The Natural History of Efavirenz Drug Induced Liver Injury

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MGHDEB001

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DECLARATION

I, Deborah Maughan, hereby declare that the research here reported is based on my original work (except where acknowledgements indicate otherwise) and that neither the whole work or part of the work has been, is being, or is to be submitted for another degree to this or any other university. This work has not been reported or published prior to registration for the above-mentioned degree.

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Signed by candidate

Deborah Maughan
24th January 2020
I would like to thank Professor Wendy Spearman for her guidance and mentorship throughout my specialist training and as an early career physician. Her example is one I can only aspire to on my journey through medicine.

I would also like to thank my family who have always supported me and encouraged me to pursue God’s purpose for my life no matter the cost.

I dedicate this to the many patients I encountered throughout this process, whose courage and willingness to participate in this research, despite there being no benefit to themselves, was truly humbling. They often expressed the hope that through their involvement, knowledge would be gained that would benefit many other people. I share that same hope.

Lastly, I remember Professor Bongani Mayosi whose kindness, humility and call to excellence in medicine for the benefit of all, especially the poor, will remain with me for the rest of my life.

Soli Deo Gloria.
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<td>AIH</td>
<td>autoimmune hepatitis</td>
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<td>ARV</td>
<td>antiretroviral</td>
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<td>AUC</td>
<td>area under curve</td>
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<td>CI</td>
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<td>Cmax</td>
<td>maximum plasma concentration</td>
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<td>minimum plasma concentration</td>
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<td>cytochrome P450</td>
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<td>DILI</td>
<td>drug induced liver injury</td>
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<td>ELISA</td>
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<td>endoplasmic reticulum</td>
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<td>FDA</td>
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<td>FDC</td>
<td>fixed dose combination</td>
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<td>HepBsAg</td>
<td>hepatitis B surface antigen</td>
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<td>Abbreviation</td>
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<td>NVP</td>
<td>nevirapine</td>
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<td>UGT</td>
<td>uridine-glucuronyl-transferases</td>
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Abstract

Background
Efavirenz (EFV), a non-nucleoside reverse transcriptase inhibitor (NNRTI), has been a component of first line antiretroviral treatment in the South African HIV/AIDS programme since 2004. Similarly, it is extensively used in ART programmes in other low and middle incomes countries. The natural history of the previously reported EFV drug induced liver injury (DILI), is unknown.

Objectives
To establish causality assessment for the drug-induced liver injury and elucidate the natural history of EFV DILI by observing a cohort of patients through documenting all the factors influencing the patterns of clinical and histological injury, the time to clinical and biochemical recovery, the associated mortality rate and to establish if any demographic or clinical factors predict poor outcomes.

Methods
Patients were prospectively included after establishing causality criteria for EFV DILI. Clinical, demographic and histological features were carefully documented from the time of presentation and through follow up. Prednisone at 0.25-0.5mg/kg was initiated at the discretion of the treating hepatologist. Risk factors for severe injury or death and time to event (full clinical recovery and full biochemical recovery) were analyzed.

Results
50 patients were included in the analysis, median age 34 (IQR 29-39) years, men significantly older than women, p=0.014. Most (92%) were female gender, and of black African ethnicity (86%). The median duration on ART at time of presentation was 6.5 months with half of the women initiating ART during pregnancy at a median gestation of 24 weeks (IQR 11 – 36). Median CD4 nadir at ART treatment initiation was 517 cells/mm³ with no significant difference (p=NS) in CD4 nadir in those pregnant or not. Median RUCAM score was 7 and of the 66% of patients who had liver biopsies, 3 histological patterns were identified: submassive necrosis (57.5%), non-specific hepatitis (36%) and mixed cholestatic hepatitis (6%). Multivariate analysis suggested predictors for the development of submassive necrosis included age >30 years [OR 0.86 (0.15-0.97), p=0.02], pregnancy [OR 6.9 (1.34 – 35.6), p=0.02]; CD4 >350 [OR, 7.1 (1.5-31.9), p=0.02] but not alcohol use [OR 1.17 (0.72-1.18); p=0.07]. For the non-specific hepatitis group, only pregnancy predicted [OR 8.7 (1.3-58.2), p=0.03]. The mortality rate was 14%, median time from admission to death was 15 days with the median duration to initial hospital discharge 33 (IQR 24 -52) days. Biochemical recovery was prolonged necessitating a follow up period of more than a year at an outpatient specialist clinic. 86% initiated protease inhibitor based ART successfully.

Conclusion
EFV DILI is a severe injury with significant inpatient mortality and morbidity requiring prolonged hospitalization and outpatient follow up.
Literature Review: Efavirenz Drug Induced Liver Injury

Introduction
Drug induced liver injury (DILI) is an important cause of acute, acute-on-chronic and, less commonly, chronic liver disease. It has an estimated incidence of 14 to 19 cases per 100,000 persons with jaundice occurring in 30% of cases.\textsuperscript{1,2} The diagnosis of DILI is dependent on establishing causality and excluding other causes of liver injury. There are three phenotypes of DILI which have distinct clinical profiles: direct (intrinsic), indirect and idiosyncratic hepatotoxicity.\textsuperscript{3}

Direct DILI is typically dose-related, predictable, reproducible in animal models and has a short latency period (hours to days) e.g paracetamol induced liver injury. Indirect DILI is caused by the action of the drug i.e induction of a new liver condition or an exacerbation of a preexisting condition, e.g. induction of immune-mediated hepatitis or worsening of hepatitis B (HBV) or C (HCV).\textsuperscript{3}

Idiosyncratic hepatotoxicity is caused by agents that have little or no intrinsic toxicity, is not dose related, is unpredictable and not reproducible in animal models with a variable latency period of days to weeks. It is rare, typically occurring after 1 in 2000 to 1 in 100,000 patient-exposures.\textsuperscript{3} Acute hepatocellular hepatitis is the most common manifestation of idiosyncratic drug induced liver injury and is marked by a latency period, which generally ranges from 5 to 90 days.\textsuperscript{3} These cases tend to be more severe and can be associated with a high mortality rate, in keeping with “Hy’s Law”, which states that jaundice and a hepatocellular injury from a drug carries a 10% mortality rate.\textsuperscript{4}

Liver disease in the context of human immunodeficiency virus (HIV) can result from a variety of aetiological factors. These include direct and indirect effects of the virus itself, opportunistic infections due to immunosuppression, immune reconstitution inflammatory syndrome (IRIS) as well medication used in the management of the disease and its complications. This results in varied biochemical and clinical presentations depending on the underlying causative pathophysiological mechanisms and patient comorbidities.\textsuperscript{5}

Non specific elevation of liver enzymes is a common complication of HIV treatment.\textsuperscript{6,7} DILI is a common cause of liver injury in HIV with frequent causative drugs including antiretrovirals (ARVs), anti-tuberculosis (TB) drugs as well as antimicrobials used in the treatment of, or prophylaxis against, various opportunistic infections. These include, amongst others, cotrimoxazole and fluconazole. DILI due to antiretroviral therapy (ART) ranges from asymptomatic elevation of liver enzymes to fulminant liver failure and death.\textsuperscript{8}

ART-induced liver failure was seen in approximately 10% of HIV patients, with life-threatening events appearing at a rate of 2.6 per 100 person years in retrospective analysis.\textsuperscript{5,9} Grade 3 and grade 4 elevations of liver enzymes occurred in between 1% and 14% of patients using combination ART.\textsuperscript{5,10} There are four major pathways of ART-associated hepatotoxicity. These include mitochondrial toxicity, hypersensitivity reactions, direct hepatotoxicity, and immune reconstitution in the presence of HBV or HCV.\textsuperscript{5}
Efavirenz (EFV) is an antiretroviral (ARV) drug that acts by noncompetitive binding to and inhibition of the HIV reverse transcriptase (RT), thereby inhibiting HIV viral replication. EFV belongs to the nonnucleoside reverse transcriptase inhibitor (NNRTI) class of ARVs together with nevirapine (NVP) which has a similar mechanism of action, but little to no structural similarity. EFV was first approved for use by the Food and Drug Administration (FDA) in the United States in 1998 and is currently used in combination with other antiretroviral agents in many first line ARV regimens, particularly in middle to low income countries, including South Africa. EFV is available generically in fixed dose combination (FDC) with emtricitabine (200 mg) and tenofovir (300 mg) under the brand names Atripla, Odimmune and Atroiza. The recommended dose of efavirenz in adults is 600 mg orally once daily.

The aim of this literature review was to explore the international and local data relating to EFV DILI. The focus was to extract data relating to clinical characteristics of the injury, associated morbidity and mortality, risk factors associated with the injury, predictors for poor outcomes, associated complications and possible molecular mechanisms involved. International trends and data were compared to limited local data available in sub-Saharan Africa. Relevant literature regarding the pharmacodynamics and pharmacogenetics of EFV particularly regarding predisposition to toxicity was also explored.

