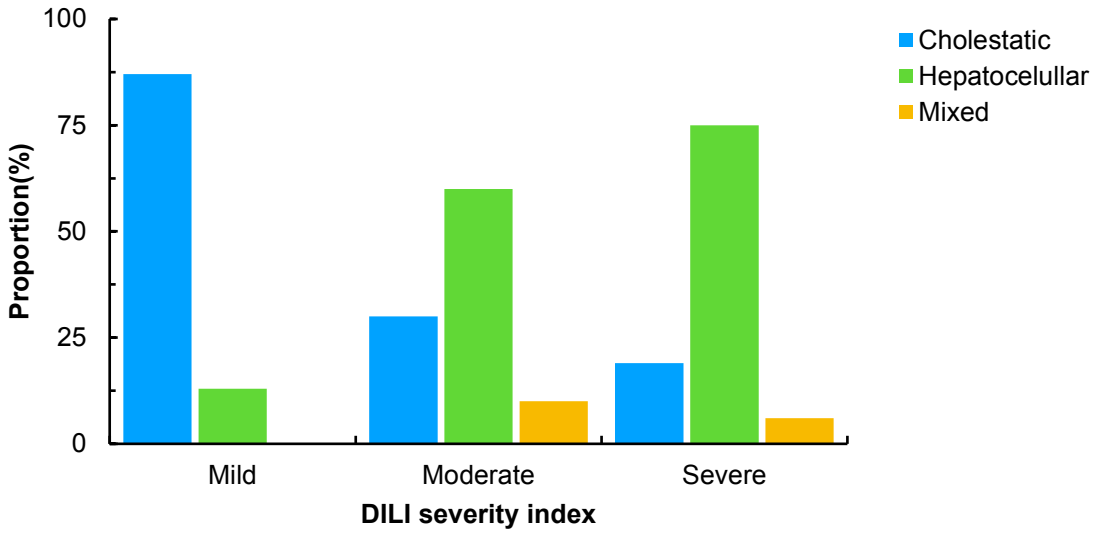

Figure 3: Comparison of expression of DILI stratified by severity grade

Table1. Baseline characteristics of patients who presented with DILI or DIRI

	DILI(n=102)	DIRI(n=32)
Age(years),median(IQR)	37(31.5-42.5)	43(35.5-51)
Sex: female,n%	66(64.7)	19(59.4)
Race: Black, n%	91(89.2)	30(93.8)
CD4 count cells/mm3, median(IQR)	57.5(24-233)	82(24.5-173)
Days in hospitals, median (IQR)	13(8-24)	14(7-23)
Receiving ART alone at presentations, n(%)	42(41.2)	20(62.5)
Days of ART, median(IQR)	214.5(38-745)	415(109-785)
ART regimen (TDF), [#] n(%)	32(31.4)	16(50)
Other Regimen ART,[$]n(%)	10(9.8)	4(12.5)
Receiving TBT alone at presentation, n(%)	17(16.7)	1(3.1)
Days of TBT, median(IQR)	30(14-45)	22(14-30)
Regimen1 TBT, [^]n(%)	14(13.7)	0(0)
Other Regimen TBT, [*]n(%)	3(2.9)	1(3.1)
Concomintant TB and ART, n(%)	43(42.2)	11(34.4)
Co-trimoxazole prophylaxis, n(%)	19(18.6)	5(15.6)
In hospital death, n(%)	13(12.7)	6(18.8)

[#]ART regimen (TDF)- Tenofivir+lamivudine/emtricitibine+Efavirenz

[$] All other ART regimens including Stavudine, Zidovudine, Abacavir based 1st line and protease inhibitor based regimen (second line)

[^]Rifafour(Rifampicin, ethambutol, isoniazid and ethambutol)

[*] Rifinah(Rifampicin and isoniazid) and MDR(multi-drug resistant regimen)

[±]Follow up for patients who survived on discharge at TLC-Themba Lethu clinic (Helen Joseph dedicated HIV

