Hepatocellular injury in children treated for rifampicin-resistant tuberculosis: incidence, aetiology and outcome

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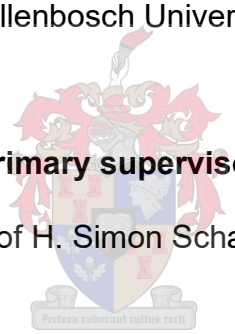

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Declaration

I, the undersigned, hereby declare that the work contained in this assignment is my original work and that I have not previously submitted it, in its entirety or in part, at any university for a degree.

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Abstract

Background: Hepatocellular injury has been commonly reported in adults on treatment for rifampicin-resistant tuberculosis (RR-TB). However, there are limited data in children.

Methods: Two observational pharmacokinetic studies of children (0-17 years) routinely treated for RR-TB were conducted in Cape Town, South Africa between October 2011 and February 2020. All hepatocellular injury adverse events (AEs; defined as elevated alanine aminotransferase [ALT]) were documented. Data were analyzed to determine the incidence, aetiology, risk factors, management and outcome of ALT elevation.

Results: A total of 217 children, median age 3.6 years at enrolment (IQR: 1.7, 7.1) were included. The median follow-up time was 14.0 months (IQR: 9.8, 17.2). Fifty-five (25.3%) developed an ALT AE. Of these, 43/55 (78%) children had 54 ALT AEs attributed to their RR-TB treatment. The incidence rate of ALT AEs related to RR-TB treatment was 22.4 per 100 person-years. Positive HIV status and having an elevated ALT at enrolment were associated with time to ALT AE attributed to RR-TB treatment, with p-values of 0.0427 and $p<0.0001$, respectively. Hepatitis A IgM was positive in 11/14 (78.6%) of grade ≥3 cases of ALT AEs. In 8/14 (57%) of severe ALT AEs, hepatotoxic drugs were stopped or temporarily interrupted. No children had a fatal or unresolved outcome.

Conclusions: Hepatocellular injury in children on RR-TB treatment is common, although usually mild; having elevated ALT early in treatment and HIV-positive status are possible risk factors for the development of hepatocellular injury on treatment. Hepatitis A was a common cause of severe ALT AE in children treated for RR-TB.

Abbreviations

Adverse event	AE
Alanine transaminase	ALT
Antiretroviral therapy	ARV
Aspartate aminotransferase	AST
Division of Acquired Immunodeficiency Syndrome	DAIDS
Drug-resistant tuberculosis	DR-TB
Drug susceptibility testing	DST
Drug-induced liver injury	DILI
Extensively drug-resistant tuberculosis	XDR-TB
Extra-pulmonary tuberculosis	EPTB
Hepatitis B surface antigen	HBsAg
Human Immunodeficiency Virus	HIV
Human leukocyte antigen	HLA
Line-probe assays	LPA
Multidrug-resistant tuberculosis	MDR-TB
Multidrug-resistant tuberculosis Pharmacokinetic study 1	MDR PK 1
Multidrug-resistant tuberculosis Pharmacokinetic study 2	MDR PK 2
Polymerase chain reaction	PCR
Pulmonary tuberculosis	PTB
Rifampicin-resistant tuberculosis	RR-TB
Total serum bilirubin	TSB
Tuberculosis	TB
Weight-for-Age-Z-score	WAZ
World Health Organization	WHO

Literature review

General introduction

Multidrug-resistant tuberculosis (MDR-TB; defined as *Mycobacterium tuberculosis* with resistance to at least both rifampicin and isoniazid) is a global threat to tuberculosis (TB) control and its treatment remains challenging. It was estimated in 2018 that 25 000-32 000 children develop MDR-TB disease annually.(1) In South Africa, the number of rifampicin-resistant TB (RRTB) cases notified (for adults and children) nearly doubled from 2007 to 2012; from 7 350 to 14 141 - the latest number notified in 2013 showing a decline to 9 791 cases.(2) In the Western Cape, South Africa, the transmission rate of RR/MDR-TB disease from adults to children in household contact has shown to be as high as 24%.(3) Studies in the Western Cape have showed an increasing incidence of RR/MDR-TB) in children from 2.3% in 1994-1998 to 6.7% in 2005-2007, and as high as 8.9% in 2007-2009. These studies also suggested that drug resistance is being transmitted to children rather than being acquired through inappropriate treatment.(4)(5)

These numbers still likely underestimate the true burden of RR/MDR-TB in children, as challenges in bacteriological confirmation remain due to the paucibacilliary nature of TB in children as well as the difficulty in obtaining appropriate specimens for bacteriology from children.(6)(7) Obtaining a respiratory specimens (sputum – induced or expectorated – or gastric aspirates) can be challenging, and even when obtained, culture confirmation (the gold standard of diagnosis) takes up to 6-8 weeks and is only confirmed in only 30-70% of cases at best.(8) Xpert MTB/RIF testing has provided for earlier diagnosis and drug susceptibility testing (DST) for rifampicin, but has a lower sensitivity of 61% in smear-negative cases when compared to mycobacterial culture.(9)

A delay in the diagnosis of RR/MDR-TB occurs often and this impacts the morbidity and mortality of the disease.(8) Clinicians should be vigilant in history taking when a child presents with signs and symptoms suggestive of TB disease, and RR/MDR-TB should be suspected when:

- the child has contact with a known adult source case who has RR/MDR-TB
- RR/MDR-TB is highly prevalent in the community in which the child resides
- the adult source case is a lost to follow-up case, retreatment case or chronic case with an unknown DST
- the child deteriorates on standard first-line treatment while being compliant on treatment
- a child's TB relapses after incomplete/incorrect TB treatment (8)

Previously, detecting drug resistance was only possible using culture-based (phenotypic) DST in which solid or liquid medium is used to assess in vitro growth of *M. tuberculosis* with exposure to a certain drug. This test could be performed qualitatively or semi-quantitatively, where quantitative tests use a single drug concentration for more basic testing, and semi-quantitative tests use different concentrations and are useful for identifying low-level or high-level resistance to drugs.(10) The main disadvantage of culture-based DST is the time delay until cultures become positive (7-12 days for liquid based tests, 6-8 weeks for solid medium based tests), as well as the availability of these tests usually being limited to central reference laboratories.(11)

In 2010 the WHO changed their policy and recommended the use of genotypic tests, such as the Xpert MTB/RIF assay, an automated PCR-based test that can detect *M. tuberculosis* complex within 100 minutes. This automated test is also shown to be cost effective when compared to conventional DST.(12) However, false negative and

false positive tests are possible – this has led to South Africa adopting a policy that endorses confirmatory testing. The test works by detecting mutations in the TB genome that can predict rifampicin resistance.(10)

In March 2017 the WHO updated their recommendation to the use of the Xpert MTB/RIF Ultra test, a test that has been shown to be more sensitive than the original Xpert MTB/RIF – especially in smear-negative culture-positive cases. This new cartridge-based test uses the existing Xpert platform. The possibly reduced specificity of this test as seen in some studies is the only potential disadvantage – with specificity being 3.2% lower than the Xpert MTB/RIF test in one study.(13)(14)

For broader drug resistance testing, the WHO has endorsed the genotypic line-probe assays (LPA). LPAs are usually limited to well-equipped laboratories with trained staff, as its performance requires a considerable amount of skill. For rifampicin and isoniazid, the GenoType MBTDR*plus* (Hain Lifescience GmbH, Nehren, Germany) is used, and for the fluoroquinolones and aminoglycosides the GenoType MTBDR*sl*-v1.0 assay of the same manufacturer is used.

Background

Once diagnosed, effective drug therapy is essential to cure RR/MDR-TB. If left untreated at least 22% of children who develop RR/MDR-TB disease will die.(1) In a recent Russian study, 90% of the 52 children with MDR-TB had successful treatment outcome (15). In a local study conducted between 2009 and 2010 in Cape Town, more than 90% of 149 children with MDR-TB were successfully treated (treatment success defined as cure or treatment completion with clinical improvement).(16) A systematic review which included 325 children who were treated for MDR-TB showed a cure rate of 81% (6) – all of these studies supports the premise that a

good outcome is possible if treated appropriately. However, compared to drug-susceptible TB, RR/MDR-TB is treated with more toxic drugs and is still treated for longer durations. Some well-known hepatotoxic drugs (isoniazid and pyrazinamide) are still often used in RR/MDR-TB treatment, isoniazid at a higher dose compared to drug-susceptible TB to overcome low-level resistance.(17) As many as 43%-75% of RR/MDR-TB strains may remain susceptible to isoniazid at appropriately higher concentrations.(18)

Changes in the 2016 WHO RR/MDR-TB guidelines provided for a 9-12 month treatment course, which is shorter than the previously recommended 18-24 month treatment course for RR/MDR-TB, but is reserved for specific cases only (see table 1 for WHO RR/MDR-TB guidelines 2016). (19)

Previous treatment guidelines for children with RR/MDR-TB according to WHO, as used during our study, is included in table 1.

Table 1

WHO RR/MDR TB treatment guidelines 2016

1. **Shorter RR/MDR-TB regimen for adults and children**

   In patients with pulmonary RR-TB or MDR-TB who were not previously treated with second-line drugs and in whom resistance to fluoroquinolones and second-line injectable agents was excluded or is considered highly unlikely, a shorter MDR-TB regimen of 9–12 months may be used instead of the longer regimens (conditional recommendation, very low certainty in the evidence).

2. **Longer RR/MDR-TB regimens for adults and children**

2a) In patients with RR-TB or MDR-TB, a regimen with at least five effective TB medicines during the intensive phase is recommended, including pyrazinamide and four core second-line TB medicines – one chosen from Group A, one from Group B, and at least two from Group C (conditional recommendation, very low certainty in the evidence). If the minimum number of effective TB medicines cannot be composed as given above, an agent from Group D2 and other agents from Group D3 may be added to bring the total to five. 2b) In patients with RR-TB or MDR-TB, it is recommended that the regimen be further strengthened with high-dose isoniazid and/or ethambutol (conditional recommendation, very low certainty in the evidence). It is recommended that any patient – child or adult – with RR-TB in whom isoniazid resistance is absent or unknown be treated with a recommended MDR-TB regimen. It could either be a shorter MDR-TB regimen, or a longer MDR-TB regimen to which isoniazid is added.

Medicines recommended for the treatment of RR-TB and MDR-TB (WHO 2016 guidelines)

Group	Medication class	Examples	Abbreviations
Group A	**Fluoroquinolones**	Levofloxacin Moxifloxacin Gatifloxacin	Lfx Mfx Gfx
Group B	**Second-line injectable agents**	Amikacin Capreomycin Kanamycin (Streptomycin)	Am Cm Km (S)

Group C	**Other core second-line agents**	Ethionamide / prothionamide Cycloserine / terizidone Linezolid Clofazimine	Eto / Pto Cs / Trd Lzd Cfz
Group D	**Add-on agents (not part of the core MDR-TB regimen)**	**D1** Pyrazinamide Ethambutol High-dose isoniazid **D2** Bedaquiline Delamanid **D3** p-aminosalicylic acid Imipenem–cilastatind Meropenem Amoxicillin-clavulanate (Thioacetazone)	 Z E Hh Bdq Dlm PAS Ipm Mpm Amx-Clv (T)

Of the recommended drugs in table 1, isoniazid, pyrazinamide, ethionamide/prothionamide, para-aminosalicylic acid and the fluoroquinolones (mainly moxifloxacin) have all been implicated in causing hepatocellular injury or drug-induced liver injury.(17) The potential hepatotoxicity of these drugs has not been studied extensively in children on RR/MDR-TB treatment.