**Methods**

A literature search was performed using PubMed, Google Scholar and Medline databases using online resources provided by the University of Cape Town. Full-text articles were identified from 2002 to 2019. The terms used for the search included “efavirenz drug induced liver injury”, “efavirenz hepatotoxicity”, “non-nucleoside reverse transcriptase (NNRTI) hepatotoxicity”, “non-nucleoside reverse transcriptase (NNRTI) drug induced liver injury”, “antiretroviral (ARV) drug induced liver injury”, “antiretroviral therapy (ART) drug induced liver injury”, “antiretroviral (ARV) hepatotoxicity”, “antiretroviral therapy (ART) hepatotoxicity”, “efavirenz pharmacogenetics”, “efavirenz pharmacokinetics”, “safety and tolerability of efavirenz” and “efavirenz safety in pregnancy”. The articles were restricted to those written in English.

Articles were imported into EndNote Desktop version X9 and categorized. Citations and referencing to articles used in the literature review were inserted using the EndNote citation plugin in Microsoft Word for Mac 2018 version 16.19. A total of 82 articles were identified for inclusion in this literature review.
Efavirenz Drug Induced Liver Injury

Incidence
DILI is a well-recognized complication of ART.\textsuperscript{11} It is a complex clinical problem which often results in hospitalization and adverse patient outcomes.\textsuperscript{12} Various patterns of liver injury are associated with different classes of ART depending on several factors including the pathway of metabolism. DILI was found to be the most common finding on liver biopsy in a cohort of 301 HIV positive patients at a tertiary hospital in Cape Town. This was attributed to various classes of ART, Cotrimoxazole and anti-tuberculosis (TB) therapy.\textsuperscript{13}

Even though hepatotoxicity is well described within the NNRTIs class of ARVS, it is NVP as opposed to EFV that has the higher reported incidence of DILI. In a recent meta-analysis comparing EFV to NVP, patients receiving NVP were more likely to experience any grade of hepatotoxicity (OR 1.5, 95% CI 1.3 – 1.8) or severe hepatotoxicity (OR 3.3, 95% CI 2.5 – 4.2) compared to patients on EFV. This hypersensitivity reaction is marked by rash, eosinophilia and hepatitis; and typically occurs early within the first three months of taking NVP.\textsuperscript{14}

A South African study where first line ART included EFV found an overall rate of 7.7 episodes of severe hepatotoxicity per 100 person-years.\textsuperscript{15} Therefore, although drug hepatotoxicity with EFV is well recognized, it is thought to occur at a frequency far less than NVP.

Clinical features
While derangement of serum aminotransferases (above five time the upper limit of normal) occurs in 1 – 8% of patients on efavirenz, clinically apparent liver injury from EFV is uncommon.\textsuperscript{16} However, several cases of EFV DILI have been described in the literature.\textsuperscript{17-26}

The mechanism of EFV is postulated to be a hypersensitivity reaction, as the liver injury is thought to be immunoallergic in nature. It is described as occurring early in therapy (within 1 – 8 weeks) with recovery rapid upon cessation of therapy. Severity ranges from mild enzyme elevation to acute hepatocellular jaundice and fulminant liver failure and death.\textsuperscript{16}

Signs of hypersensitivity are less common than with nevirapine hepatotoxicity, but symptoms can include rash, fever, and eosinophilia.\textsuperscript{17,19} The biochemical pattern of liver injury is variable, typically cholestatic or mixed, but occasionally hepatocellular. Hepatocellular injuries tend to be more severe and often associated with submassive necrosis.

Histopathological features
In South Africa, however, 3 distinct clinicopathological patterns of Efavirenz-induced liver injury were described in 2016 in the largest cohort of patients (81) with EFV DILI.\textsuperscript{27} Histological patterns of EFV DILI are immuno-allergic in nature and in standardizing EFV DILI histological patterns of injury reporting, the following 3 patterns were noted:

**Submassive necrosis (SMN):** zonal/panzonal necrosis with inflammatory cell infiltrates composed of lymphocytes, plasma cells, and conspicuous eosinophils
Nonspecific hepatitis (NSH): portal and/or lobular inflammation particularly in zone 3 with/without cholate stasis in zone 1, with inflammatory cells including lymphocytes and eosinophils.

Mixed cholestatic hepatitis (MCH): combination of portal tract inflammation/interface hepatitis with inflammatory cells, including lymphocytes and eosinophils with marked zone 3 bilirubinostasis and a ductular reaction.

One histological pattern, submassive necrosis, was associated with significant morbidity and mortality. The biochemical, clinical and histological features of this novel injury indicated that this was a severe injury requiring prolonged hospitalization and predictors of risk included female gender, higher CD4 nadir at drug initiation and younger age. 27

Molecular Mechanisms of EFV DILI

The molecular mechanism responsible for the development of EFV DILI is largely poorly understood. Mitochondrial toxicity is known to be a potential mechanism of DILI. 28 In the context of ART, this was largely attributed to the nucleoside reverse transcriptase inhibitors (NRTIs) on the basis of inhibition of mitochondrial DNA polymerase gamma (Pol-γ), the enzyme responsible for mitochondrial DNA (mtDNA) replication. 29

NNRTIs also cause mitochondrial toxicity by a mechanism independent of mitochondrial DNA replication. 30 Recent work has provided insight into the possible molecular basis for EFV DILI and potential mechanisms of NNRTI mitochondrial toxicity, highlighting features of mitochondrial dysfunction in hepatocytes exposed to EFV.

In vitro, clinically relevant concentrations of EFV reduce hepatocyte cellular proliferation and viability in a concentration dependent manner through an acute mitotoxic effect. Mitochondrial function is affected through the induction of oxidative stress as well as an increase in mitochondrial mass. Hepatocyte apoptosis is triggered by the intrinsic pathway. These effects are partially reversed by antioxidant therapy, which is suggestive of a role of reactive oxygen species (ROS) generation. 31

Specifically, EFV compromises mitochondrial function by reducing mitochondrial respiration through direct inhibition of Complex 1. This leads to a decrease in ATP production and mitochondrial membrane potential, and an increase in ROS generation together with other metabolic dysfunction including lipid accumulation. 32

Furthermore, EFV has a significant effect on the expression of certain stress and toxicity genes in human hepatocytes. Up-regulation of Cytochrome P450, family 1, subfamily A, polypeptide 1 is indicative of oxidative stress. Increased expression of several genes related to oxidative stress and damage, including Methalothionein 2A, Heat shock 70 kDA protein 6, Growth differentiation factor 15 and DNA-damage-inducible transcript 3, also occurs. 33

Mitochondrial dysfunction is a major cause of autophagy, which is a mechanism of mitochondrial quality control and is generally a cytoprotective response. The process of autophagy initially promotes cell survival, however if a threshold is exceeded, autophagy overload or stress occurs. Clinically relevant concentrations of EFV induce
autophagy and in particular mitophagy i.e. the degradation of mitochondria by autophagy, in human hepatic cells.34

The understanding of the role of autophagy in liver pathophysiology, especially regarding DILI, is still limited. However, it is thought that autophagy promotes cell survival in response to liver injury including drug toxicity. In the context of EFV DILI, autophagy is thought to be a rescue mechanism that promotes hepatocyte survival.35

While moderate levels of EFV activate autophagy, higher concentrations exceed this threshold promoting “autophagic stress”. Pharmacological inhibition of autophagy worsens the toxic effects of EFV, highlighting the role of autophagy as a rescue mechanism enabling cell survival.36

The endoplasmic reticulum (ER) is also vulnerable to the effects of EFV. ER stress markers, including C/EBP-homologous protein and glucose-regulated protein 78 are up-regulated in hepatocytes at clinically relevant doses of EFV. EFV also increases cytosolic calcium content and induces morphological changes in the ER indicative of ER stress, which results in an “unfolded protein response”. This response is attenuated in cells with altered mitochondrial function.37

Hepatocytes that lack functional mitochondria (rho°) are less vulnerable to the effects of EFV. EFV-treated rho° cells display less mitochondrial toxicity such as superoxide production and altered mitochondrial mass compared to EFV-treated cells with functional mitochondria.38

However, the hypothesis that EFV hepatotoxicity is related only to mitochondrial toxicity does not match the histopathological findings found in these patients. These findings include a marked inflammatory cell infiltrate including lymphocytes, plasma cells and eosinophils as well as varying degrees of hepatocyte necrosis.27 This is indicative of an immunological mechanism as the predominant cause of EFV DILI as opposed to direct mitochondrial injury.