Table2. Clinical presentation of patients who presented with DILI expressed by treatment type

	Total(n=102)	ART alone(n=42)	TBT alone(n=17)	ART and TBT(n=43)
Expression of liver function				
Cholestatic, n(%)	61(59.8)	21(50)	12(70.6)	28(65.1)
Hepatocellular, n(%)	38(37.3)	19(45.2)	4(23.5)	15(34.9)
Mixed, n(%)	3(2.9)	2(4.8)	1(5.9)	0(0)
Severity of DILI				
Mild, n(%)	60(58.8)	24(57.14)	9(52.94)	27(62.79)
Moderate, n(%)	10(9.8)	3(7.14)	1(5.88)	6(13.95)
Severe, n(%)	32(31.4)	15(35.71)	7(41.18)	10(23.26)
In hospital death, n(%)	13(12.75)	3(7.14)	4(23.53)	6(13.95)

Table3. Clinical presentation of patients who presented with DIRI expressed by treatment type[#]

	Total(n=31)[*]	ART alone(n=20)	ART and TB(n=11)
Laboratory values, median(IQR)			
Creatinine micromol/L[^]	155(120-525)	142(126-525)	223(120-525)
Urine protein creatinine ratio	0.217(0.097-0.329	0.256(0.107-0.325)	0.18(0-0.782)
Severity of DIRI			
Stage1, n(%)	17(54.84)	12(60)	5(45.45)
Stage2, n(%)	1(3.23)	0(0)	1(9.09)
Stage3, n(%)	13(41.94)	8(40)	5(45.45)
In hospital death, n(%)	6(19.4)	5(25)	1(9.09)

[#] DIRI due to TBT alone excluded from analysis due to small numbers (n=1)
[^] Creatinine on admission

Table4: Unadjusted and adjusted Odds ratios for risk factors for DILI

		Unadjusted odds rato		Adjusted odds ratio	
		(95% CI)	p-value	(95% CI)	p-value
Age	<35 years	1.00(Reference)		1.00(Reference)	
	>=35 years	0.70(0.46-1.05)	0.085	0.53(0.32-0.87)	0.013
Sex	Male	1.00(Reference)		1.00(Reference)	
	Female	1.83(1.19-2.82)	0.006	2.18(1.27-3.74)	0.005
LOHS #	<10 days	1.00(Reference)		1.00(Reference)	
	>=10 days	1.95(1.25-3.06)	0.003	2.4(1.4-4.2)	0.003
Total Bilirubin	<=60 micromol/L	1.00(Reference)		1.00(Reference)	
	>60 micromol/L	1.64(0.93-2.91)	0.089	1.58(0.88-2.84)	0.126

#LOHS- Length of hospital stay

Table5: Unadjusted and adjusted Odds ratios for risk factors for DIRI					
		Unadjusted odds rato		Adjusted odds ratio	
		(95% CI)	p-value	(95% CI)	p-value
Age	<35 years	1.00(Reference)		1.00(Reference)	
	>=35 years	1.99(0.85-4.64)	0.112	1.82(0.76-4.32)	0.177
LOHS [#]	<10 days	1.00(Reference)		1.00(Reference)	
	>=10 days	1.91(0.87-4.16)	0.105	2.07(0.93-4.62)	0.075
Creatinine micromol/L[$]	<200	1.00(Reference)		1.00(Reference)	
	200-300	3.06(0.98-9.56)	0.054	3.17(1.01-9.97)	0.048
	>300	7.89(3.65-17.08)	<0.001	8.02(3.68-17.50)	<0.001