Drug-induced liver injury (DILI) is a diagnosis of exclusion, often only made after diagnostic probing for other causes are negative. It usually occurs within weeks to months of initiation of a drug, and its diagnosis is supported by resolution when the drug(s) is discontinued/withdrawn. A more than two-fold rise in serum alanine transferase (ALT) after re-challenge with the suspected offending agent also points to the diagnosis.(20)(21) In a study of 125 Indian adults, the incidence for hepatocellular injury on MDR-TB treatment was 2%. (22) Very little data is available on the incidence of hepatocellular injury on RR/MDR-TB treatment in children. Various biochemical markers for identifying potential hepatotoxicity exist of which ALT remains the gold standard. Although ALT is not an entirely specific marker for hepatocellular injury, it remains more specific than AST (aspartate aminotransferase) – which can also indicate damage to myocytes. (23) Total serum bilirubin (TSB) is also considered a marker for cholestasis secondary to hepatocellular injury, and in acute liver injury, is considered a better marker for severity of disease than ALT.(24) However, it is mostly used as a marker for hepatobiliary pathology, and it can also be increased due to haemolysis.(23)

Isoniazid is metabolized through acetylation by N-acetyl transferase-2 (NAT-2). Acetyl-isoniazid is metabolized to the toxic mono-acetyl hydrazine (MAH) and to non-toxic diacetyl hydrazine and other metabolites. MAH damages tissues by the production of free radicals. The duration of exposure to MAH can be affected by acetylator status, but the influence of acetylator status on toxicity of isoniazid is controversial. The most recent studies suggest that slow acetylators experience more severe transaminase elevations than fast acetylators.(25) When RR/MDR-TB is treated, higher doses of isoniazid may be use in cases with isoniazid resistance conferred by *inhA* promoter region mutations – these mutations confer a low-level

isoniazid resistance which may be overcome by increasing the dose.(26)(27)(28) However, doses exceeding 15 to 20mg/kg has been associated with a higher risk of hepatocellular injury in children.(29)(30)

The clinical presentation of isoniazid hepatocellular injury may range from asymptomatic, to abdominal pain, nausea and vomiting as well as constitutional symptoms. Fever and rash can also be seen but is less common; overt jaundice, dark urine and clay-coloured stools are late signs. Signs of liver failure – coagulopathy with bruising and bleeding, oedema from hypoalbuminemia and changes of level of consciousness due to hypoglycaemia are signs of life-threatening toxicity and fulminant liver failure.

The timing of toxicity due to isoniazid usually is within weeks to months of initiation – one study in adults showing a median time of 16 weeks.(31) Most cases of isoniazid hepatocellular injury recover after discontinuation, although this may take days to several weeks. (32)(21)

Pyrazinamide is metabolized by liver microsomal amidase into its active metabolite, pyrazinoic acid. This in return is hydroxylated by xanthine oxidase into 5-hydroxypyrazinoic acid (5-OH-PA). Animal studies have suggested that this metabolite (5-OH-PA) is mainly responsible for pyrazinamide's hepatotoxic effect.(33) Its hepatotoxicity is likely more severe than isoniazid according to studies in adults, and a large study done in over 3000 adults showed that adding pyrazinamide to drug susceptible TB therapy (isoniazid and rifampicin) increases the odds of hepatocellular injury by 2.8. (34)(35)(36) The use of rifampicin and pyrazinamide as preventive therapy in an adult study had higher rates of hepatocellular injury that when using isoniazid as preventive therapy for latent drug-susceptible TB.(36) Hepatocellular injury caused by pyrazinamide is dose related

and idiosyncratic.(37) Pyrazinamide can also cause hypersensitivity with eosinophilia and liver injury or granulomatous hepatitis.(38)(39)

Ethionamide and Prothionamide are thioamides. These are prodrugs which inhibit mycolic acid production and ultimately leads to cell wall lysis in the *M. tuberculosis* cell wall. They are important components to RR/MDR-TB treatment especially due to their ability to cross the blood brain barrier and are therefore also used in tuberculous meningitis. They are structurally similar to isoniazid and when mutations in the *inh*A promoter region exist, they share resistance (co-resistant). Significant hepatocellular injury occurs in approximately 2% of adult patients on ethionamide and prothionamide – while asymptomatic transient elevated transaminases occur more commonly. Gastrointestinal intolerability remains the most common adverse event with the use of the thionamides – these symptoms can overlap with the symptoms of hepatocellular injury.(26)

The **fluoroquinolones** have a low risk of hepatocellular injury, with a reported incidence on <1 per 100 000.(40). Moxifloxacin and levofloxacin are considered safe in the management of TB DILI caused by first-line drugs. Elevation in transaminases are mostly reversible and can occur in 2 to 3% of cases.(41)(42) The mechanism for fluoroquinolone hepatocellular injury is thought to be a hypersensitivity reaction – with eosinophilia being a hallmark.(43) Levofloxacin is eliminated as an unchanged drug in the urine, whereas moxifloxacin is partially metabolized in the liver (approximately 50%) and the rest excreted unchanged in stool and urine.(44)(45)(46)

Hypersensitivity to **para-aminosalicylic acid (PAS)** is well described.(47) Hepatocellular injury is included in this reaction – the reaction includes a systemic response and can even cause a clinical picture similar to infectious mononucleosis.(48)

Risk factors for hepatocellular injury on TB treatment

There are multiple potential risk factors for developing hepatocellular injury on TB treatment. Most data are from patients on first-line TB treatment. A Japanese study found that children aged less than 5 years are particularly at risk for DILI.(49) Other studies have followed to show no difference in risk related to age groups.(50) A poor nutritional state and hypo-albuminaemia has also been shown to increase the risk for hepatocellular injury in adults.(51) A recent Indonesian study evaluated 1424 children treated as inpatients for TB – they showed that 3.5% of children developed hepatocellular injury. However, when looking at age, sex, nutritional status, type of TB and comorbid diseases as risk factors, none were found statistically significant.(52) The NAT-2 gene is responsible for isoniazid metabolism – polymorphism in this gene can lead to a slow, medium or fast acetylation status. A Japanese study evaluating adult patients showed an odds ratio of 4.32 between NAT-2 slow acetylators and hepatocellular injury risk. A much older study conducted in 256 children in 1986 failed to demonstrate an association between acetylation phenotype and the risk of developing hepatocellular injury. Some specific genetic variants and HLA types have been shown to be potentially linked to risk of developing hepatocellular injury – but in resource-limited settings like South Africa this might not be pragmatic to investigate for in all patients. However, genetic differences between race groups and ultimately differences in the metabolism of TB medication and risk for drug toxicity is known to play a role.(25)(53)(54)(55)(56)(57)(58) Abnormal liver function tests at baseline is also a well-known risk factor to developing hepatocellular injury in adults.(59)(60)

Viral hepatitis has been implicated as possible cause or contributing factor of presumed DILI in patients on anti-tuberculosis therapy in various studies looking at first-line anti-TB drug studies.(61) Since the addition of hepatitis B vaccine to the South African expanded programme on immunisation (EPI) schedule, the rates of HBsAg positive infants have dropped dramatically. In a large study from the Eastern Cape, South Africa, including 2299 infants prior to the introduction of hepatitis B immunisation, 8.1% of 0-6 month old infants and 8.9% in 7-12 month old infants tested positive for HBsAg respectively - a high rate likely influenced by the impact of HIV infection.(62) (63) Subsequent to the introduction of hepatitis B vaccine in 1995, the positivity rate for HBsAg have dropped significantly to <2% in children with an unknown HIV status, and 2.7% in HIV-positive children. (62)(63)(64)(64)

In developing countries like South Africa, the incidence of hepatitis A remains high, and hepatitis A vaccine is not included in the national EPI schedule. In a study conducted in the Western Cape at Tygerberg Hospital, the median incidence of hepatitis A for children aged <13 year from 2001 to 2004 was 45.4/100 000/year, a significantly high incidence, above the threshold incidence for diseases usually included in the vaccination schedule.(65) The likely reason for this is that hepatitis A is usually considered a self-limiting disease in children and the prognosis in most cases is favourable. However, the incidence of hepatitis A is difficult to estimate using hospital data, and although it is estimated that 1% of patients develop fulminant hepatitis, this is likely an over estimation.(66)

However, there is no routine screening for viral hepatitis and specifically hepatitis A in children admitted for TB treatment or RR/MDR-TB treatment, and neither is a vaccine to hepatitis A currently routinely considered in these children. Differentiating

between drug-induced and viral hepatitis is not possible on clinical grounds alone. It has been noted in adult studies that the hepatocellular injury caused by viral aetiology is usually more delayed, transaminases are raised more significantly and takes longer to recover than in drug-induced hepatocellular injury. (67)
Hepatitis A may be significant contributor to hepatitis seen in children on RR/MDR-TB treatment and could influence patient management including earlier screening, patient isolation and admission policies, and consideration for vaccination of at patients at risk.

The impact of HIV

HIV status can play a significant role in the incidence of hepatocellular injury in patients on RR/MDR-TB treatment. It is a shared adverse effect for nevirapine, ritonavir-boosted protease inhibitors, and anti-TB drugs such as isoniazid and pyrazinamide.(68) However, when looking at available literature (including adult studies) very little data are available.(69) In an adult study evaluating 52 HIV-positive individuals on MDR-TB treatment, up to 17.3% of patients developed hepatitis and in a third of these patients antiretroviral (ARVs) or anti-TB drugs had to be discontinued.(70) The thionamides have an increased risk of hepatocellular injury when used in combination with efavirenz, nevirapine, tipranavir and darunavir. Hepatic CYP450 enzymes are responsible for metabolizing a variety of drugs and the combination of ARVs and anti-TB drugs could influence drug concentrations due to either enzyme induction or inhibition. When hepatic impairment is present, it is best to monitor drug concentrations more closely – especially when ethionamide is used in combination with other hepatotoxic drugs, as this increases the risk for hepatocellular injury greatly. (71)

The DAIDS Adverse Event (AE) grading table provides an AE severity grading scale ranging from grades 1 to 5 with descriptions for each AE based on the following general guidelines:

• Grade 1 indicates a mild event

• Grade 2 indicates a moderate event

• Grade 3 indicates a severe event

• Grade 4 indicates a potentially life-threatening event

• Grade 5 indicates death (*Note*: This grade is not specifically listed on each page of the grading table).

Table 2. DAIDS Adverse Event (AE) grading table description of grades (72)

PARAMETER	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
Clinical adverse event **NOT** identified elsewhere in the grading table	Mild symptoms causing no or minimal interference with usual social & functional activities with intervention not indicated	Moderate symptoms causing greater than minimal interference with usual social & functional activities with intervention indicated	Severe symptoms causing inability to perform usual social & functional activities with intervention or hospitalization indicated	Potentially life-threatening symptoms causing inability to perform basic self-care functions with intervention indicated to prevent permanent impairment, persistent disability, or death

Table 3. DAIDS (the division of AIDS) grading table for alanine transaminase (ALT), aspartate transaminase (AST) and total serum bilirubin (TSB)

Parameter	Grade 1 (mild)	Grade 2 (moderate)	Grade 3 (severe)	Grade 4 (potentially life-threatening)
AST	1.25 to <2.5 X ULN*	2.5 to < 5 X ULN	5 to <10 X ULN	≥10 X ULN
ALT	1.25 to <2.5 X ULN	2.5 to < 5 X ULN	5 to <10 X ULN	≥10 X ULN
Total bilirubin, high (age >28 days)	1.1 to < 1.6 X ULN	1.6 to <2.6 X ULN	2.6 to <5 X ULN	≥5 X ULN

*ULN is the upper limit of normal

Very little data is available on the current management guidelines for hepatocellular injury on RR/MDR-TB treatment in children. This study aims to contribute to this.(73)

Aims

Main aim: To investigate the incidence, severity and potential risk factors associated with the development of hepatocellular injury (hepatotoxicity) in children routinely receiving treatment for RR/MDR-TB.

Secondary aims: To confirm causality of the severe cases of hepatocellular injury (hepatotoxicity) in children on RR/MDR-TB treatment, as well as to describe the outcome and management.

Objectives

1. To determine the incidence, timing and severity of hepatocellular injury (hepatotoxicity) in children on RR/MDR-TB treatment
2. To analyze whether certain risk factors are associated with developing hepatocellular injury in children on RR/MDR-TB treatment.
3. To determine the cause where possible in severe cases of hepatocellular injury in children on RR/MDR-TB treatment.
4. To determine whether hepatocellular injury in children on RR/MDR-TB treatment leads to treatment interruption.
5. To describe the management and outcome in severe cases of hepatocellular injury in children on RR/MDR-TB treatment.

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Publication-ready Manuscript

The following manuscript has been prepared for submission to the Pediatric Infectious Diseases Journal. The journal's aims and scope, as well as author guidelines are attached as Appendix B.

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None to declare

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Abstract

Background: Hepatocellular injury has been reported commonly in adults on rifampicin-resistant and multidrug resistant tuberculosis (RR/MDR-TB) treatment. However, there are limited data in children.

Methods: Two pharmacokinetic studies of children (0-17 years) routinely treated for RR/MDR-TB were conducted in Cape Town, South Africa between October 2011 and February 2020. Hepatocellular injury adverse events (AEs; defined as elevated alanine aminotransferase [ALT]) were documented serially. Data were analysed to determine the incidence, aetiology, risk factors, management and outcome of ALT elevation.