**Pharmacokinetics of EFV**

EFV is a potent NNRTI that noncompetitively inhibits the HIV-1 RT. It binds directly and reversibly to the catalytic site of the RT enzyme and interferes with viral RNA to DNA-directed polymerase activities. This renders RT unable to convert viral RNA into DNA.39

EFV is well-absorbed after oral administration and attains peak plasma concentrations (Cmax) after 3 to 5 hours. EFV has a long serum half-life ranging from 52 to 76 hours following single oral doses. Auto-induction results in a half-life of 45 hours following long term administration. This allows for once daily administration. Steady-state plasma concentrations are achieved between 6 to 10 days.40 Increases in Cmax and area under the plasma concentration-time curve (AUC) are dose proportional for 200, 400, and 600 mg EFV doses.

EFV is highly bound (>99%) to plasma albumin. It crosses the blood-brain barrier and adequate concentrations have been reported in the CSF.41 The pharmacokinetics (PK) of EFV has not been studied in renal insufficiency. However, the impact should be minimal as less than 1% of a dose is excreted unchanged in the urine. Safety of EFV in
patients with significant underlying liver disorders has not been established and caution is recommended in patients with mild to moderate liver disease. The use of EFV is contraindicated in patients with severe hepatic impairment.

EFV is metabolised by the cytochrome P450 (CYP)-enzyme system, primarily by the CYP3A4 and CYP2B6 isoenzymes to inactive hydroxylated metabolites which are subsequently glucuronidated. The oxidative inactive metabolites are excreted into bile and urine. In human liver microsomes, EFV undergoes primary oxidative hydroxylation to 8-hydroxy EFV (major) and 7-hydroxy EFV (minor) and secondary metabolism to 8,14-dihydroxy EFV. The contribution of N-glucuronidation to overall clearance of EFV, however, appears minimal. Hence, there are three hydroxylated EFV metabolites: 8-hydroxy-EFV, 8,14-dihydroxy-EFV and 7-hydroxy-EFV.

CYP2B6 is the most important enzyme in the metabolism of EFV to both the 8-hydroxy and 7-hydroxy metabolites. Other enzymes that metabolise EFV to a lesser extent are CYP3A4, CYP3A5, CYP2A6, CYP2C9 and uridine-glucuronyl-transferases (UGT). Metabolites are excreted in the bile and urine with <1% appearing as unchanged drug in the urine. CYP2B6 is the principal catalyst of EFV sequential hydroxylation, being involved in the formation of the secondary metabolite 8,14-dihydroxy-EFV. EFV can also be hydroxylated to 7-hydroxy-EFV by CYP2A6, a minor pathway of EFV metabolism accounting for approximately 20% of overall EFV metabolism in vitro.

**Pharmacogenetics of EFV**

Genetic polymorphisms of enzymes involved in the EFV metabolism pathway have been extensively investigated for association with PK parameters, clinical outcomes and adverse events, such as its well established central nervous system (CNS) toxicity. As CYP2B6 is responsible for most of EFV metabolism, the impact of its genetic polymorphisms on EFV toxicity, particularly neurological toxicity, is significant and has been extensively studied including in sub-Saharan African population groups. The CYP2B6 516G→T single nucleotide polymorphism (SNP) has been shown to predict for increased plasma efavirenz exposure. This CYP2B6 516TT genotype is more common in Africans and African Americans than in Caucasians. Additional polymorphisms CYP2B6 983T→C and CYP2B6 15582C→T also predict increased plasma efavirenz exposure, however these are less common than the CYP2B6 516TT genotype in African population groups. The CYP2B6 983C allele is found almost exclusively with African ancestry. Polymorphisms in other genes of enzymes apart from CYP2B6, including CYP2A6 genes have also been infrequently reported to be associated with efavirenz concentrations. Polymorphisms in CYP2B6 that predict higher efavirenz plasma concentrations predispose to efavirenz-mediated neurotoxicity and patients with CYP2B6 slow-metabolizer genotypes also have higher CSF efavirenz exposure. In vitro studies have implicated efavirenz and especially its metabolite 8-hydroxy-efavirenz in neuronal toxicity.

Although a clear association with CYP2B6 genetic polymorphisms and EFV induced neurotoxicity exists, the same cannot be said of cases of EFV hepatotoxicity. Apart from an isolated case report of suspected EFV DILI (with corresponding histopathology) in a patient with CYP2B6 516G→T genotype, CYP2B6 polymorphisms have not been shown to be a definitive risk factor for the development of EFV DILI.
Side effect profile of EFV
One of the most common side effects of EFV is neurotoxicity. These CNS side effects include insomnia, abnormal dreams, impaired concentration, amnesia, and hallucinations. These symptoms typically occur early in the course of therapy and can resolve spontaneously within a month despite continued use. However, some patients will have to discontinue the drug due to persistent symptoms. CNS toxicity has been reported more frequently in adult and paediatric patients with high EFV trough plasma concentrations >4 mcg/ml and as described relates to the “slow metabolizer” CYP2B6 genotype common in sub-Saharan Africa. Other CNS neuropsychiatric effects include mood disorders, psychosis and suicidality. A late manifestation of EFV induced ataxia and encephalopathy has also been described. Skin rashes, in the form of maculopapular eruptions, have also been described. This occurs more commonly in children and typically presents within the first two weeks after EFV initiation. Resolution is typical within one month of continued EFV use.

EFV use in the South African context
Sub-Saharan Africa has the highest prevalence of HIV/AIDS in the world with an estimated overall HIV prevalence rate of approximately 12.6% among the South African population. In 2016, there were 7.1 million people living with HIV, among whom 56% were accessing antiretroviral therapy (ART). Among pregnant women living with HIV, more than 95% were accessing treatment to prevent mother-to-child transmission (MTCT).

EFV has formed part of the South African national first line ART since 2004. Subsequently with the introduction of FDC in 2015, EFV became the favored first line NNRTI when significant governmental policy and treatment regimen changes occurred to facilitate this change. Data from various case reports indicated an association of first trimester efavirenz exposure with central nervous system congenital anomalies. This resulted in a recommendation by the United States FDA in 2005 to avoid using efavirenz-based regimens in the first trimester of pregnancy due to concern for teratogenicity when EFV was changed from category C to category D. Birth defects included neural tube defects such as meningomyelocele and anophthalmia.

However, subsequent systematic reviews found no evidence of increased risk of congenital anomalies associated with first-trimester exposure to efavirenz. As a result, in 2012, World Health Organisation (WHO) recommended that efavirenz can be included as part of preferred first-line therapy in pregnant women and women of childbearing age. Importantly, all HIV positive pregnant women were prioritized for immediate ART initiation with EFV as first line therapy as the WHO had deemed it safe for use in pregnancy despite initial concerns.

As a result, all HIV positive pregnant or breastfeeding women are currently commenced on lifelong ART with EFV as part of the first-line regimen regardless of CD4 count or gestation of the pregnancy.

In general, it is unclear if pregnancy independently increases the risk of developing ART hepatotoxicity. In particular, EFV pharmacokinetics does not appear to be altered in a clinically significant way. It is well known that the pharmacokinetics of many drugs are affected by physiological changes induced by pregnancy. For EFV, it has been shown that this is further affected by the pharmacogenetic profiles of the
population studied. In a Nigerian study, the combined effects of pregnancy and CYP2B6 polymorphisms showed significant differences in EFV pharmacokinetics based on CYP2B6 516G>T genotypes. The most significant differences were seen in patients with the CYP2B6 516GG genotype with the EFV Clearance (Cl/F) increasing by 100% (P = 0.0013) and the minimum concentration (Cmin) was reduced by 61.6% (P=0.0027) during pregnancy compared with postpartum.75

Since 2014 the South African national ARV guidelines incrementally increased the initiating CD4 count first from 200 to 350, then 500, until the current “test and treat” strategy that recommends initiating ARV therapy irrespective of CD4 count.76 This was based on the findings of two pivotal randomized controlled trials (RCTs) published in 2015: strategic timing of antiretroviral therapy (START) and TEMPRANO ANRS 12136 (early antiretroviral treatment and early isoniazid prophylaxis against tuberculosis in HIV-infected adults) that showed significant individual clinical benefit from commencing ART immediately in patients with CD4 counts higher than 500 cells/ul.77,78

The WHO guidelines prior to the recent recommendation of Dolutegravir (DTG) in combination with a nucleoside reverse-transcriptase inhibitor (NRTI) backbone as the preferred first-line ART regimen,79 recommended efavirenz-based first-line ART, with dosing options of 600 mg or 400 mg available.80 EFV 600 mg has been the preferred dose in first-line ART as 400 mg was not available in FDCs. However, the ENCORE1 study demonstrated that 400mg is non-inferior to 600mg dose of EFV at both 48 weeks and 96 weeks in terms of virological suppression and immune reconstitution. Importantly, the 400mg arm had 10% less adverse events, including hepatotoxicity, compared to the 600mg arm. However, this randomized controlled trial was designed mainly to address potential cost saving measures that a lower dose would achieve. Importantly, it excluded all pregnant women and patients who were taking TB treatment concurrently with EFV-containing ART.81