[#]LOHS- Length of hospital stay
[$] Creatinine on admission

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CHAPTER 3 APPENDICES

3.1 Data collection form

Race
1-Black
2-White
3-Coloured
4-Other
ARV Regimen on admission
1-First-Line ART: TDF+3TC/FTC+EFV/NVP
2-First-Line ART: D4T+3TC+EFV/NVP
3-First-Line ART: AZT+3TC+EFV/NVP
4-First-Line ART: ABC+3TC+EVF/NVP
5-Second-Line ART: 2NRTIs+b(boosted)PI (LPV/r)
6-Second Line ART: 2NRTIs+bPI(ATV/r)
7-Third-line and Salvage ART: any regimen containing Second-generation NNRTIs
Rilpivirine or Etravirine, the Integrase Inhibitor Raltegravir, the PI Darunavir
8-Pre-ART(not yet on ARV's)
TB Regimen
1-Rifafour
2-Rifinah
3-MDR
4-Single agent identified: Isoniazid(INH)
5-Single agent identified: Ethambutol(EMB)
6-Single agent identfied:Rifampicin (RIF)
7-Not on TB treatment
eGFR(Baseline/Admission/Discharge/Demise)
1 : > 65
2: 30-65
3: 10-30
4: <10 or HD
5: unknown
Hepatitis status
1-HepB Pos(Acute)
2-HepB Pos(Chronic)
3-HepC Pos
4-HepB and C Serology-ve
5-Unknown
LFTs: Transaminase(AST,ALT) elevation
1: Normal/near normal(<1.5x elevated) transaminase levels
2: >normal but ≤ 2-5 x rise in either ALT/AST or both
3: ≥5x rise in ALT/AST or both
4: Transaminase levels unknown
LFTs: Hyperbilirubinaemia(Total Bilirubin, TB)
1-None
2- > normal TB but ≤ 2-5x rise above normal
3- ≥5x rise in TB
4- TB levels unknown
LFTs: Clinical and Biochemical Expression of Liver Dysfunction
1-normal liver function
2- Cholestatic Pattern (Predominant ALP and GGT rise with/without increase in T.Bilirubins
>>any proportion increase of Transminases
3- Hepatitic Pattern(Predominant ALT and AST rise compared with other liver indices)
4- Mixed Pattern (Enzyme and Tbilirubin levels increased proportionally)
5- Any of the above patterns (1-3) together with an INR > 2.5 and/or clinical signs of
liver failure
6-Liver biochemistry unknown
Outcomes: Length of hospital stay(LOS)
1: LOS < 7days
2: LOS 8-14 days
3: LOS 15-30 days
4: LOS ≥ 30days or ≥ one month
5: LOS 'unknown'

Patient demographics				Treatment			Renal Function	
Entry no	Age	Gender	Race	ARV regimen	TB regimen	Combination(ARV+TB)	Baseline creatinine	Creatinine(discharge/demise)

Hepatic Function				Outcomes
Baseline Hep status	Transaminase level	Hyperbilirubinaemia	Clinical/Biochemical Expressio	LOS(length of hospital stay)

3.2 Ethics clearance

R14/49 Dr Lifa Dhlamini

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M160410

NAME: (Principal Investigator)	Dr Lifa Dhlamini
DEPARTMENT:	Internal Medicine Helen Joseph Hospital Right to Care
PROJECT TITLE:	Drug-Related Toxicity of HIV Patients on Antiretroviral and Tuberculosis Therapy at Helen Joseph Hospital: A Retrospective Study of Referrals to the Infectious Infectious Disease Unit, January 2013 - June 2015 January 2013- June 2015
DATE CONSIDERED:	06/05/2016
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Dr Prudence Ive and Dr Dave Spencer
APPROVED BY:	Professor P. Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	23/05/2016

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04 JUL 2016
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This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