Results: A total of 217 children, median age 3.6 (IQR: 1.7, 7.1) years at enrolment were included. The median follow-up time was 14.0 months (IQR: 9.8, 17.2). Fifty-five (25.3%) developed an ALT AE. Of these, 43/55 (78%) patients had 54 ALT AEs attributed to their RR/MDR-TB treatment. The incidence rate of ALT AEs related to RR-TB treatment was 22.4 per 100 person-years. Positive HIV status and having an elevated ALT at enrolment were associated with time to ALT AE attributed to RR/MDR-TB treatment, with p-values 0.0427 and $p<0.0001$, respectively. Hepatitis A IgM was positive in 11/14 (78.6%) of severe (grade ≥3) cases of ALT AEs. In 8/14 (57%) severe ALT AEs, hepatotoxic drugs were stopped or temporarily interrupted. None had a fatal or unresolved outcome.

Conclusions: Hepatocellular injury in children on RR/MDR-TB treatment is common, although usually mild; having elevated ALT early in treatment and HIV-positive status are possible risk factors. Hepatitis A was a common aetiology of severe ALT AE in children treated for RR/MDR-TB.

Introduction

Globally in 2019 there were an estimated 465,000 (95% confidence interval 400,000–535,000) incident cases of rifampicin-resistant (RR) tuberculosis (TB); 78% had multidrug-resistant TB (MDR; i.e. disease caused by *Mycobacterium tuberculosis* resistant to both isoniazid and rifampicin).(1) Of these, an estimated 25,000-30,000 are children.(2) In a recent hospital-based surveillance study in the Western Cape, South Africa, the prevalence of MDR-TB in children with bacteriologically confirmed TB was high at 8.0% of 587 children, with a further 2.2% that had rifampicin mono-resistant TB.(3) If left untreated, the mortality of children with RR/MDR-TB is high (2); however, successful treatment outcomes of 81-90% have been documented with appropriate therapy under routine care conditions.(4)(5)(6) Compared to drug-susceptible TB, RR/MDR-TB treatment requires more drugs with worse safety profiles for longer durations, increasing the risk for drug-related adverse effects. The 2016 World Health Organisation (WHO) drug-resistant (DR)-TB guidelines recommended a 9-11 month RR/MDR-TB treatment regimen, which is shorter than the previously recommended 18-24 month regimens, but this is reserved for specific RR/MDR-TB cases only.(7) Some well-known hepatotoxic drugs (isoniazid and pyrazinamide) are still often used in RR/MDR-TB treatment, and isoniazid is used at a higher dose compared to drug-susceptible TB to overcome low-level resistance often seen with *inhA* promotor region mutation conferring isoniazid resistance.(8)(9)(10) Up to 43-75% of RR/MDR-TB strains may remain susceptible to isoniazid at appropriately high concentrations.(11) Of the recommended drugs used to treat RR/MDR-TB in children, isoniazid, pyrazinamide, ethionamide/prothionamide, para-aminosalicylic acid and the fluoroquinolones (mainly moxifloxacin) have all been implicated in

causing hepatocellular injury.(8) An elevated alanine aminotransferase (ALT) level above the normal reference range for age and sex is considered the best biomarker for hepatocellular injury.(12) Drug-induced liver injury (DILI) is a potential cause for hepatocellular injury and is also a diagnosis of exclusion, often only made after negative evaluation for other aetiologies. In the STREAM adult MDR-TB trial, 7.8% of 423 patients developed grade 3-5 hepatobiliary disorders on MDR-TB treatment.(13) There is limited data on the incidence of hepatocellular injury in children on RR-TB treatment.

Identifying patients at risk of developing DILI may enable clinicians to do targeted monitoring for liver toxicity. However, most studies that aimed to identify risk factors for DILI included adults with drug-susceptible TB only. A recent study found that 3.5% of 1424 children treated as inpatients for drug-susceptible TB developed hepatocellular injury, defined either as jaundice, raised total serum bilirubin (>1.5mg/dL) and/or 3-5 fold rise of serum ALT above normal values. However, when assessing age, sex, nutritional status, type of TB and comorbid diseases as risk factors, none were found to be associated with hepatocellular injury.(14) In adults, hypoalbuminaemia and poor nutritional status have been associated with increased risk for hepatocellular injury.(15) Some studies have suggested that children aged <5 years are more at risk, but other smaller studies report conflicting results.(14)(16)(17) Abnormal liver function tests at baseline is a well-described risk factor for developing hepatocellular injury in adults treated for TB.(18)(19) Viral hepatitis has also been implicated as a possible contributing factor to presumed DILI in patients on first-line antituberculosis therapy and in adults on RR-TB treatment. (20)(21)(22)(23)

HIV infection may increase the risk of developing hepatocellular injury in patients on RR-TB treatment, as antiretroviral agents (ARVs) such as nevirapine and ritonavir-

boosted protease inhibitors share this potential adverse effect with RR-TB treatment;(24) however, little data are available.(25) In an adult study evaluating 52 HIV-positive individuals on MDR-TB treatment, 17.3% of patients developed hepatocellular injury, and in a third of these, ARVs or anti-TB drugs had to be permanently discontinued.(26)

The aim of this study was to determine the frequency, aetiology and outcome of hepatocellular injury in children on RR-TB treatment.

Methods

Study population, data collection

This study included data from two prospective observational pharmacokinetic studies of children routinely treated for RR-TB in Cape Town, South Africa between October 2011 and February 2020. Children aged 0-17 years, HIV-negative and HIV-positive, were enrolled at Tygerberg Hospital, Brooklyn Chest Hospital and Brewelskloof Hospital and were followed until treatment completion. Most children were already on treatment at the time of enrolment with study eligibility allowing children to be on 2-12 weeks of treatment at enrolment. Routine South African and international WHO guidance was used in addition to drug susceptibility testing to construct individualized treatment regimens. Bedaquiline and delamanid became available in 2018 and were used in some participants. Adverse events (AEs) were documented at serial visits using standard case report forms and investigations; further investigations were completed where clinically indicated. ALT and total bilirubin were assessed at study enrolment, monthly for the first 6 months, and every 2 months thereafter while on treatment.

Definitions of hepatocellular injury

Hepatocellular injury was defined as a raised ALT value above the normal levels as per the South African National Health Laboratory Service (NHLS) reference values for age and sex. All ALT adverse events (ALT AEs) were graded according to the Division of Acquired Immunodeficiency Syndrome (DAIDS) table for grading the severity of adult and pediatric adverse events (Corrected Version 2.1).(27) Additional data captured from the NHLS database and patient folders were used in the analysis including date of AE, associated symptomatology, TB and/or ARV treatment interruption and likely association with TB treatment.(27)(12) Severe hepatocellular injury was defined as Grade 3 or higher as per DAIDS grading.

Hepatocellular injury (or ALT AE) was classified as either potentially drug-induced liver injury (DILI) related or caused by any other aetiologies. The first group was termed broadly as “possible, probable or definite” ALT AEs related to RR-TB treatment, as to include all cases of ALT AEs which were not definitely caused by alternative aetiologies, e.g. proven viral hepatitis, as DILI remains a diagnosis of exclusion and not all cases of ALT AEs (especially mild cases) were investigated for an alternative diagnosis by further testing.

Elevated ALT values captured at enrollment were not considered or included as ALT AEs, and patients who subsequently developed ALT AEs had to have normalized their ALT values between enrolment and the ALT AEs for it to be included as an ALT AE. Some patients had recurring ALT AEs, and ALT values had to normalize between events to be counted separately. In cases where patients had serially raised ALT values with no recovery, it was counted as a single event.

Data analysis

Clinical and demographic variables at enrolment were described using summary statistics. Continuous variables were not normally distributed and hence were summarized using medians and interquartile ranges (IQR). Categorical variables were reported using frequencies and percentages. Weight-for-age Z-scores (WAZ) were calculated using the British 1990 growth reference. The clinical and demographic variables at enrolment were stratified by ALT status (patients with any ALT AE vs. patients without ALT AE). Potential risk factors evaluated included age, sex, HIV status, nutritional status (WAZ<-2.0 vs WAZ>-2.0) and ALT at enrolment (elevated ALT vs normal ALT). The incidence rates for ALT AEs were calculated per person-time of observation, and ALT AEs were presented by DAIDS grade.

Person-time was calculated from the enrolment study assessment until the treatment completion date, or the last available study visit for patients who did not complete the study per protocol. Time to hepatocellular injury was analyzed from enrolment assessment up until the date of hepatocellular injury. Patients were censored at the time of treatment completion or at the date of last study assessment. Log-rank tests were used to assess if any of the potential risk factors were associated with time to any ALT AE, time to any Grade 3 or higher ALT AE, time to any ALT AE at least possibly related to RR-TB, or time to any Grade 3 or higher ALT AE at least possibly related to RR-TB. A p-value <0.05 was considered statistically significant. Kaplan-Meier curves were plotted for time to any ALT AE at least possibly related to RR-TB treatment, time to ALT AE Grade 3 or higher at least possibly related to RR-TB treatment, time to any ALT AE at least possibly related to RR-TB by HIV status and time to any ALT AE at least possibly related to RR-TB by ALT at enrolment status. The data was analyzed using Stata 16.0 special edition software (StataCorp. 2019. *Stata Statistical Software:* Release 16. College Station, TX; StataCorp LP.).

The frequencies and percentages of ALT AEs requiring treatment interruption or permanent TB drug discontinuation were calculated and stratified by grade of ALT AEs. Treatment interruption was defined as omission of any potentially hepatotoxic RR-TB medication of 2 days or more. The proportion of patients with ALT AEs and concurrent symptoms and signs (new onset vomiting, abdominal pain/tenderness and/or jaundice) was reported using frequencies and percentages. In children with severe liver injury/AE, a viral panel for antibodies against hepatitis A, B and C were performed to exclude alternative diagnoses; positive hepatitis A IgM was used to indicate active hepatitis A. The proportion of children with laboratory-confirmed viral hepatitis is reported. The proportion of patients who developed hepatitis A during in-patient stay (nosocomial infection) was calculated; this was defined as hepatitis A occurring from between 2 weeks from admission and up to 6 weeks post discharge, given the hepatitis A incubation period of 2-6 weeks.

Ethical considerations

Ethics approval was obtained from Stellenbosch University Health Research Ethics Committee for both MDR-PK 1 and MDR-PK 2 studies as well as for this sub-study (S19/09/183). Written informed consent was obtained from parents/legal guardians and assent from children >7 years of age.

Results

A total of 217 children were included, median age 3.6 years (IQR: 1.7, 7.1) at enrolment; 148/217 (68.2%) were below 5 years of age, and 34/217 (15.7%) were children living with HIV (Table 1). The median time between treatment initiation and study enrolment was 25 days (IQR: 15, 43). The 217 children contributed a total of

241 person-years of observation time to AE assessment, with a median follow-up of 14.0 months (IQR: 9.8, 17.2) (Table 2). Fifty-five (25.3%) of the 217 children developed a total of 67 ALT AE after enrolment, with events recurring in 11 patients. The median age at the time of first ALT AE was 4.4 years (IQR 1.81, 6.66). Of the 55 children with an ALT AE, 43 children had 54 ALT AEs assessed as possibly, probably, or definitely related to RR-TB treatment. The incidence rate of ALT AEs possibly, probably or definitely related to RR-TB treatment was 22.4 per 100 person-years. Mild events (Grade 1 or 2 ALT AE) comprised 79% of all events in our study. Of the 217 children, 14 (6.5%) had a severe ALT AE, none of which were HIV positive.

Figure 1a displays the Kaplan-Meier survival estimate for all hepatocellular events attributed possibly, probably or definitely to RR/MDR-TB treatment. Three patients had severe ALT AE attributed to RR-TB treatment, and these episodes occurred within the first 90 days of enrolment (Figure 1b). For any ALT AE attributed to RR-TB treatment, HIV status and elevated ALT at enrolment were associated with time to ALT AE (p-value=0.0427 (Figure 1c) and p-value<0.0001 (Figure 1d), respectively). For an ALT AEs, elevated ALT at enrolment was associated with time to ALT AE, (p-value <0.0001). No other risk factors had statistically significant associations as tested for any ALT AE, any severe ALT AE, as well as ALT AE associated with treatment, including severe ALT AE associated with treatment.