These two exclusions have proven important in the South African context where TB prevalence is high. The HIV Clinicians Society of South Africa’s response to the ENCORE1 study has been to highlight the potential issues with drug-drug interactions between Rifampicin (RIF) and/or Isoniazid (INH) with EFV, as well as the concern regarding adequate dosing of EFV in pregnancy. Since no studies have been done using the reduced dose in pregnant women or patients on rifampicin-based TB treatment, guidelines had not recommended the routine use of the lower 400mg dose in first-line ART.82

This recommendation was further supported by the outcome of a systematic review of the pharmacokinetics (PK) of efavirenz in patients on anti-TB therapy. When EFV was used at 600mg dosing, RIF and INH had a negligible effect on EFV plasma concentrations. However, recent studies using EFV at 400 mg dosage have also shown similar provisional results. Although this may indicate that using a lower EFV dose is safe with the coadministration of anti-TB therapy, the full report is yet to be published and further studies particularly in high TB/HIV burden countries are warranted.83

Conclusion
Hepatotoxicity caused by EFV is well documented post initial drug marketing. The clinical features are quite varied, with some case reports of hypersensitivity responses
similar to NVP, and others a more delayed presentation of an idiosyncratic drug reaction.

While molecular mechanisms have been postulated suggesting direct mitochondrial toxicity and autophagy as the predominant mechanism of injury, these in vitro studies are not in keeping with clinical or histopathological features described in cases of known EFV DILI.

The safety of EFV for use in patients with chronic liver disease has not been established, but it is contraindicated in patients with severe hepatic impairment. Risk factors for developing the injury in the sub-Saharan African population include female gender, high CD4 count nadir at drug initiation and younger age. While CYP2B6 polymorphisms are well established as causative in the aetiology of the neuropsychiatric side effects of EFV, this is yet to be established as causal in EFV DILI. Apart from isolated case reports, there are no cohort studies or case series that detail the full natural history of the injury. Although steroid therapy is advised for the immunoallergic phenotype of injury, the exact duration of therapy is not well-described.

Thus, information regarding the natural history of EFV DILI in a prospective cohort study would provide new insight into this injury and provide important clinical information regarding time to clinical and biochemical injury, associated morbidity and mortality and could possibly identify further risk factors for this injury. This is important as EFV is likely to still be used particularly in women of reproductive age, despite the expected introduction of dolutegravir as first line therapy in the national ART program due to the concern around teratogenicity of dolutegravir.
References


The Natural History of Efavirenz Drug Induced Liver Injury

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Abstract

Background
Efavirenz (EFV), a non-nucleoside reverse transcriptase inhibitor (NNRTI), has been a component of first line antiretroviral treatment in the South African HIV/AIDS programme since 2004. Similarly, it is extensively used in ART programmes in other low and middle incomes countries. The natural history of the previously reported EFV drug induced liver injury (DILI), is unknown.

Objectives
To establish causality assessment for the drug-induced liver injury and elucidate the natural history of EFV DILI by observing a cohort of patients through documenting all the factors influencing the patterns of clinical and histological injury, the time to clinical and biochemical recovery, the associated mortality rate and to establish if any demographic or clinical factors predict poor outcomes.

Methods
Patients were prospectively included after establishing causality criteria for EFV DILI. Clinical, demographic and histological features were carefully documented from the time of presentation and through follow up. Prednisone at 0.25-0.5mg/kg was initiated at the discretion of the treating hepatologist. Risk factors for severe injury or death and time to event (full clinical recovery and full biochemical recovery) were analyzed.

Results
50 patients were included in the analysis, median age 34 (IQR 29-39) years, men significantly older than women, \( p=0.014 \). Most (92%) were female gender, and of black African ethnicity (86%). The median duration on ART at time of presentation was 6.5 months with half of the women initiating ART during pregnancy at a median gestation of 24 weeks (IQR 11 – 36). Median CD4 nadir at ART treatment initiation was 517 cells/mm\(^3\) with no significant difference (\( p=\text{NS} \)) in CD4 nadir in those pregnant or not. Median RUCAM score was 7 and of the 66% of patients who had liver biopsies, 3 histological patterns were identified: submassive necrosis (57.5%), non-specific hepatitis (36%) and mixed cholestatic hepatitis (6%). Multivariate analysis suggested predictors for the development of submassive necrosis included age >30 years [\( \text{OR} 0.86 (0.15-0.97), p=0.02 \)], pregnancy [\( \text{OR} 6.9 (1.34 – 35.6), p=0.02 \)]; CD4 >350 [\( \text{OR}, 7.1 (1.5-31.9), p=0.02 \) but not alcohol [\( \text{OR} 1.17 (0.72-1.18), p=0.07 \).

For the non-specific hepatitis group, only pregnancy predicted [\( \text{OR} 8.7 (1.3-58.2), p=0.03 \)]. The mortality rate was 14%, median time from admission to death was 15 days with the median duration to initial hospital discharge 33 (IQR 24-52) days. Biochemical recovery was prolonged necessitating a follow up period of more than a year at an outpatient specialist clinic. 86% initiated protease inhibitor based ART successfully.

Conclusion
EFV DILI is a severe injury with significant inpatient mortality and morbidity requiring prolonged hospitalization and outpatient follow up.
**Introduction**

Sub-Saharan Africa carries a considerable HIV/AIDS burden, the highest in the world. South Africa, an epicentre for HIV/AIDS, has an estimated adult HIV prevalence of approximately 12.6%. In 2016, an estimated 7.1 million South Africans were living with HIV, of whom 56% were accessing antiretroviral therapy (ART). More than 95% of HIV positive pregnant women were accessing treatment to prevent mother-to-child transmission (MTCT) of HIV.

Efavirenz (EFV), a non-nucleoside reverse transcriptase inhibitor (NNRTI), has formed part of the South African national first line ART since 2004. Subsequently with the introduction of a fixed dose combination (FDC) formulation with tenofovir and emtricitabine, EFV became the favored first line NNRTI in 2015. A change in South African government policy necessitated the treatment program change. Importantly, HIV positive pregnant women were prioritized for immediate ART initiation with EFV based first line therapy when the World Health Organization (WHO) deemed it safe for use in pregnancy after initial concerns of EFV use in pregnancy. As a result, all HIV positive pregnant or breastfeeding women are currently commenced on lifelong ART with EFV as part of the first-line regimen regardless of CD4 count or gestation of the pregnancy in many programmes, especially in low and middle income countries.

Since 2014, the South African national ARV guidelines incrementally raised the initiating CD4 count: first from 200 to 350, then to 500, eventually to the current “test and treat” strategy that recommends initiating ARV therapy irrespective of CD4 count. This was based on the findings of two pivotal 2015 randomized controlled trials: the strategic timing of antiretroviral therapy (START) and TEMPRANO ANRS 12136 (early antiretroviral treatment and early isoniazid prophylaxis against tuberculosis in HIV-infected adults) studies that demonstrated significant individual clinical benefit from commencing ART immediately in patients with CD4 counts higher than 500 cells/mm³.

Several known side effects of EFV are well documented, most notably the neuropsychiatric adverse effects including insomnia, depression and the more recently recognized manifestation of EFV encephalopathy. These side effects are thought to be dose-related and can be attributed to the higher prevalence of the so-called “slow metabolizer” phenotypes where cytochrome P450 2B6 (CYP2B6) genotypic polymorphism combinations result in elevated plasma EFV concentrations in HIV-infected African black adults and children consequent to slower EFV metabolism.

The WHO guidelines until recently have recommended efavirenz-based first-line ART, with dosing options of 600 mg or 400 mg available. EFV 600 mg has been the preferred dose in first-line ART as 400 mg is not currently available in FDCs. However, EFV 400 mg demonstrated non-inferior efficacy with improved tolerability in ENCORE 1. Given that no studies have been done using the reduced dose in pregnant women or patients on rifampicin-based Tuberculosis (TB) treatment, guidelines to date have not recommended the routine use of the lower 400mg dose in first-line ART.