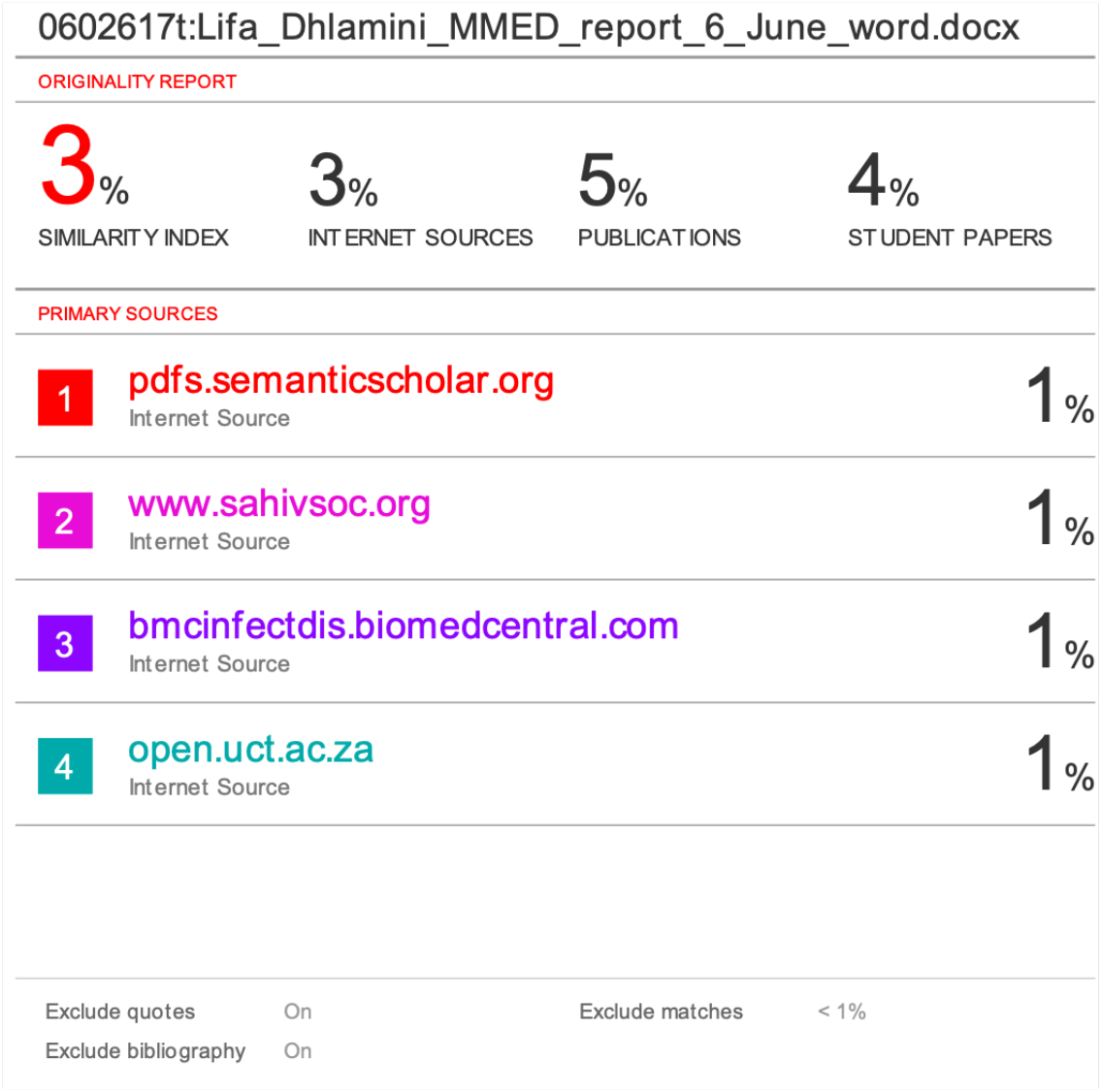
To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 10004,10th floor, Senate House/2nd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand. I/We fully understand the the conditions under which I am/we are authorised to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated,from the research protocol as approved, I/we undertake to resubmit to the Committee. **I agree to submit a yearly progress report.** The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. in this case, the study was initially review in April and will therefore be due in the month of April each year.

Principal Investigator Signature

Date 04/07/16

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

3.3 Turn-it- in Report



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