Hepatitis A was confirmed in 11/14 (78.6%) children with severe ALT AEs, of which 9/11 (82%) were possibly nosocomial infections. Two patients with non-severe ALT AE also tested positive for hepatitis A. Eight of 14 (57%) children with severe ALT AEs had documented treatment interruption of more than 48 hours or regimen adjustments; 6 of these 8 had positive (IgM) hepatitis A serology. Only 2/14 (14%)

children with severe hepatotoxicity had any symptoms documented. No children with severe ALT AEs were living with HIV. Of the 9 cases with a severe ALT AE with possible nosocomial-acquired hepatitis A, 6 had overlapping time periods for their admissions at one long-stay TB hospital.

Table 3 lists the anti-tuberculosis drugs used at study enrolment.

Symptoms were only reported in 2/14 of the patients with severe ALT AE, and none of the patients with mild ALT AE had any reported symptoms or treatment interruption. The management and outcome of children with severe ALT AEs was variable and are described in Table 4. In the management of severe cases, the general first step was to discontinue all potentially hepatotoxic drugs, including isoniazid, pyrazinamide, ethionamide and para-aminosalicylic acid (PAS); of the 11 cases that had adequate documentation, all cases had successful reintroduction of medication, with regimen adjustments in 5 of these patients. Overall, even in the severe ALT AE group, all patients recovered and did well.

Discussion

We found that the occurrence of hepatocellular injury defined as ALT AEs in children on routine RR-TB treatment regimens, prior to the widespread access of novel drugs and regimens including bedaquiline and delamanid, was common. Of 217 patients on RR-TB therapy, 25% developed ALT AEs, and of these, 78% had events assessed as possibly treatment related. Severe ALT AE was found in 21% of all events of which the majority was due to hepatitis A. In a study of 244 children on isoniazid preventive therapy, 44% developed elevated transaminases, supporting our study's finding, as many of the children on RR-TB treatment in our study were receiving high-dose isoniazid.(28) All children routinely underwent laboratory safety

evaluations, regardless of symptoms. In our study, only children with severe ALT AEs were investigated for other aetiologies of hepatocellular injury through viral antibody testing to hepatitis A, B and C. It is interesting to note that several patients in our study had recurring mild episodes of ALT AEs during RR-TB treatment. Only one child with a severe ALT AE, had a recurring mild ALT AE – the other 11 children with recurring events had mild ALT AE.

Our study supports the finding that abnormal ALT especially in the first month of treatment initiation increases the risk for ALT AEs on RR-TB treatment, including for ALT AEs that were assessed as related or unrelated to RR-TB treatment.(18)(19) HIV infection should also be considered a risk factor, as ALT AEs attributed to RR-TB treatment were significantly associated with children living with HIV. However, in our study, none of the 14 children with severe ALT AEs were living with HIV, in contrast to adult data.(26)

Since the introduction of hepatitis B vaccine to the South African expanded programme on immunisation (EPI) schedule in 1995, the rates of HBsAg-positive infants have dropped dramatically, and not one of the 14 severe cases in our study had ALT AEs caused by hepatitis B.(29)(30)(31) However, in TB-endemic settings, the incidence of hepatitis A remains high, and hepatitis A vaccination is not included in the EPI. In a study conducted at Tygerberg Hospital in the Western Cape, South Africa, the median incidence of hepatitis A for children aged <13 year from 2001 to 2004 was 45.4/100,000/year, a high incidence above the threshold incidence for diseases usually included in the international EPI vaccination schedule.(32) Screening for viral hepatitis and specifically hepatitis A is not routine in children admitted for TB treatment and hepatitis A vaccination is not considered for these children despite its availability. Nosocomial transmitted infections in children have

been previously reported in the same clinical context and occur commonly.(33) Various studies describe the co-existence of chronic viral hepatitis, such as hepatitis B and C, and the risk for hepatocellular injury in patients on drug-susceptible and RR-TB treatment. We could not identify studies describing the occurrence and influence of viral hepatitis A in patients on RR-TB treatment.(20)(21)(33) In our study, ALT AEs caused by hepatitis A contributed to, or likely caused treatment interruption or regimen adjustment in 6/8 (75%) of severe cases requiring treatment interruption. Hepatitis A vaccine, which is licensed for children aged >12 months, is effective in preventing the disease in >95% of fully vaccinated children, and it should also be used routinely to provide protection after exposure and to help prevent outbreaks in high-risk populations.(33) Two patients with non-severe ALT AE also tested positive for hepatitis A, although children with mild AEs did not routinely have viral hepatitis panels done; because we did not screen all children for hepatitis A, we may have underestimated the number of children affected. Nine patients with severe ALT AE in our study likely acquired this infection nosocomial, and this is supported by the fact that 6 of these patients had overlapping time periods for their admissions at the same long-stay hospital, signifying possible nosocomial spread of hepatitis A. Only 3 of the 14 children with severe ALT AEs had negative tests for hepatitis A, suggesting that drug-induced severe ALT AEs in children on RR-TB treatment is rare. Notably, in the group with severe ALT AEs, most children were on RR-TB treatment including first-line anti-TB drugs, such as isoniazid and pyrazinamide. On the other hand, bedaquiline and delamanid, two of the new drugs with the potential for DILI were used in only a few children in this study and we were unable to comment on their risk of ALT AEs in children (Table 3).

Symptoms were reported infrequently, which may be due the challenge of eliciting symptoms in young children, inadequate documentation or the occult nature of ALT AEs. Our data suggest that ALT testing early in the course of RR-TB treatment could help predict which patients are most at risk for an ALT AE. Our study was limited in that these ALT values at enrolment were not taken before treatment initiation. However, most patients were asymptomatic and had mild events. In only 8/217 (3.7%) children, hepatotoxic drugs were interrupted or stopped. Six of the eight patients who had treatment interruption with severe ALT AE had no documented symptoms; these ALT AEs might have been missed if routine testing was not done. Treatment regimens for the 217 children were varied and multiple potentially hepatotoxic drugs were used in each regimen. Analyzing the attribution of individual drugs to an ALT AE was not possible.

In conclusion, despite the large number of potentially hepatotoxic drugs used in children with RR-TB, severe ALT AEs were uncommon and outcomes from these ALT AEs were good. Hepatitis A was a common cause of severe ALT AEs in our study, and possible nosocomial transmission in one long-stay hospital contributed significantly to these events. Patients with hepatitis A tolerated hepatotoxic drugs well.

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Figures and tables

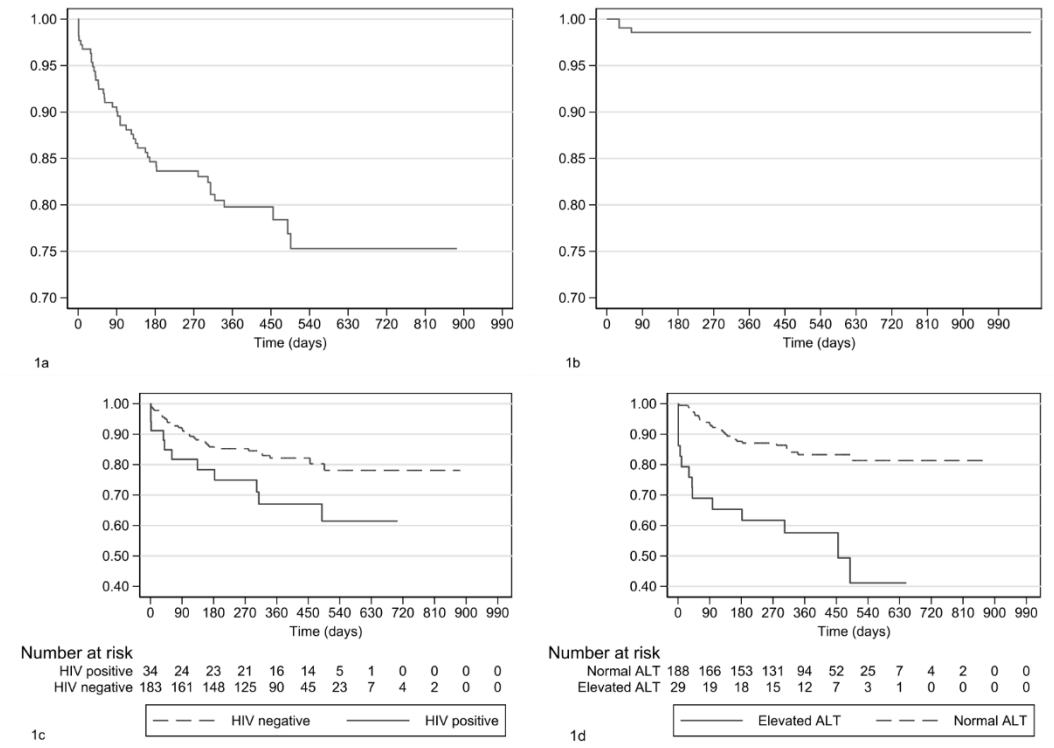

Figure 1 legends

Figure 1:

Figure 1a: Kaplan-Meier event estimate for all hepatocellular events attributed possibly, probably or likely to RR-TB treatment

Figure 1b: Kaplan-Meier event estimate for all severe (grade 3 or more) hepatocellular events attributed possibly, probably or likely to RR-TB treatment

Figure 1c: Kaplan-Meier event estimate for HIV positive and HIV negative patients with ALT AE attributed to RR-TB treatment

Figure 1d: Kaplan-Meier event estimate for patients with elevated and normal enrollment ALT with ALT AE attributed to RR-TB treatment

Table 1: Demographic and clinical characteristics of children on rifampicin-resistant tuberculosis treatment

	All Patients (n=217)	Patients with any ALT AE (n=55)	Patients without ALT AE (n=162)
Median age in years at enrollment (IQR)	3.6 (1.7, 7.1)	4.1 (1.6, 6.0)	3.1 (1.8, 8.2)
Age at enrolment			
0-2 years (%)	72 (33.2)	19 (34.5)	53 (32.7)
2-5 years (%)	76 (35.0)	18 (32.7)	58 (35.8)
5-10 years (%)	32 (14.7)	12 (21.8)	20 (12.3)
10-18 years (%)	37 (17.1)	6 (10.9)	31 (19.1)
Male gender (%)	108 (49.8)	30 (54.5)	78 (48.1)
Ethnicity			
Black (%)	118 (54.4)	30 (54.5)	88 (54.3)
Mixed race (%)	99 (45.6)	25 (45.5)	74 (45.7)
TB disease type [N=216]			
PTB only (%)	168 (77.8)	39 (70.9)	129 (80.1)
EPTB only (%)	15 (6.9)	7 (12.7)	8 (5.0)
PTB and EPTB (%)	33 (15.3)	9 (16.4)	24 (14.9)
HIV-positive (%)	34 (15.7)	11 (20.0)	23 (14.2)
Median weight-for-age-Z-score (IQR) [n=215]	-1.09 (-1.88, -0.39)	-1.08 (-1.88, -0.29)	-1.09 (-1.90, -0.45)
Weight-for-age-Z-score <-2.0 (%) [n=215]	50 (23.3)	13 (23.6)	37 (23.1)
Abnormal enrolment ALT (%)	29 (13.4)	16 (29.1)	13 (8.0)
Median time (days) between TB initiation and enrolment (IQR)	25 (15, 43)	22 (15, 44)	26 (14, 43)

Table 2: Hepatocellular injury adverse events in children on rifampicin-resistant tuberculosis treatment

Adverse Event	Adverse event by grade							Adverse effects possibly, probably, definitely attributed to RR-TB treatment by grade						
	# of patients with event	Grade 1	Grade 2	Grade 3	Grade 4	total # of events	Event Rate (per 100 person-yrs)	# of patients with event	Grade 1	Grade 2	Grade 3	Grade 4	total # of events	Event Rate (per 100 person-yrs)
LAB AEs														
Bilirubin	1	0	0	0	1	1	0.41	0	0	0	0	0	0	0.00
ALT	55	40	13	5	9	67	27.8	43	39	12	3	0	54	22.4

LAB AEs = laboratory adverse events; RR-TB = rifampicin-resistant tuberculosis; ALT = alanine aminotransferase
217 patients followed for a median time of 14.0 months (IQR: 9.8, 17.2 months)
Total person years = 241.3

Table 3: TB drugs administered at study enrolment (N=212)