Even though hepatotoxicity is well described within the non-nucleoside reverse transcriptase inhibitors class of antiretrovirals (ARVS), it is nevirapine (NVP) as opposed to EFV that has the higher reported incidence of drug induced liver injury (DILI). This well described hypersensitivity reaction is marked by rash, eosinophilia.
and hepatitis (a DRESS phenomenon) and typically occurs early within the first three months of taking NVP.\textsuperscript{13}

Drug hepatotoxicity with EFV was well recognized but occurred at a frequency far less than NVP. Furthermore, there were no particular characteristics ascribed to EFV hepatotoxicity. Although well recognised, the liver injury is not well described in particular with regards to natural history. In South Africa, however, 3 distinct clinicopathological patterns of Efavirenz-induced liver injury were described in 2016. One histological pattern in particular, submassive necrosis, was associated with significant morbidity and mortality. The biochemical, clinical and histological features of this novel injury indicated that this was a severe injury requiring prolonged hospitalization and predictors of risk included female gender, higher CD4 nadir at drug initiation and younger age.\textsuperscript{14}

Very few data exists in the literature with respect to EFV DILI. A few case reports describe strong causality with respect to suspected EFV DILI with little documented with respect to the natural history and clinical manifestations of the injury.\textsuperscript{15-18}

We elected to prospectively document the natural history of this novel injury noting all clinical and histological characteristics, management and clinical outcomes in terms of its morbidity and mortality.

Methods

We conducted an observational, prospective cohort study of patients presenting to the Division of Hepatology/Liver Clinic at Groote Schuur Hospital and identified as meeting causality criteria for EFV DILI from October 2014 until July 2016. Patients were included if they met strict causality assessment utilizing the RUCAM (Roussel Uclaf Causality Assessment Method) causality tool as the standard.\textsuperscript{19} The RUCAM scale involves a scoring system that categorizes the suspicion of a DILI into "definite or highly probable" (score >8), "probable" (score 6-8), "possible" (score 3-5), "unlikely" (score 1-2) and "excluded" (score \leq 0). Ethics approval was obtained from the Faculty of Health Sciences Human Research Ethics Committee at the University of Cape Town (HREC REF 593/2018).

The identified causality criteria for EFV DILI included: a temporal relationship to drug exposure and clinical disease, excluding acute viral hepatitis (hepatitis A, B, C and E); negative autoantibodies/elevated total immunoglobulin G and histology not compatible with autoimmune hepatitis (AIH); radiological exclusion of biliary and vascular obstruction; exclusion of alcohol/herbal toxins; observing the effects of drug de-challenge and a histological injury pattern compatible with a DILI. For viral hepatitis, hepatitis E was assessed by means of an in-house hepatitis E PCR test rather than serology, given the low sensitivity and specificity of many commercial kits.\textsuperscript{20} Hepatitis A IgM, hepatitis B surface antigen and core IgM and hepatitis C antibody (Abbot ARCHITECT, Chicago, Illinois, USA), were screened to exclude acute hepatitis A, B and C.

Patients were prospectively identified and once confirming causality for EFV DILI, relevant clinical and demographic data were collected on admission at the time of enrollment if informed consent was obtained. At the discretion of the treating hepatologist, with sepsis excluded, patients were initiated on oral prednisone at 0.25 –
0.5 mg/kg dose - starting at a low dose and titrating according to response. Once biochemical normality was achieved, progressive weaning of steroids occurred until complete discontinuation, at which time patients were discharged from the clinic.

Patients’ clinical course and time to clinical and biochemical recovery was documented up to at least 12 months after initial presentation. Outpatient follow up occurred at the Liver Clinic, Groote Schuur Hospital. All relevant biochemical data was documented. Clinical recovery was defined as clinically stable for discharge and follow-up as an outpatient. Biochemical recovery was defined as a return of liver profile to normal or to the patient’s baseline before or at start of Efavirenz-based ART. Cumulative dose of immunosuppressive therapy was extracted from the JAC Medicines Management System. The JAC system is utilized at over thirty hospital sites in the Western Cape and is South Africa’s first regional-scale deployment of an interconnected hospital pharmacy system that manages the hospital’s services electronically.

As part of standard of care in assessing causality and requirement for corticosteroid therapy (presence of immune-allergic pattern of injury with inflammation), liver biopsies (including transjugular biopsies) were performed except where severe or uncorrectable coagulopathy precluded a safe procedure. Histological patterns of EFV DILI are immuno-allergic in nature and in standardizing EFV DILI histological patterns of injury reporting, the following 3 patterns were noted:

1. **Submassive necrosis (SMN):** zonal/panzonal necrosis with inflammatory cell infiltrates composed of lymphocytes, plasma cells, and conspicuous eosinophils.
2. **Nonspecific hepatitis (NSH):** portal and/or lobular inflammation particularly in zone 3 with/without cholate stasis in zone 1, with inflammatory cells including lymphocytes and eosinophils.
3. **Mixed cholestatic hepatitis (MCH):** combination of portal tract inflammation/interface hepatitis with inflammatory cells, including lymphocytes and eosinophils with marked zone 3 bilirubinostasis and a ductular reaction.

All 3 histological patterns of injury had eosinophils as part of the inflammatory infiltrate.

### Statistical analysis

Statistical analyses were performed using STATA statistical software, version 11.0. Demographic and clinical characteristics were summarized with descriptive statistics. Mean ± standard deviation was used for normally distributed data and median with interquartile ranges for non-parametric data. Continuous variables were compared using either student t-test (parametric) or Wilcoxon rank-sum test (non-parametric). Categorical variables were presented as frequencies and percentages and compared using Pearson’s X² or Fisher’s exact test as appropriate. For laboratory data that included more than two groups, the Kruskal-Wallis was utilized for non-parametric data. Categorical variables were presented as frequencies and percentages and compared using Pearson’s X² or Fisher’s exact test as appropriate.

Multivariate analysis was performed on risk factors found significant on univariate analysis to identify factors predictive of various patterns of liver injury. Time to event (full clinical recovery and full biochemical recovery) were analyzed at 3, 6, 9 and 12 months using the Kaplan-Meier method. Factors associated with time to event were
analyzed using Cox Proportional Hazards regression. All regression estimates were presented with 95% confidence intervals (CI). All P-values considered significant at p≤ 0.05.

**Results**

50 patients who met causality criteria for EFV DILI were prospectively included in the analysis and follow up. The baseline demographic characteristics of patients are listed in **Table 1**. Notably, most patients (92%) were female gender, and of black African ethnicity (86%). Median age was 34 (IQR 29-39) years, men significantly older than women, p=0.014, although notably the n-value for men was small. Median BMI was 25.4 (IQR 22.7-30.5) kg/m², ranging from normal to overweight BMI.

The median duration on ART at time of presentation was 6.5 months, but the range was wide (IQR 5 -10 months), highlighting the long latency period to presentation with this drug injury. Half of the women initiated ART during pregnancy at a median gestation of 24 weeks (IQR 11 – 36). Patients where ART was initiated in pregnancy presented a median 3 (IQR 2-6) months after delivery. Notably, the median CD4 nadir at ART treatment initiation was 517 cells/mm³ with no significant difference (p=NS) in CD4 nadir in those pregnant or not.

With reported alcohol use, 28% drank alcohol although this typically did not exceed more than 4 units of alcohol per week.

Of importance, autoimmune serology was positive in 9 patients, with one patient anti-nuclear antibody and 8 anti-smooth muscle antibody positive. The titers were all very low at ≤1:100. Significantly, liver biopsies were not compatible with an autoimmune hepatitis pattern of injury.

Two patients were coinfected with hepatitis B, both HBe-antigen negative and an undetectable hepatitis B DNA viral load at presentation. Hepatitis C antibody was detected in no patients.

For causality, the median RUCAM score was 7 – placing it in the probable category – with a RUCAM range of 6 – 9. Of the 50 patients, 6 (12%) had received isoniazid preventive therapy, with 1 patient previously on cotrimoxazole prophylaxis. The clinical course, histological findings and temporal relationship with drug use and clinical onset, were not compatible with isoniazid nor cotrimoxazole induced liver-injuries. Given the severity of the injury, EFV was not rechallenged on any patients. Thus, the RUCAM score did not reflect the three potential additional points from a failed EFV rechallenge.

Of the 66% (n=33) of patients who had liver biopsies, 3 histological patterns were identified: submassive necrosis [n=19 (57.5%)], non-specific hepatitis [n =12 (36%)]) and mixed cholestatic hepatitis [n=2 (6%)]. All 3 of these patterns were associated with grade 4 ALT elevation (using the Modified AIDS Clinical Trial Group grading).21

The most severe injury, clinically, biochemically, and histologically, was submassive necrosis. At presentation (**Table 3**), jaundice was significantly greater with this pattern of injury compared to the non-specific hepatitis or mixed cholestatic hepatitis pattern respectively; total bilirubin 272; 93; 87 umol/l, respectively, p = 0.012. This occurred
in the absence of a significant difference in ALT presentation in the 3 patterns, 713; 927; 510 U/L, respectively, p=NS. Similarly, liver synthetic dysfunction at presentation, as measured by the international normalized ratio was significantly greater in the submassive necrosis group compared with the other 2 patterns of injury groups (1.72 vs. 1.19; p <0.0001)

On multivariate analysis, predictors for the development of submassive necrosis included age >30 years [OR 0.86 (0.15-0.97), p=0.02], pregnancy [OR 6.9 (1.34 – 35.6), p=0.02]; CD4 >350 [OR, 7.1 (1.5-31.9), p=0.02] but not alcohol use [OR 1.17 (0.72-1.18); p=0.07]. For the non-specific hepatitis group, only pregnancy predicted [OR 8.7 (1.3-58.2), p=0.03].

42 patients were treated with steroids. The median duration of prednisone was 11 (IQR 8-16) months with a cumulative prednisone dose of 3198 (IQR 1 898 – 4 670) milligrams. Ten patients had episodes of in-hospital sepsis, 3 were culture positive and 7 were culture negative where a presumptive diagnosis based on clinical criteria was made. Steroid-induced diabetes occurred in 5 (10%) patients. During follow up, 86% (37/43) were restarted successfully on protease inhibitor based ART and 70% (30/43) were virologically suppressed at last visit.

No patients met clinical criteria for tuberculous immune reconstitution inflammatory syndrome (TB IRIS). Two patients were diagnosed with EFV encephalopathy concurrently with their presentation with drug-induced liver injury. Both had EFV levels >20mg/L (therapeutic range 1–4 mg/L). Despite the immune-allergic nature of the liver histological pattern, no patients had any skin rash associated with EFV drug induced liver injury.

The mortality rate was 14%, all deaths occurring in the first 15 days after presentation. The median duration of admission or time to initial discharge from hospital was 33 (IQR 24 -52) days (Table 2). The median time to biochemical recovery (discharge from liver clinic) was 574 (IQR 239 – 728) days. Graphs 1a, 1b and 1c denote the trend in recovery of parameters of the liver profile. On univariate and multivariate analysis, there were no significant predictors for death. The Kaplan-Meier probability of death within 30 days was 10%. All deaths occurred in the patients who were not biopsied where coagulopathy precluded a safe procedure reflecting the severity of the liver injury.

There were also no significant predictors for time to full biochemical recovery on both univariate and multivariate analysis.

**Discussion**

We report the first study documenting the natural history of EFV drug induced liver injury. Several important features have emerged that allow for better insight into the clinical understanding and management of patients. The consistently long latency period of 6 months from initiation of the efavirenz based ART to presentation, complicates clear guidance on surveillance of at risk patients. Furthermore, phenotypically, unlike nevirapine, there were no features of a hypersensitivity or DRESS type syndrome observed with the liver injury. This compounds the difficulty in the detection of this drug injury. Jaundice is a universal presenting feature and its
presence should prompt clinicians to immediately terminate therapy until confirmation of the DILI.

Previous data suggested several predictors for the submassive necrosis pattern of injury, including female gender, CD4 count and younger age. Our study confirmed the finding of younger age and a higher baseline CD4 count. The predominance of female gender in this prospective cohort, is however intriguing. Female gender has long been suggested as a risk factor for DILI. However, this was questioned in a study from Iceland where female gender was not shown to be a risk factor for the development of DILI. This further emphasises the need for local studies addressing the risk factors for DILI. In our study, the association with initiating the drug in pregnancy, was another predictive factor, with almost half of the patients starting ART in pregnancy. These two parameters, gender and pregnancy are compelling, but we are unable to establish for bias given that HIV screening and treatment initiation is universal as part of antenatal care. Hence, pregnant women are routinely screened and immediately linked to ART if HIV positive, irrespective of CD4 count. Further studies are required to understand the significance of the association of EFV DILI and pregnancy as it may have important programmatic implications for ARV programs using EFV as 1st line therapy in at risk populations. This may be a factor to be considered in recommending DILI surveillance in pregnant and postpartum women initiated on EFV in pregnancy, and whether EFV is even the best agent to be initiated in pregnancy.

Although male patients were small in number, their CD4 count at ART initiation was significantly lower than females, in keeping with known HIV/AIDS epidemiology. At lower CD4 counts, males developed the mixed cholestatic-hepatitic pattern of injury. This is understandable given our finding that a high baseline CD4 count is a clear risk factor for the submassive necrosis pattern of injury.

The morbidity and mortality with EFV DILI is substantial, characterized by the need for a prolonged hospital admission (median 38 days) and requiring a follow up period of more a year at an outpatient specialist clinic.

The mortality rate of 14% is significant and is in keeping with “Hy’s Law”, stating that jaundice and a hepatocellular injury from a drug results in at least a 10% mortality rate. Death occurred within the first 2 weeks of admission in all patients with most of these deaths occurring early. These patients had an uncorrectable coagulopathy that precluded biopsy but very likely had submassive necrosis as their pattern of injury.

The identification of this novel liver injury in 2016 did not lead to any significant programmatic changes in South Africa’s national ART guidelines. However, increased awareness of the injury was important in order to identify patients earlier and stop EFV appropriately. To this end, the Health Department acted to educate clinicians about the novel aspects of EFV DILI and the importance of improved pharmacovigilance. Likewise the regulator, the South African Health Products Regulatory Authority (SAHPRA), amended package inserts for efavirenz containing ART.

As a consequence of the marked mixed inflammatory cell immune-allergic pattern of injury on liver biopsy, initiation of 0.25mg – 0.5mg/kg of prednisone was standard of
care, unless a contraindication precluded its immediate use. Weaning of corticosteroids with monitoring of liver enzymes commenced between 3-6 months and in most patients was completed at 12 months, after initiation of new protease inhibitor based ART.

Although positive autoimmune serology was present, albeit at low titres, in nine patients, the clinico-pathological correlation for autoimmune hepatitis was weak. Firstly, anti-smooth muscle antibodies can be non-specifically detectable in the setting of significant liver necrosis, as in submassive necrosis. Secondly, the histological findings were highly compatible with DILI and not autoimmune hepatitis including an inflammatory cell infiltrate composed of mostly lymphocytes and eosinophils and scanty plasma cells. Lastly, patients were all successfully weaned off steroid therapy in a relatively short space of time, an unlikely occurrence in autoimmune hepatitis. Biochemical stability was maintained off immunosuppression and the majority of patients were successfully rechallenged on a protease inhibitor (PI) based regimen.

Further understanding of this liver injury, particularly the mechanisms at play in its aetiology, is crucial to identifying at-risk patients. These mechanisms are likely multifactorial including genetic, possibly related to CYP2B6 polymorphisms, as well as immunological, given the higher CD4 counts in patients with the most aggressive histological and clinical patterns of injury. Exactly what these factors are, almost certainly related to both HLA and non-HLA haplotype polymorphisms or variants, warrant further study. Also, with the number of patients accessing ART in a population with an identifiable genetic predisposition to the “slow metaboliser” phenotype of CYP2B6 polymorphisms, genetic studies are necessary to potentially identify tests that could easily identify at-risk patients for EFV DILI in sub-Saharan Africa.

In 2017, the Health Department reported that the integrase inhibitor, dolutegravir, was to replace efavirenz as part of first-line ART. The newly formulated fixed dose combination with an integrase inhibitor promised not only a better tolerated first line regimen but also significant cost savings for the national ART program. The benefits of dolutegravir include not only significant cost-savings, but also proven efficacy in the treatment of HIV, fewer treatment discontinuations due to adverse events and a high barrier to the development of resistance. However, subsequent concerns regarding reported teratogenicity of dolutegravir, specifically neural tube defects, has delayed this important programmatic change which is now planned for 2020. The WHO’s response to date has been to revert to their 2016 guidelines which recommend an efavirenz-based regimen for women who intend becoming pregnant or are not using effective contraception, to avert the potential period of risk for the development of neural tube defects. SAHPRA have also issued recommendations on the use of dolutegravir among women of reproductive age. It is thus likely that future national guidelines may advise against the use of dolutegravir in pregnancy, meaning EFV will remain the ARV of choice in pregnancy.

Considering this, it remains important to further establish and define risk factors for this injury and continue to advocate for improved pharmacovigilance particularly for high risk groups especially pregnant women. It is important to note that the hepatotoxicity of EFV was not initially appreciated at the time of initial registration of the drug in the late 1990’s. This emphasises the need for continued drug surveillance post drug marketing, especially in population groups not included in the initial drug trials. This
phenomenon is not unique to efavirenz, but holds true for many newer therapies, seldom trialed in low and middle income countries in the initial registration studies.