	Total* (n=212) (%)	Patients with any ALT AE (n=55) (%)	Patients without ALT AE (n=157) (%)
Isoniazid	197 (92.9)	52 (94.5)	145 (92.4)
Pyrazinamide	208 (98.1)	52 (94.5)	156 (99.4)
Ethionamide	182 (85.8)	48 (87.3)	134 (85.4)
Ethambutol	193 (91.0)	50 (90.9)	143 (91.1)
Para-aminosalicylic acid (PAS)	61 (28.8)	19 (34.5)	42 (26.8)
Terizidone	202 (95.3)	52 (94.5)	150 (95.5)
Amikacin	128 (60.4)	38 (69.1)	90 (57.3)
Kanamycin	3 (1.4)	1 (1.8)	2 (1.3)
Capreomycin	10 (4.7)	4 (7.3)	6 (3.8)
Clarithromycin	1 (0.5)	0 (0.0)	1 (0.6)
Amoxicillin/Clavulanate	4 (1.9)	3 (5.5)	1 (0.6)
Moxifloxacin	47 (22.2)	14 (25.5)	33 (21.0)
Levofloxacin	131 (61.8)	36 (65.5)	95 (60.5)
Ofloxacin	33 (15.6)	10 (18.2)	23 (14.6)
Linezolid	34 (16.0)	9 (16.4)	25 (15.9)
Clofazimine	52 (24.5)	11 (20.0)	41 (26.1)
Delamanid	6 (2.8)	1 (1.8)	5 (3.2)
Bedaquiline	15 (7.1)	3 (5.5)	12 (7.6)

*TB drugs administered at study enrolment was only adequately documented in 212 of 217 patients

TABLE 4: Summary of the management of severe cases of hepatocellular injury in children on rifampicin-resistant tuberculosis treatment (N=14)

Grade[a]	Hepatitis A IgM [b]	ALT at AE	Symptoms	Treatment interruption duration (days)[c]	Management/Outcome
3	negative	196	Pruritus and anorexia	Treatment regimen adjusted	INH and PZA permanently discontinued
3	negative	189	none	4	PZA permanently discontinued, PAS and ETO temporarily withheld
3	positive	275	none	0	Undocumented
3	positive	174	none	0	Undocumented
3	negative	140	none	1[c]	ICU admission with ARDS due to viral LRTI, drugs withheld as precaution for 1 day
4	positive	872	none	0	Treatment likely continued, not well documented
4	positive	391	none	5	INH permanently discontinued (deemed unnecessary), PAS and co-trimoxazole withheld 5 days
4	positive	739	none	3	All drugs withheld until viral hepatitis panel*** result positive then restarted
4	positive	748	none	1[c]	All drugs withheld until viral hepatitis panel result positive then restarted
4	positive	456	none	5	INH, ETO, PZA temporarily stopped, restarted when viral hepatitis panel result positive
4	positive	379	none	Treatment completed on stop date	Undocumented
4	positive	898	none	19	INH, ETO, PZA temporarily stopped, restarted when viral hepatitis panel result positive
4	positive	1787	Nausea/Vomiting	14	Moxifloxacin changed to levofloxacin, INH and PAS temporarily stopped
4	positive	455	none	Treatment regimen adjusted	INH permanently discontinued

a. DAIDS grading for ALT value: Grade 1: 1.25 to <2.5 X ULN (upper limit of normal); Grade 2: 2.5 to < 5 X ULN; Grade 3: 5 to <10 X ULN; Grade 4: ≥10 X ULN[34]

b. Viral hepatitis panel included serology for hepatitis A, B, C, but no cases tested positive for active hepatitis B or C

c. Treatment interruption was regarded as significant if ≥2 days

ALT= alanine transaminase; AE = adverse event; INH = isoniazid; PZA = pyrazinamide; ETO = ethionamide; ICU = intensive care unit; ARDS = acute respiratory distress syndrome; LRTI = lower respiratory tract infection; PAS = para-aminosalicylic acid

APPENDIX A – Research Protocol

RESEARCH PROTOCOL

TITLE:

Incidence, causes and outcome of management in cases of hepatocellular injury in paediatric patients on multidrug-resistant tuberculosis therapy

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Purpose of study

The aim of this study is to describe the incidence, severity and risk factors of hepatocellular injury in children on multidrug-resistant anti-tuberculosis treatment. Additional risk factors for severe (grade 3 or more) cases of hepatocellular injury will be sought, and further investigations into the causality of these severe cases will be explored to attempt to distinguish other causes from true drug induced liver injury (DILI). Lastly, the management as well as the outcomes of this sub-group of severe cases will be described.

Background

Multidrug-resistant tuberculosis (MDR-TB) is defined as resistance to at least both rifampicin and isoniazid, two of the most effective anti-tuberculosis drugs. It is estimated that up to 32 000 children developed MDR-TB globally in 2010 (86). More recently, a mathematical modelling study projected that in 2014 up to 5 million children were infected with isoniazid mono-resistant tuberculosis, 2 million with MDR-TB, and 100 000 with extensively drug-resistant (XDR)-TB (i.e. MDR plus resistance to fluoroquinolones and a second-line injectable agent) globally of which 58 000, 25 000 and 1200 were likely to have developed the disease, respectively. (87). In South Africa, the number of rifampicin-resistant TB (RR-TB)/MDR-TB cases notified (for adults and children) nearly doubled from 2007 to 2012; from 7350 to 14 141 - the latest number notified in 2013 showing a decline to 9 791 cases. (2). In the Western Cape, South Africa, the transmission rate of MDR-TB disease from adults to children in household contact has shown to be as high as 24%. (3) In 2009, a study showed an increasing incidence of drug-resistant TB (DR-TB) in the Western Cape in children from 2.3% to 6.7% from 1994-1998 to 2005-2007, respectively. This study also suggested that the drug resistance is being transmitted in the community rather than being acquired. (4)

These numbers still likely underestimate the true burden of DR-TB in children as diagnostic challenges remain due to the paucibacilliary nature of TB in children.(6)(7). Obtaining a respiratory sample (sputum or gastric aspirate) can be challenging, and even when obtained, culture

confirmation (the gold standard of diagnosis) takes up to 6-8 weeks and is only confirmed in 30-70% of cases at best.(8). Xpert MTB/RIF testing has provided for earlier diagnosis and drug susceptibility testing (DST) for rifampicin, but still has a low sensitivity of 61% in smear-negative cases when compared to cultures. (9)

A delay in the diagnosis of DRTB is often the case and this impacts the morbidity and mortality of the disease.(8) Clinicians should be vigilant in history taking when a child presents with signs and symptoms suggestive of TB disease, and DR-TB should be suspected when:

- the child has contact with a known adult source case who has DR-TB
- DR-TB is highly prevalent in the community that the child resides
- the adult source case is a lost to follow-up case, retreatment case or chronic case with an unknown DST
- the child deteriorates on standard treatment while being compliant on first-line treatment
- a child's TB relapses after incomplete/incorrect TB treatment (8)

Previously, detecting drug resistance was only possible by culture-based DST – a phenotypic test, where agar or liquid medium is used to assess in vitro growth of the TB organism with exposure to a certain drug. This test could be performed qualitatively or semi-quantitatively, where quantitative tests uses a single drug concentration for more basic testing, and semi-quantitative tests uses difference concentrations and are useful for picking up low level or high level resistance to drugs.(10) The main drawback of DST is the time delay (7-12 days for liquid based tests, weeks for solid medium based tests), as well as the availability of these tests usually being limited to central reference laboratories.(11)

In 2010 the WHO changed their policy and recommended the use of a genotypic test, the Xpert MTB/RIF test, providing results within 100 minutes. This automated PCR based test is also shown to be cost effective when compared to conventional DST. (12) However, false negative tests and false positive tests are possible – this has led to South Africa adopting a policy that endorses

confirmatory testing. The test works by detecting mutations in the TB genome that can predict rifampicin resistance. (10)

In March 2017 the WHO once again changed their recommendation to the use of the Xpert MTB/RIF Ultra test, a test that has been shown to be more sensitive than the Xpert MTB/RIF test – especially in smear-negative culture-positive cases. This new cartridge-based test can still be used in the previous Xpert equipment. The reduced specificity of this test is the only drawback – with specificity being 3.2% lower than the Xpert MTB/RIF test. (13)

For broader drug resistance testing, the WHO has endorsed line-probe assays (or LPA). LPAs are usually limited to tertiary care settings and reference laboratories, as its performance requires a considerable amount of skill. For rifampicin and isoniazid, the GenoType MBTDRplus is used, and for the fluoroquinolones, aminoglycosides and ethambutol the GenoType MTBDRsl-v1.0 assay is used.

Once diagnosed, effective drug therapy is essential to cure RR/MDR-TB. If left untreated at least 22% of children who develop MDR-TB could die.(1) In a recent Russian study, 90% of the 52 children with MDR-TB followed up had successful treatment outcome (15). In a local study conducted between 2009 and 2010 in Cape Town, more than 90% of children with MDR-TB were successfully treated.(16) A systematic review which included 325 children who were treated for MDR-TB showed a cure rate of 81% (6) – which supports the premise that a good outcome is possible. However, compared to drug-susceptible TB, RR/MDR-TB is treated with more toxic drugs and is also treated for a longer duration. Some well-known hepatotoxic drugs (isoniazid and pyrazinamide) are used at higher doses in RR/MDR-TB treatment regimens. (17).

Changes in the 2016 WHO DR-TB guidelines provide for a 9-12 month treatment course, which is shorter than the previously recommended 18-24 month treatment course, but is reserved for specific cases only (see table 1 for WHO RR/MDR-TB guidelines 2016). (19)

Previous treatment guidelines for children with RR/MDR-TB according to WHO, as used during our study, is included in table 1.

Table 1

WHO RR/MDR TB treatment guidelines 2016

3. **Shorter RR/MDR-TB regimen for adults and children**

   In patients with pulmonary RR-TB or MDR-TB who were not previously treated with second-line drugs and in whom resistance to fluoroquinolones and second-line injectable agents was excluded or is considered highly unlikely, a shorter MDR-TB regimen of 9–12 months may be used instead of the longer regimens (conditional recommendation, very low certainty in the evidence).

4. **Longer RR/MDR-TB regimens for adults and children**

   2a) In patients with RR-TB or MDR-TB, a regimen with at least five effective TB medicines during the intensive phase is recommended, including pyrazinamide and four core second-line TB medicines – one chosen from Group A, one from Group B, and at least two from Group C (conditional recommendation, very low certainty in the evidence). If the minimum number of effective TB medicines cannot be composed as given above, an agent from Group D2 and other agents from Group D3 may be added to bring the total to five. 2b) In patients with RR-TB or MDR-TB, it is recommended that the regimen be further strengthened with high-dose isoniazid and/or ethambutol (conditional recommendation, very low certainty in the evidence). It is recommended that any patient – child or adult – with RR-TB in whom isoniazid resistance is absent or unknown be treated with a recommended MDR-TB regimen. It could either be a shorter MDR-TB regimen, or a longer MDR-TB regimen to which isoniazid is added.

Medicines recommended for the treatment of RR-TB and MDR-TB

Group A	**Fluoroquinolones**	Levofloxacin Moxifloxacin Gatifloxacin	Lfx Mfx Gfx
Group B	**Second-line injectable agents**	Amikacin Capreomycin Kanamycin (Streptomycin)	Am Cm Km (S)
Group C	**Other core second-line agents**	Ethionamide / prothionamide Cycloserine / terizidone Linezolid Clofazimine	Eto / Pto Cs / Trd Lzd Cfz
Group D	**Add-on agents (not part of the core MDR-TB regimen)**	**D1** Pyrazinamide Ethambutol High-dose isoniazid **D2** Bedaquiline Delamanid **D3** p-aminosalicylic acid Imipenem–cilastatin Meropenem Amoxicillin-clavulanated	 Z E Hh Bdq Dlm PAS Ipm Mpm Amx-Clv

		(Thioacetazone)	(T)

Of these drugs, isoniazid, pyrazinamide, ethionamide, prothionamide, para-aminosalicylic acid and the fluoroquinolones (moxifloxacin especially) have all been implicated in causing jaundice or hepatotoxicity (17). The hepatotoxicity of these drugs have not been studied extensively in children on RR/MDR-TB treatment – a lack of data exists to which this study will contribute.
Drug induced liver injury (DILI) is a diagnosis of exclusion, often only made after diagnostic probing comes back negative. It usually occurs within weeks to months of initiation of a drug, and its diagnosis is supported by the resolution with withdrawal of the drug. A more than two-fold rise in serum alanine transferase (ALT) after re-challenge with the suspected offending agent also points to the diagnosis. (20)(21)

Isoniazid is metabolized through acetylation by N-acetyl transferase (NAT-2). Acetyl-isoniazid is metabolized to the toxic mono-acetyl hydrazine (MAH) and to non-toxic diacetyl hydrazine and other metabolites. MAH damages tissues by the production of free radical generation. The duration of exposure to MAH can be affected by acetylator status, but the influence of acetylator status on toxicity of isoniazid is controversial. The most recent studies suggest that slow acetylators experience more severe transaminase elevations than fast acetylators. (25) When RR/MDR-TB is treated, often higher doses of INH is used mainly in cases with INH resistance conferred by *inhA* promoter region mutations- these mutations cause a low-level INH resistance which can be overcome by increasing the dose.(26)(27)(28) However, doses exceeding 15 to 20mg/kg has been associated with a higher risk of hepatotoxicity. (29)(30)
The clinical presentation of isoniazid hepatotoxicity may range from being asymptomatic, to abdominal pain, nausea and vomiting as well as constitutional symptoms. Fever and rash can also be seen but is less common, and overt jaundice, dark urine and clay coloured stools are very late signs. Signs of liver failure – coagulopathy with bruising and bleeding, oedema from

hypoalbuminemia and changes of level of consciousness due to hypoglycaemia are signs of life threatening toxicity and fulminant liver failure.