The cohort size is relatively small and can be considered a study limitation, however for a DILI, the numbers are significant. The cohort also represents a single centre study, although it is a regional and supraregional referral centre. The study was not significantly powered to be able to identify any significant predictors for death or prolonged time to recovery. However, given the fact that the natural history of EFV DILI has never been described, it provides important insight into the severity of the liver injury both in morbidity and mortality.

**Conclusion**

EFV DILI is a novel and severe injury associated with both significant morbidity and mortality. Hospital admission and outpatient follow up is prolonged with need for adjunctive steroid therapy. CD4 counts >350 cells/mm³, younger age and ART initiated in pregnancy were predictive for development of the severe submassive necrosis histological pattern of injury. EFV is likely to still be used particularly in women of reproductive age, despite the expected introduction of dolutegravir as first line therapy in the national ART program. Furthermore, the drug will likely remain in use in other low and middle income countries for the foreseeable future. It is therefore vitally important to further elucidate the mechanisms underlying the injury as well as improve pharmacovigilance particularly in pregnant women on ART.
References


   http://dx.doi.org/10.4102/sajhivmed.v18i1.776.

6. INSIGHT START Study Group, Lundgren JD, Babiker AG, et al. Initiation of antiretroviral therapy in early asymptomatic HIV infection.  


10. World Health Organization Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection June 2016,  


Table 1: Baseline Demographic Data

<table>
<thead>
<tr>
<th>Parameters</th>
<th>n = 50</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>34 [29-39]</td>
<td>p=0.014</td>
</tr>
<tr>
<td>Male</td>
<td>34 [29-38]</td>
<td></td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>43 [39-49]</td>
<td></td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black African</td>
<td>43 (86%)</td>
<td></td>
</tr>
<tr>
<td>Mixed Ancestry</td>
<td>7 (14%)</td>
<td></td>
</tr>
<tr>
<td><strong>Body Mass Index (kg/m²)</strong></td>
<td>25 [22-33]</td>
<td></td>
</tr>
<tr>
<td><strong>ARVS initiated in pregnancy</strong></td>
<td>23 (46%)</td>
<td></td>
</tr>
<tr>
<td>Months Post-Partum at Presentation</td>
<td>3 [2-6]</td>
<td></td>
</tr>
<tr>
<td><strong>CD4 (cells/mm³)</strong> ^</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnant (n = 23)</td>
<td>517 [348-722]</td>
<td>p = NS</td>
</tr>
<tr>
<td>Non pregnant (n = 27)§</td>
<td>553 [424-792]</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>424 [301-658]</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>539 [354-780]</td>
<td></td>
</tr>
<tr>
<td><strong>Duration on ART (months):</strong></td>
<td>6 [5-10]</td>
<td></td>
</tr>
<tr>
<td><strong>Alcohol consumption</strong> # n (%)</td>
<td>14 (28%)</td>
<td></td>
</tr>
<tr>
<td><strong>HBsAg Positive n (%)</strong></td>
<td>2 (4%)</td>
<td></td>
</tr>
<tr>
<td>HBV core IgM Positive</td>
<td>0/2</td>
<td></td>
</tr>
<tr>
<td>HBV Surface Antigen Positive</td>
<td>2/2</td>
<td></td>
</tr>
<tr>
<td>HBV Viral Load &lt;20 IU/ml</td>
<td>2/2</td>
<td></td>
</tr>
<tr>
<td><strong>HCV Antibody Positive n (%)</strong></td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td><strong>HEV IgM Antibody Positive n (%)</strong></td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>HEV PCR Positive</td>
<td>0/1</td>
<td></td>
</tr>
<tr>
<td><strong>Autoimmune serology</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antinuclear antibody positive n (%)</td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Anti-smooth muscle antibody positive n (%)</td>
<td>8 (16%)</td>
<td></td>
</tr>
<tr>
<td>Titre 1:100</td>
<td>8 (16%)</td>
<td></td>
</tr>
<tr>
<td>Anti-liver kidney microsomal type 1 antibody</td>
<td></td>
<td></td>
</tr>
<tr>
<td>positive n (%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>Medication use n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------------------------------------</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| Cotrimoxazole (Bactrim)             | 1 (2%)  
| Isoniazid (INH) prophylaxis         | 6 (12%)  
| Anti-TB drugs (Rifafour)            | 3 (6%)  
| Fluconazole                         | 0 (0%)  
| Herbal/Traditional                  | 3 (6%)  
| Other                               | 1 (2%)  

*Data expressed as median and interquartile range [IQR]

# Defined as consuming any alcohol >1 unit per week

$ Included 4 males

^ CD4 nadir

**Abbreviations:** ARVS, antiretrovirals; ART, antiretroviral therapy; HBsAg, Hepatitis B surface antigen; HBV, Hepatitis B; HCV, Hepatitis C; HEV, Hepatitis E
### Table 2: Clinical features & Outcome

<table>
<thead>
<tr>
<th>Clinical features:</th>
<th>n = 50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jaundice</td>
<td>47 (94%)</td>
</tr>
<tr>
<td>Skin Rash</td>
<td>0</td>
</tr>
<tr>
<td>EFV Encephalopathy</td>
<td>2 (4%)</td>
</tr>
<tr>
<td>Clinical criteria for TB immune reconstitution inflammatory syndrome (IRIS)</td>
<td>0</td>
</tr>
<tr>
<td>Median RUCAM score (range)</td>
<td>7 (6-9)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Outcome</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Recovery n (%)</td>
<td>37 (74%)</td>
</tr>
<tr>
<td>Median duration of admission days (time to clinical recovery)</td>
<td>33 (24 – 52)</td>
</tr>
<tr>
<td>Median duration of outpatient follow up (time to biochemical recovery)</td>
<td>574 (239 – 728)</td>
</tr>
<tr>
<td>Death n (%)</td>
<td>7 (14%)</td>
</tr>
<tr>
<td>Time to death (days)</td>
<td>15 (10-162)</td>
</tr>
<tr>
<td>Lost to follow up n (%)</td>
<td>6 (12%)</td>
</tr>
<tr>
<td>Median Cumulative dose of Prednisone in mg (IQR)</td>
<td>3 198 (1 898 – 4 670)</td>
</tr>
<tr>
<td>Duration on steroids (prednisone) (months)</td>
<td>11 (8 – 16)</td>
</tr>
</tbody>
</table>

#### Complications
- Sepsis (culture positive) n (%) | 3 (6%)
- Sepsis (culture negative) n (%) | 7 (14%)
- Steroid induced Diabetes Mellitus n (%) | 5 (10%)

#### Current ARV regimen:
- Emtricitabine/Tenofovir/Ritonavir/Atazanavir | 16
- Emtricitabine/Tenofovir/Lopinavir/Ritonavir | 20
- Lamivudine/Abacavir/Lopinavir/Ritonavir | 1
- Unknown (lost to follow up) | 6

Abbreviations: EFV, Efavirenz; IRIS, Immune Reconstitution Inflammatory Syndrome; RUCAM, Roussel Uclaf Causality Assessment Method; TB, Tuberculosis
Table 3: Laboratory parameters of the three histological patterns of efavirenz-related drug-induced liver injury (33 liver biopsies performed)

<table>
<thead>
<tr>
<th>Pattern of Injury</th>
<th>Submassive necrosis (n = 19)</th>
<th>Non-specific hepatitis (n=12)</th>
<th>Mixed cholestatic hepatitis (n=2)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total bilirubin</td>
<td>272 (210-317)</td>
<td>93 (63-276)</td>
<td>87</td>
<td>P=0.012</td>
</tr>
<tr>
<td>Conjugated bilirubin</td>
<td>165 (117-228)</td>
<td>67 (16-156)</td>
<td>48</td>
<td>P=0.0054</td>
</tr>
<tr>
<td>ALT</td>
<td>713 (470-1445)</td>
<td>927 (389-1174)</td>
<td>510</td>
<td>P=NS</td>
</tr>
<tr>
<td>AST</td>
<td>1055 (571-1966)</td>
<td>876 (374-1403)</td>
<td>729</td>
<td>P=NS</td>
</tr>
<tr>
<td>ALP</td>
<td>296 (173-389)</td>
<td>170 (131-203)</td>
<td>271</td>
<td>P=0.0082</td>
</tr>
<tr>
<td>GGT</td>
<td>229 (143- 596)</td>
<td>264 (142-538)</td>
<td>615</td>
<td>P=NS</td>
</tr>
<tr>
<td>INR</td>
<td>1.72 (1.5-2.28)</td>
<td>1.19 (1.1-1.4)</td>
<td>1.13</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>CD4 a</td>
<td>553 (415-743)</td>
<td>538 (258-665)</td>
<td>205</td>
<td>P=0.033</td>
</tr>
</tbody>
</table>

Laboratory parameter (laboratory reference range). Data are expressed as median and interquartile ranges.
Total and Conjugated Bilirubin, (umol/L); ALT, alanine aminotransferase (U/L); AST, aspartate aminotransferase (U/L); ALP, alkaline phosphatase (U/L); GGT, g-glutamyl transferase (U/L); INR, international normalized ratio.

a Nadir CD4 cell count at the initiation of ART.
<table>
<thead>
<tr>
<th>Factor</th>
<th>Non-specific hepatitis</th>
<th>Submassive necrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>P-value</td>
</tr>
<tr>
<td>Age &gt;30 years</td>
<td>0.5 (0.1-3.1)</td>
<td>0.4</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>8.7 (1.3-58.2)</td>
<td>0.03</td>
</tr>
<tr>
<td>CD4&gt;350</td>
<td>5.6 (0.9-32.6)</td>
<td>0.06</td>
</tr>
<tr>
<td>Alcohol use</td>
<td>0.17 (0.03-1.6)</td>
<td>0.1</td>
</tr>
</tbody>
</table>
Graph 1a: Trends in Recovery of Liver Profile: Median liver profile parameters

Total and Conjugated Bilirubin, (umol/L); ALT, alanine aminotransferase (U/L); AST, aspartate aminotransferase (U/L); ALP, alkaline phosphatase (U/L); GGT, gamma-glutamyl transferase (U/L); INR, international normalized ratio
**Graph 1b: Median liver enzyme profiles over 1 year of follow up**

ALT, alanine aminotransferase (U/L); AST, aspartate aminotransferase (U/L); ALP, alkaline phatase (U/L); GGT, g-glutamyl transferase (U/L)
Graph 1c: Median Total and Conjugated bilirubin over 1 year of follow up

Total Bilirubin (umol/L)  Conjugated Bilirubin (umol/L)

<table>
<thead>
<tr>
<th>Months</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Bilirubin (umol/L)</td>
<td>250</td>
<td>200</td>
<td>150</td>
<td>100</td>
<td>50</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conjugated Bilirubin (umol/L)</td>
<td>250</td>
<td>200</td>
<td>150</td>
<td>100</td>
<td>50</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendix 1: Instructions to authors (South African Medical Journal)

Research

Guideline word limit: 4 000 words

Research articles describe the background, methods, results and conclusions of an original research study. The article should contain the following sections: introduction, methods, results, discussion and conclusion, and should include a structured abstract (see below). The introduction should be concise – no more than three paragraphs – on the background to the research question, and must include references to other relevant published studies that clearly lay out the rationale for conducting the study. Some common reasons for conducting a study are: to fill a gap in the literature, a logical extension of previous work, or to answer an important clinical question. If other papers related to the same study have been published previously, please make sure to refer to them specifically. Describe the study methods in as much detail as possible so that others would be able to replicate the study should they need to. Results should describe the study sample as well as the findings from the study itself, but all interpretation of findings must be kept in the discussion section, which should consider primary outcomes first before any secondary or tertiary findings or post-hoc analyses. The conclusion should briefly summarise the main message of the paper and provide recommendations for further study.

Select figures and tables for your paper carefully and sparingly. Use only those figures that provided added value to the paper, over and above what is written in the text.

Do not replicate data in tables and in text.

Structured abstract

- This should be 250-400 words, with the following recommended headings:
  - **Background:** why the study is being done and how it relates to other published work.
  - **Objectives:** what the study intends to find out
  - **Methods:** must include study design, number of participants, description of the intervention, primary and secondary outcomes, any specific analyses that were done on the data.
  - **Results:** first sentence must be brief population and sample description; outline the results according to the methods described. Primary outcomes must be described first, even if they are not the most significant findings of the study.
  - **Conclusion:** must be supported by the data, include recommendations for further study/actions.

- Please ensure that the structured abstract is complete, accurate and clear and has been approved by all authors.
- Do not include any references in the abstracts.
Main article

All articles are to include the following main sections: Introduction/Background, Methods, Results, Discussion, Conclusions.

The following are additional heading or section options that may appear within these:

- Objectives (within Introduction/Background): a clear statement of the main aim of the study and the major hypothesis tested or research question posed
- Design (within Methods): including factors such as prospective, randomisation, blinding, placebo control, case control, crossover, criterion standards for diagnostic tests, etc.
- Setting (within Methods): level of care, e.g. primary, secondary, number of participating centres.
- Participants (instead of patients or subjects; within Methods): numbers entering and completing the study, sex, age and any other biological, behavioural, social or cultural factors (e.g. smoking status, socioeconomic group, educational attainment, co-existing disease indicators, etc) that may have an impact on the study results. Clearly define how participants were enrolled, and describe selection and exclusion criteria.
- Interventions (within Methods): what, how, when and for how long. Typically for randomised controlled trials, crossover trials, and before and after studies.
- Main outcome measures (within Methods): those as planned in the protocol, and those ultimately measured. Explain differences, if any.

Results

- Start with description of the population and sample. Include key characteristics of comparison groups.
- Main results with (for quantitative studies) 95% confidence intervals and, where appropriate, the exact level of statistical significance and the number need to treat/harm. Whenever possible, state absolute rather than relative risks.
- Do not replicate data in tables and in text.
- If presenting mean and standard deviations, specify this clearly. Our house style is to present this as follows:
  - E.g.: The mean (SD) birth weight was 2 500 (1 210) g. Do not use the ± symbol for mean (SD).
- Leave interpretation to the Discussion section. The Results section should just report the findings as per the Methods section.

Discussion

Please ensure that the discussion is concise and follows this overall structure – subheadings are not needed:

- Statement of principal findings
- Strengths and weaknesses of the study
- Contribution to the body of knowledge
• Strengths and weaknesses in relation to other studies
• The meaning of the study – e.g. what this study means to clinicians and policymakers
• Unanswered questions and recommendations for future research

Conclusions

This may be the only section readers look at, therefore write it carefully. Include primary conclusions and their implications, suggesting areas for further research if appropriate. Do not go beyond the data in the article.

Tables

• Tables should be constructed carefully and simply for intelligible data representation. Unnecessarily complicated tables are strongly discouraged.
• Large tables will generally not be accepted for publication in their entirety. Please consider shortening and using the text to highlight specific important sections, or offer a large table as an addendum to the publication, but available in full on request from the author
• Embed/include each table in the manuscript Word file - do not provide separately as supplementary files.
• Number each table in Arabic numerals (Table 1, Table 2, etc.) and refer to consecutively in the text.
• Tables must be cell-based (i.e. not constructed with text boxes or tabs) and editable.
• Ensure each table has a concise title and column headings, and include units where necessary.
• Footnotes must be indicated with consecutive use of the following symbols: * † ‡ § ¶ || then ** †† ‡‡ etc.

References

NB: Only complete, correctly formatted reference lists in Vancouver style will be accepted. Reference lists must be generated manually and not with the use of reference manager software. Endnotes must not be used.

• Authors must verify references from original sources.
• Citations should be inserted in the text as superscript numbers between square brackets, e.g. These regulations are endorsed by the World Health Organization,[2] and others.[3,4-6]
• All references should be listed at the end of the article in numerical order of appearance in the Vancouver style (not alphabetical order).
• Approved abbreviations of journal titles must be used; see the List of Journals in Index Medicus.
• Names and initials of all authors should be given; if there are more than six authors, the first three names should be given followed by et al.
• Volume and issue numbers should be given.
• First and last page, in full, should be given e.g.: 1215-1217 not 1215-17.
Wherever possible, references must be accompanied by a digital object identifier (DOI link). Authors are encouraged to use the DOI lookup service offered by CrossRef:

- On the Crossref homepage, paste the article title into the ‘Metadata search’ box.
- Look for the correct, matching article in the list of results.
- Click Actions > Cite
- Alongside ‘url =’ copy the URL between { }.
- Provide as follows, e.g.: https://doi.org/10.7196/07294.937.98x
Appendix 2: Ethics approval

17 September 2018

HREC REF: 593/2018

A/Prof W Spearman
Hepatology
K-Floor, OMB

Dear A/Prof Spearman

PROJECT TITLE: THE NATURAL HISTORY OF EFAVIRENZ DRUG INDUCED LIVER INJURY (SUB-STUDY LINKED TO S30/2012) (MMed Candidate - Dr D. Maughan)

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee.

It is a pleasure to inform you that the HREC has formally approved the above-mentioned study.

Approval is granted for one year until the 30 September 2019.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period. (Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

Please quote the HREC REF in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator must obtain appropriate institutional approval, where necessary, before the research may occur.

The HREC acknowledge that the student, Dr Deborah Maughan will also be involved in this study.

Yours sincerely

Signature Removed

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE
Federal Wide Assurance Number: FWA000001637
Institutional Review Board (IRB) number: IRB000001938
This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical

The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.