The timing of toxicity due to isoniazid usually is within weeks to months of initiation – one study in adults showing a median time of 16 weeks. (31) Most cases of isoniazid hepatotoxicity recover after discontinuation, although this may take days to several weeks. (32)(21)

Pyrazinamide can also damage the liver by free oxygen radical generation – however, the mechanisms of injury between isoniazid and pyrazinamide can overlap. Pyrazinamide can cause hypersensitivity with eosinophilia and liver injury or granulomatous hepatitis. (38)(39)

Ethionamide and Prothionamide are thioamides. These are prodrugs which inhibit mycolic acid production and ultimately leads to cell wall lysis in the mycobacterium tuberculosis cell wall. They are an important component to RR/MDR-TB treatment especially due to their ability to cross the blood brain barrier and are used in tuberculous meningitis. They are structurally similar to INH and when mutations in the *inh*A promoter region exist, they share resistance. Significant hepatotoxicity occurs roughly in only 2% of patients on ethionamide and prothionamide – while asymptomatic transient elevated transaminases occur more commonly. Gastrointestinal intolerability remains the most common adverse event with the use of the thionamides – these symptoms can overlap with the symptoms of hepatotoxicity.(26)

The **fluoroquinolones** have a low risk of hepatotoxicity, with a reported incidence on <1 per 100 000.(40). Moxifloxacin and levofloxacin are considered safe in TB drug induced hepatitis. Elevation in transaminases are mostly reversible and can occur in 2 to 3% of cases. (41)(42) The mechanism for fluoroquinolone hepatotoxicity is thought to be a hypersensitivity reaction – with eosinophilia being a hallmark. (43) Levofloxacin is eliminated as an unchanged drug in the urine, whereas moxifloxacin is partially metabolized in the liver (approximately 50%) and the rest excreted unchanged in stool and urine.(44)(45)(46)

Hypersensitivity to **para-aminosalicylic acid (PAS)** is well described.(47) Hepatocellular injury is included in this reaction - the reaction includes a systemic response and can even cause a clinical picture similar to infectious mononucleosis.(48)

Risk factors

Various potential risk factors for DILI exist. A Japanese study found that children aged less than 5 years are particularly at risk. (49) Other studies have followed to show no difference in risk related to age groups.(50) A poor nutritional state and hypo-albuminaemia has also been shown to increase the risk for DILI in adults.(51) A more recent Indonesian study published in 2017 looked at 1424 children treated as inpatients for tuberculosis – they showed that 3.5% of children developed DILI. However, when looking at age, sex, nutritional status, type of TB and comorbid diseases as risk factors, none were found statistically significant. The N-acetyltransferase 2 (NAT 2) gene is responsible for INH metabolism – polymorphism in this gene can lead to a slow, medium or fast acetylation status. A Japanese study looking at adult patients showed an odds ratio of 4.32 between NAT 2 slow acetylators and DILI risk. A much older study conducted in 256 children in 1986 failed to demonstrate an association between acetylation phenotype and the risk of developing hepatotoxicity. Some specific genetic variants and HLA types have been shown to be potentially linked to risk of developing DILI – but in resource limited settings like South Africa this might not be pragmatic to investigate for in all patients. However, genetic differences between race groups and ultimately differences in the metabolism of tuberculosis medication and risk for drug toxicity is known to play a role .(25) (53) (54)(55)(56)(57)(58) Abnormal liver function tests at baseline is also a well-known risk factor to developing DILI in adults.(59)

Viral hepatitis has been implicated as possible cause or contributing factor of presumed drug induced hepatitis in patients on anti-tuberculosis therapy in various studies looking at first-line anti-tuberculosis drug studies.(61) Since the addition of hepatitis B vaccine to the South African EPI vaccination schedule, the rates of HBVsAg positive babies have dropped dramatically, from 8.1%

in 0-6 month old babies and 8.9% in 7-12 month old babies to 0.0% in children with an unknown HIV status, and 2.7% in HIV positive children. (62)

However, in developing countries like South Africa, the incidence of hepatitis A is high, and the vaccine for hepatitis A is not included in the national immunisation schedule (EPI) for children. In a study conducted in the Western Cape at Tygerberg Hospital, the median incidence of hepatitis A for children aged <13 year from 2001 to 2004 was 45.4/100 000/year, a significantly high incidence, above the threshold incidence for diseases usually included in the vaccination schedule (65). The likely reason for this is that Hepatitis A is usually considered a self-limiting disease in children and the prognosis in most cases is favourable – only 1% develop fulminant hepatitis.(66) Despite this, viral hepatitis and specifically hepatitis A is not screened for routinely in children admitted for TB treatment or RR/MDR-TB treatment, and neither is a vaccine to hepatitis A considered in these children. Differentiating between drug-induced and viral hepatitis is not possible on clinical grounds alone. It has been noted in adult studies that the hepatocellular injury caused by viral aetiology is usually more delayed, transaminases are raised more significantly and takes much longer to recover than in drug-induced hepatitis. (67)

The question needs to be asked whether hepatitis A is contributing significantly to hepatitis seen in children on RR/MDR-TB treatment; if this is significant, it could influence patient management: sooner screening, patient isolation and admission policies, as well as consideration for vaccination of at patients at risk.

The impact of HIV

HIV status can play a significant role in the incidence of hepatocellular injury in patients on RR/MDR-TB treatment. It is a shared side effect for nevirapine, ritonavir-boosted protease inhibitors, isoniazid and pyrazinamide.(68) However, when looking at available literature (including adult studies) very little data is available. (69) In an adult study looking at 52 HIV positive individuals on MDR TB treatment, up to 17.3% of patients developed hepatitis and in a third of

these patients anti-retrovirals or anti-tuberculous drugs had to be discontinued.(70) The thiodamines have an increased risk of hepatotoxicity when used in combination with evavirenz, nevirapine, tipranavir and darunavir. Hepatic CYP450 enzymes are responsible for metabolizing a variety of drugs and the combination of anti-retrovirals and anti-tuberculous drugs could influence drug levels due to either enzyme induction or inhibition. When hepatic impairment is present, it is best to monitor drug levels more closely – especially when ethionamide is used in combination with other hepatotoxic drugs, as this increases the risk for hepatocellular injury greatly. (71)

The DAIDS Adverse Event (AE) grading table provides an AE severity grading scale ranging from grades 1 to 5 with descriptions for each AE based on the following general guidelines:

• Grade 1 indicates a mild event

• Grade 2 indicates a moderate event

• Grade 3 indicates a severe event

• Grade 4 indicates a potentially life-threatening event

• Grade 5 indicates death (*Note*: This grade is not specifically listed on each page of the grading table).

Table 2(72)

PARAMETER	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
Clinical adverse event **NOT** identified	Mild symptoms causing no or minimal	Moderate symptoms causing greater	Severe symptoms causing	Potentially life-threatening symptoms

elsewhere in the grading table	interference with usual social & functional activities with intervention not indicated	than minimal interference with usual social & functional activities with intervention indicated	inability to perform usual social & functional activities with intervention or hospitalization indicated	causing inability to perform basic self-care functions with intervention indicated to prevent permanent impairment, persistent disability, or death

Very little information is available on the current management guidelines for hepatocellular injury on RR/MDR TB treatment in children. This study aims to contribute to this.(73)

Study objectives

Among children routinely treated for RR/MDR-TB:

Primary study objective:

1. To characterize the incidence, severity, and attribution to RR/MDR-TB treatment of hepatocellular injury

Secondary study objectives are:

1. To characterize the risk factors for developing hepatocellular injury

2. To describe the incidence, causes, management and outcome of severe cases of hepatocellular injury (grade 3 or more)

Methods

Study design

This study will be a retrospective analysis on prospectively collected data.

Study setting

It will derive its data from two MDR PK studies described below which were conducted in Cape Town. Children aged 0-17 were recruited at two sites (TCH and BCCH) in Cape Town, Western Cape province and followed up until treatment outcome.

The first study (MDR PK1) enrolled 276 children aged 0-15years over a period of 3.5 years – from October 2011 to October 2015- this included 276 children on RR/MDR-TB treatment or RR/MDR-TB preventive therapy. Both HIV-positive children (approximately 30%) as well as HIV-negative children were enrolled, and the HIV-positive children also included a control group children not on RR/MDR-TB treatment to be able to compare drug levels. The children on RR/MDR-TB treatment (approximately 140) were followed up until treatment completion or withdrawal from the study, and these will be included in this specific sub-study. Measures were taken to ensure that enough children under the age of two years were enrolled.

At baseline, HIV disease status, TB disease, anthropometric measures, symptoms of toxicity and certain biochemistry levels were measured (ALT, total bilirubin). Nat2 genotyping was done at an accredited lab and the patients were classified into the following acetylator types: homozygous fast (FF), heterozygous fast (FS) or homozygous slow (SS). CXR were taken in most cases when clinically indicated. TB drug dosing and adherence, TB culture and drug susceptibility testing were also monitored, and in HIV positive children, CD4 and viral load. Some of these parameters/measurements were repeated on monthly to two monthly basis and monitored throughout the treatment phase.

The schedule of events for this study is as follows:

Table 5. Schedule of events (serial follow-up in children with RR/MDR disease only)

Months following enrolment.

NOTE: ALL research measures are in bold and highlighted in yellow, other investigations are all routinely part of clinical care; all required blood volumes are indicated. PK sampling will not always co-coordinated with routine clinical sampling.

All children	**Baseline[1]**	**1**	**2**	**3**	**4**	**5**	**6**	**8**	**10**	**12**	**14**	**16**	**18/treatment completion**	**Ongoing**
HIV status*	•													
PK TB drugs[1*]	•													
12-lead electrocardiogram[6]														
TB disease status	•													
Anthropometric status *	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Toxicity (signs, symptoms, history) *	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Audiology	•	•	•	•	•	•	•			•			•	
Biochemistry (ALT, Total bili, creatinine, and K+ (0.5 ml)*	•		•		•		•			•			•	•

TSH, T4/T3 (0.8 ml)*	•		•		•		•	•	•	•		•	
Serum; TB biomarkers	•	•	•				•					•	
Haematology (FBC, diff) 0.5 ml[2*]	•	•	•		•		•			•		•	
Ophthalmology evaluation[7]	•	•	•				•					•	
TB exposure history*	•												
NAT1/2 Genotype (cell pellet)	•												
AG susceptibility Genotype	•												
TB drug dosing and adherence *During admission, chart review and outpatients*	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Concurrent other medication*	•	•	•	•	•	•	•		•		•		•	•
CXR	•		•		•		•	•	•	•	•	•	•	•
TB culture and DST	•	•	•	•	•	•	•		•	•	•		•	
Urine (bag)*	•	•												

HIV-infected (including HIV-infected controls not on TB therapy)													
PK cART*	•												
cART adherence*	•	•	•	•	•	•	•	•	•	•	•	•	•	
CD4 count and viral load*	•						•			•			•	

*[1]Baseline: at first study visit. [2]FBC required if the child is on linezolid, initially monthly and then if stable 2 monthly; to be completed in all HIV-infected children. Baseline FBC all disease, 6monthly for HIV+. Repeat FBC for initial abnormal result 6 months. [3]As per national programme, CD4 and VL every 6 months. ** in HIV-infected children on TB therapy and concurrent HIV-infected controls without TB therapy (monthly cART). All serial measures in children with DR disease only. TB drug PK to be repeated if TB regimen changes; [5] reassessed in MDR contacts as appropriate; [6] 12-lead ECG to be done on PK sampling day at 3 hours after drug dosing; [7]perform at first opportunity if already past some scheduled evaluations*

All serial measures only in children with disease ; Audiology is routinely monitored monthly while on injectable drugs e.g. amikacin e.g. 8/12 PRN; last audiology test will be 6/12 after stopping injectables

CD4 count and HIV viral load are routine at baseline and at 6 –monthly intervals

Note: HIV-infected controls without TB therapy will only have ART PK sampling and baseline measures indicated with an asterisk*

Children on PAS will not have additional TB drug sampling completed based on total blood volume requirements for research.

The second study (MDR PK2) was slightly different in design and also included children aged 0-17 years on RR/MDR and XDR TB treatment. It included 100 children, and ran over the course of approximately 5 years, enrolling patients from September 2015 to November 2017. Similarly it recorded baseline measures (e.g. HIV status, TB diagnosis, anthropometric status, CXR and CD4 and viral load in HIV infected individuals). For adverse event monitoring, various blood test were done including ALT and AST at allocated intervals.

The schedule for the second study was as follows:

(visit in weeks)	**BL**	**2**	**4**	**8**	**12**	**16**	**20**	**24**	**32**	**40**	**48**	**56**	**64**	**72[1]**	**Fnl**
General study measures															
HIV status	•														
Clinical symptoms/signs	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
TB disease status, type and severity	•														
TB exposure history	•														
TB drug dosing and adherence	•		•	•	•	•	•	•	•	•	•	•	•	•	
Concurrent other medication	•		•	•	•	•	•	•		•		•		•	
Anthropometric status	•		•	•	•	•	•	•	•	•	•	•	•	•	•
CXR	•			•		•		•			•			•	•
cART adherence* (HIV-infected only)	•		•	•	•	•	•	•	•	•	•	•	•	•	
CD4 count and viral load* (HIV-infected only)	•							•			•			•	

TB treatment outcome															•
PK sampling															
PK: Intensive sampling	•			•											
PK: Sparse sampling (<2y)											•				•
Genotype (cell pellet from PK)	•			•							•				•
Adverse events monitoring															
Clinical AE assessment	•		•	•	•	•	•	•	•	•	•	•	•	•	•
12-lead ECG (triplicate)	•			•											
Audiology[2]	•		•	•	•	•	•	•			•			•	•
ALT, albumin, creatinine, and K+ (0.5 ml)	•			•		•		•			•			•	•
TSH, FT4/FT3 (0.8 ml)	•			•		•		•	•	•	•			•	•
FBC with diff (0.5 ml)[3]	•							•							•
CPK, AST, lactate, FBC (Lzd only)[4]	•		•	•	•	•	•	•	•	•	•	•	•	•	•
Visual assessment (Lzd only)	•		•	•	•	•	•	•	•	•	•	•	•	•	•
Formal ophthalmology assessment	•		•	•				•							•
Treatment Response															
Bacteriology, DST	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Clinical markers of treatment response (symptoms, CXR, anthropometrics)	•	•	•	•		•		•			•			•	•

Study bacteriology (respiratory samples, stool, including GeneXpert)	•	•	•	•		•		•							
Serum; TB biomarkers (1 ml)	•		•	•				•							•
C-reactive protein	•		•	•				•							•
Urine; TB biomarkers (bag)*	•		•												
Acceptability/Bioavailability															
Acceptability assessments	•			•				•							
PK: Additional crossover intensive PK (subset only)	•														

LZD = linezolid; [1] Any additional follow-up visits will follow the similar pattern of 2 monthly evaluations, with more intensive monitoring 6 monthly; [2] Schedule of audiology for those who received injectable medications; those not receiving injectables will have audiology at baseline only; stop audiology monitoring earlier if injectable drugs are stopped, with last audiology test at least 6 months after stopping injectables [3] FBC at baseline and final assessment for all patients, and at additional time points for HIV-infected children as per standard of care; 6 months if initial FBC abnormal; [4] safety monitoring for those on Lzd only

Data collection

For both the studies from which study will derive its data, data were collected by research nurses, research counsellors and study physicians. Clinical data was stored in a safe place, coding of all confidential data including HIV test results, PK and other data, and patients' personal information, and blinding of laboratory staff to all personal patient information was done. Patient data were entered into a password-controlled database with restricted access limited to the principal investigators, data manager and study coordinator only. The results of toxicity monitoring (e.g.

liver function, hearing, thyroid function tests) was be made available to the attending routine clinical physician.

For this study, data will be derived from the existing database, and only patients with RR/MDR TB disease placed on RR/MDR TB treatment will be included. INH mono-resistance will not be considered as part of this analysis, and where patients' diagnosis were incorrect (e.g. where they later proved to not have TB disease or to rather have drug sensitive TB possibly due to false positive test results) will be excluded from the analysis. In cases where additional information might be required which is not included in the database, a folder review will be conducted at the appropriate site.

Hepatocellular injury will be graded according to the DAIDS (the division of AIDS) grading table as raised liver enzyme enzymes, including specifically ALT(alanine aminotransferase), AST(aspartate transaminase) and total serum bilirubin.(82)

Parameter	Grade 1 (mild)	Grade 2 (moderate)	Grade 3 (severe)	Grade 4 (potentially life-threatening)
AST	1.25 to <2.5 X ULN*	2.5 to < 5 X ULN	5 to <10 X ULN	≥10 X ULN
ALT	1.25 to <2.5 X ULN	2.5 to < 5 X ULN	5 to <10 X ULN	≥10 X ULN
Total bilirubin, high (age >28 days)	1.1 to < 1.6 X ULN	1.6 to <2.6 X ULN	2.6 to <5 X ULN	≥5 X ULN

*ULN is the upper limit of normal

The normal reference ranges of NHLS (the National Health Services Laboratory) will be used. There is no South African data for the normal reference ranges of normal liver enzymes in children, but reference ranges in other countries have been published. (88–92)

Statistical analysis

The baseline demographics and clinical characteristics will be summarized using frequencies and percentages for categorical variables. For continuous variables, if normally distributed, the means and standard deviations will be displayed; if not normally distributed, median and interquartile ranges will be displayed. Weight-for-age Z-score (WAZ) and height-for-age Z-score (HAZ) will be calculated using British 1990 growth reference.

The frequency of hepatocellular injury will be reported by DAIDS grade. This will be defined as raised ALT/raised AST as per NHLS normal values. The proportion of patients with a concurrently raised total bilirubin will also be described. The cumulative incidence rates for hepatocellular injury will be calculated per person-time of observation. Person-time will be calculated from the baseline study assessment until the treatment completed or the last available study visit for patients that did not complete the study per protocol. The median time to hepatocellular injury AE and corresponding IQR will be reported. The Kaplan Meier curve denoting time to any hepatocellular injury AE and time to Grade 3 or more hepatocellular injury AE will be displayed.

We will describe the proportion of patients who had hepatocellular injury which required treatment interruption or treatment discontinuation. The proportion of patients with hepatocellular injury who also had concurrent symptoms and signs (new onset vomiting and abdominal pain/tenderness and jaundice) will be reported – as opposed to those who did not develop symptoms and signs. The proportion of patients who did develop new onset vomiting (i.e. symptoms) but not hepatitis will also be reported.

The incidence rate for hepatitis A in this group will be described.

Risk factors will include age, sex, ethnicity, HIV status, ART status (patient on ART at time of hepatocellular injury vs not on ART), in-patient status vs. out-patient status, nutritional status, ALT status at baseline (abnormal ALT vs normal ALT). Where data available, HBsAg status (HBsAg positive vs HBsAG negative), hepatitis A status andNAT2 genotype acetylator status (FF vs FS vs

SS) and vaccination status (whether up to date/not up to date or undocumented) will also be counted as risk factors. The association between these variables and the occurrence of DILI will be assessed using survival analysis. Log rank tests will be done for each of the risk factors to determine if they are associated with time to hepatocellular injury. $P<0.05$ will be considered statistically significant.

Specific risk factors for severe cases (grade3-4) will be calculated similarly as above. In some cases further investigations might have been performed to exclude alternative diagnoses (viral, autoimmune) as possible alternative or contributing factors – this study will also describe (where possible) the outcome of these investigations in severe cases.

Finally, this study will aim to describe the management and outcome of severe cases.

Ethics considerations

Human subjects' involvement, characteristics

The primary studies that this study will derive its data from was conducted in a population with high rates of drug-resistant TB disease. The prevalence of culture confirmed drug-resistant TB in children was 6.8% in 2011. The PK (pharmacokinetics) of second line TB drugs has not been studied in this population before. Drug toxicity related to RR/MDR-TB treatment, which often implicates prolonged duration of treatment, has not been studied in this population group. The primary studies were prospective hospital based non-interventional studies where PK of anti-TB drugs and ART were measured as well as toxicity of drugs. This sub-study will focus on hepatocellular injury specifically, and will aim to identify which children are particularly at risk of developing hepatocellular injury.

Children with RR/MDR or XDR TB are usually initially hospitalized and treatment duration is typically 12-18 months long, depending on the specific severity and disease spectrum. This treatment is offered free of charge in the public health care sector. Treatment outcome for these children is usually positive (clinical or bacteriological cure in >90%). Treatment outcome data was collected, including bacteriological conversion, where present.

HIV co-infection is common (15-20% of all childhood TB cases are HIV co-infected), and HIV positive children were fast tracked for ART (antiretroviral therapy). The care for these patients were also free of charge.

Risks to the subjects

The primary procedures done for these two primary studies (PK of TB drugs and ART) is not part of routine care of children with RR/MDR-TB and/or HIV. Data obtained from these measures did not directly impact the clinical care of participants but it would be used to inform clinical treatment guidelines in the future.

Overall, the following requirements were considered when determining sample size and sampling schedule: 1) To characterize PK for a range of key TB with differing PK parameters using drug concentrations measured in the same samples and to accurately describe PK across a range of ages and covariates. This approach utilizes minimal blood volumes for PK and other sampling. Children may experience pain associated with phlebotomy. This was reduced by using topical anaesthetic cream (Emla). Only venous samples were collected. An indwelling catheter was inserted in a peripheral vein on the morning of the evaluation. A maximum of 3 attempts were made to insert the catheter. Minimum volume assays were used. Arterial blood specimens were not obtained. Nursing staff were trained by an experienced paediatrician to perform phlebotomy. Additionally, distracters such as puppets, toys, and music or pin wheels were used to minimize the amount of stress experienced by children. The cost of these distracters was built into estimated phlebotomy costs.

Blood volumes taken did not exceed those recommended by the CMRC IRB. Children weighing less than 5kg and a haemoglobin <8 were excluded in the first primary study for this reason. In the second study, children less than 2.5kg were excluded. A child's total blood volume is 80ml/kg. Internationally accepted and NIH research guidelines regarding study involvement of children dictate that no child should provide >3% of total blood volume, per 24 hour period (see www.irb.pitt.edu/Guidance/Bloodwithdrawpediatrics.pdf). In a 2.5kg child, the participant with the

lowest potential body weight recruited in our study, this amounts to 6mL (2.5 x 80 mL x 0.03) per study visit and in a 5 kg child this amounts to 12ml. In the first study, only children >5kg were included and only 6-8mls of bloods was taken. In the second study, blood volumes collected in infants <12 months of age were limited to 6mL per study visit, which will be most relevant to the subset of children in our proposed study who are on linezolid.

International guidelines further specify that the cumulative withdrawal over an 8-week period shall not exceed 10% of the circulating blood volume. In a 2.5 kg child this amounts to 25mL over 8 weeks and in a 5 kg child this amounts to 40mls. The first study limited sampling to 8.5ml over any given 8 week period, and the second study obtained a maximum of 8-10 ml over any given 8-week period for this study. Thus, venous blood specimens provided by study participants adhered to the recommended guidelines. These blood volumes were culturally acceptable and feasible to obtain in the study population. High rates (>90%) of successful phlebotomy in participants <5 years were experienced.

Phlebotomy for assessment of toxicity was combined with PK where possible. Where toxicity screening has already been completed as part of routine care, those data would be included in the study without subjecting the child to further phlebotomy.

Serial follow-up will be completed in children on RR/MDR treatment until the end of treatment completion as per our schedule of evaluations.

Other measures and procedures (toxicity monitoring, TST, CXR, sputum collection) were routinely completed in children with DR-TB disease, with very limited patient risk. As part of research participation, children were monitored in a more standard manner during their entire duration of TB therapy. Treatment response was routinely assessed in children with DR disease throughout therapy including weight measures, CXR and gastric aspirates/sputum and DST to assess treatment success.

All TB drugs given to children were components of recommended standard of care in the study setting.

Recruitment and informed consent

All children presenting to the two study hospitals (Tygerberg Children's Hospital and Brooklyn Chest Hospital) were approached by the study teams and offered information about the study in an attempt to recruit children. The research team contacted the child's parent of legal guardian to obtain informed consent.

South African law defines an adult as older than 18 years. Since all study subjects were below this age and therefore considered minors, written informed consent was obtained from parents/legal guardians for study participation as well as blood tests for PK sampling and liver enzymes. Consent forms were translated into English, Xhosa and Afrikaans, and back-translated to English to ensure clarity, in accordance with the South African Good Clinical Practise Guidelines. HIV testing was consented for separately and additionally with the appropriate pre- and post-test counselling. Provision was made for unexpected HIV positive results (including confirmatory ELISA or PCR testing), and newly diagnosed HIV-infected children were referred to a social worker and paediatric services at the Tygerberg HIV Family clinic. Children with a known positive HIV status were not tested again. Consent was also obtained for genotyping relevant to TB drug toxicity and metabolism in the second primary study. Assent was attained where age-appropriate, and consent/assent were obtained in the home language of the study participant or parent/legal guardian. Provisions were made for illiterate adults. Parents/guardians were allowed to disclose their child's HIV test only at their own discretion. Research counsellors were verbally fluent in the local languages (Xhosa, Afrikaans and English) and also familiar with the local culture values and norms.

The first study enrolled children ≤15 years of age. This study only enrolled children on treatment for >2 weeks and <8 weeks.

The second study enrolled children ≤17 years of age.

Inclusion and exclusion criteria for the second study were as follows:

Inclusion criteria

HIV-infected and uninfected children routinely started on 2nd line TB drugs for DR-TB treatment will be consecutively screened for enrolment. The following children will be included.
1. Known HIV status or willingness to undergo HIV testing is status us unknown
2. Aged 0-17 years
3. Routinely started on 2nd-line treatment for DR-TB (MDR, rifampicin mono-resistance, Pre-XDR and XDR TB) with a regimen that includes levofloxacin, moxifloxacin, or linezolid
4. Written informed consent/assent as age-appropriate
5. On TB treatment for <12 weeks.
Exclusion criteria
1. Informed consent/assent for participation not obtained
2. Lab-documented anaemia (Hb <8 g/dl) (may be deferred)
3. Body weight <2.5 kg (may be deferred).
4. Serious chronic illness, such as clinically significant structural cardiac disease, chronic lung, renal or liver disease
Enrolment will be deferred for any acute illness such as respiratory compromise, severe dehydration, or renal impairment, which may compromise safe participation in the study. PK Sampling will be deferred until on TB treatment for ≥ 2 weeks but <12 weeks, and if HIV infected on ARVs for ≥2 weeks and for acute illness. Children with DR-TB meningitis will not be excluded; when CSF is being routinely collected for clinical purposes, we will opportunistically sample CSF for levofloxacin, moxifloxacin, and linezolid concentrations.

Following study enrolment, baseline clinical and demographic data was obtained. All study measures were conducted in the examination and procedure rooms of Tygerberg Children's Hospital and Brooklyn Chest Hospital for Chest Diseases PK research unit, with adequate clinical facilities available. Tygerberg Children's Hospital is adjacent to the Stellenbosch Faculty of Heath Sciences, and Brooklyn Chest Hospital is 20 km from the faculty. A study physician completed clinical assessment for study purposes, and research nurses also assisted with obtaining study measures in children, including HIV testing and PK sampling. The medical care of children participating in these studies were not adversely affected if guardians declined participation.

Protection against risk

Both the primary studies were submitted to the institutional review boards of Stellenbosch University, University of Cape Town, the University of California and San Francisco and the Western Cape Department of Health. The IRBs reviewed the study protocols, consent forms, the data collection forms and questionnaires. Adult consent involved a full length written consent. Informed consent for participating minors were given by a parent or legal guardian. Assent was completed in all children ≥7 years of age, based on South Africa guidelines. Consent documents were translated and reverse translated from English into the local languages (Xhosa and Afrikaans) by a professional translator employed at the Stellenbosch University Language School who holds a Master's degree with proficiency in Xhosa, Afrikaans and English.

The studies were conducted in full conformity with the up to date revision of the Declaration of Helsinki (1989) and/or with the International Conference for Harmonization Good Clinical Practise regulations and guidelines.

Both HIV and TB are associated with stigma in the study setting- however, due to the hospital based nature of these studies this was not a major problem. Patient confidentiality was maintained at all times by the research team. For all study purposes, language (Xhosa, English or Afrikaans, where relevant) and concepts that are locally relevant and understood were used. Input from local hospital health care workers and families were obtained when drafting study documents and

health care staff who are familiar with the study population and sensitive to their needs were employed and recruited to reduce cultural barriers. Ongoing research in these 2 hospitals has provided valuable insights into locally acceptable cultural norms and practice.

Site preparation has been established through ongoing paediatric RR/MDR-TB research conducted by at both Tygerberg and Brooklyn Hospital for Chest Diseases, where the medical superintendent is regularly consulted and study results regularly disseminated. No financial incentives were be offered to participants, apart from necessary reimbursement of transport costs as appropriate throughout the study to help enable parents to visit their children in hospital.

Confidentiality

Data was be collected by research nurses, research counsellors and study physicians. Study staff maintained standard procedure to ensure patient confidentiality. This included storage of all clinical data in a safe place, coding of all confidential data including HIV test results, PK and other data, and patients' personal information, and blinding of laboratory staff to all personal patient information. Patient data was entered into a password-controlled database with restricted access limited to the principal investigators, data manager and study coordinator only. The results of toxicity monitoring (e.g. liver function, hearing, thyroid function tests) was made available to the attending routine clinical physician.

Potential benefits of the proposed research to human subjects and others

There were no direct benefits to study participants of either of these primary studies. The standard of care of study participants related to both TB and HIV were of equal or higher quality compared to care routinely available through public health facilities. For example, complete standard serial clinical monitoring for potential toxicities related to TB drugs and ARV were conducted. Results of these routine investigations, e.g. hearing tests, were made available to the attending routine care team.

All children enrolled in these studies already received routine clinical care for DR disease and HIV care as per local and international guidelines. All children with TB were notified to the local health

authorities as per South African guidelines. All HIV-infected children newly diagnosed were referred for care to the most proximate routine public HIV clinic. TB disease status assessment and related measures were completed in accordance with local and international diagnostic guidelines. No routine TB disease diagnostic procedures or treatment responses measures were completed solely for study purposes. Follow-up procedures (e.g. thyroid function, liver and renal function) are recommended but not always implemented in standard fashion. TB and ARV PK results will not be provided, as these are not included in South African TB guidelines and were viewed as a research measure. All results provided to the routine health care providers had study unique identifiers removed.

Importance of the knowledge to be gained

Study results were disseminated to all relevant role players, including local clinicians, hospital personnel, academic colleagues and public health authorities; ongoing feedback was provided regarding interim study results. Feedback was provided to the study hospitals and personnel on a bi-annual basis, at scheduled meetings. The investigators presented their findings at local and international scientific meetings and in international peer-reviewed scientific journals. The anticipation for both studies was that data from these studies on the PK of 2ndline drugs for DR-TB in children would impact on the clinical care of young and HIV-infected children with regards to: 1) appropriate dosing, 2) drug interactions and 3) monitoring and future prevention of toxicity. The findings may form scientific foundation for recommendations and policy in children in resource-limited settings with high burdens of TB and HIV, where rational guidelines and allocation of resources are urgently needed. The potential gained from this knowledge exceeded the minimal risk of participation.

Voluntary withdrawal

Participation in these studies was entirely voluntary and the parent/legal guardian or participant had the right to withdraw participation at any stage. Withdrawal from the studies did affect their routine clinical care.

Inclusion of children

The study populations (children aged ≤15 years for the first study and children ≤ 17 years of age for the second study with DR-TB disease) has been selected because there is limited data on PK of key 2^{ndline} line TB drugs in children with and without HIV co-infection and this group of children has a potential high risk of toxicities associated with the current treatment regimens, which need to be optimized. The studies could only be completed in a population with high rates of DR disease and high HIV prevalence in children. These populations do not exist in the US and are highly prevalent in South Africa. Young children (<5 years) and HIV-infected children have the most variable drug exposure with TB drugs including 2^{ndline} line TB drugs and need to specifically targeted for inclusion in PK and safety studies. Children 0-2 years and also infants (< 12 months of age) were specifically included using a stratified enrolment approach. No infants were excluded from participation since data in these paediatric subpopulations are extremely limited and are urgently needed. Only infants below 5 kg in the first study and infants below 2.5 kg weight in the second study, will be excluded, given the challenges with blood volumes for the proposed research measures.

The study teams were composed of clinicians with expertise in the study of paediatric DR-TB TB and HIV. The study site has extensive experience conducting paediatric TB clinical and PK research. Study staff are trained to be sensitive to the special needs of children according to their ages. Consent of parents or guardians and assent of children ≥ 7 years of age was routinely obtained for study participation. Sample size considerations were completed to ensure that the targeted recruitment goal would support meaningful analysis and answer the study questions in relevant age groups, including questions regarding formulation acceptability in children and their caregivers.

Knowledge gained from the more optimal use of key 2^{ndline} line TB drugs in children will also inform the evaluation of novel TB drugs and regimens in children in future. Children have traditionally been excluded from research on novel TB drugs but changing regulatory frameworks in the US

and in Europe now require appropriate paediatric evaluation of such novel entities. The hope is that knowledge gained from these two primary studies may greatly improve the treatment of DR-TB in children of representative ages, with and without HIV co-infection, in resource-limited settings, and globally.

Ethics approval

This study proposal will be submitted to the Health Research Ethics Committee of the Faculty of Medical and Health Sciences of Stellenbosch University, for review and approval for MMed purposes – however, the two primary studies it will derive its data from, have already been approved by ethics.

Study limitations

This study is limited in that is derived its data from two studies that were planned and purposed rather for PK sampling than the specific aim to establish the incidence and risk factors for hepatocellular injury. However, adequate information should be available from study measurements to make valuable contributions despite these limitations.

Budget

The cost involved for this study is negligible as its data will be largely derived from two fully funded studies without additional cost.

Budget Item	**Cost (Rand)**
Assistance with data collection	Funded, Desmond Tutu TB Centre (DTTC)
Assistance with database development	Funded, DTTC
Assistance with data entry	Funded, DTTC
Stationery and photocopying	R500
TOTAL	**R500**

Data collection and Management

For the primary studies, standard questionnaires were designed and an Access-based relational database developed. All original data source documents was transported to DTTC on a daily basis, and captured through dual-entered validated real-time procedures. Data entry and quality control was done by a data entry technician supervised by a data manager. A study-specific QA officer completed QC and ongoing QA processes. Data was stored on a central SQL server that is backed up daily; access is limited to the data management team and study PIs. A patient follow-up tracking system was built into the database to optimize longitudinal data collection and minimize missing data. The database manager was responsible for supervision of data entry, generating daily missing data reports, and weekly recruitment and follow-up summaries. Weekly database meetings were held between the data and lab team study coordinator and the on-site PI.

For this specific sub-study, data has been captured and stored in REDCap database with secured access limited to authorized personnel involved in the two primary studies.

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APPENDIX B – Instructions for Authors

The Pediatric Infectious Disease Journal (PIDJ)

Online Submission and Review System

Author Resources
Instructions for Authors (this page)
Reprint Ordering
Permissions Requests

SCOPE

The Pediatric Infectious Disease Journal is a peer-reviewed, multidisciplinary journal directed to physicians and other health care professionals who manage infectious diseases of childhood.

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Journal article
1. Trujillo M, Correa N, Olsen K, et al. Cefprozil concentrations in middle ear fluid. *Pediatr Infect Dis J*. 2000;19:268—270.

Book chapter
2. Grose C. Bacterial myositis and pyomyositis. In: Feigin RD, Cherry JD, eds. *Textbook of Pediatric Infectious Diseases*. 4th ed. Philadelphia: Lippincott Williams & Wilkins; 1998:704—708.

Entire book
3. Nelson JD, Bradley JS. *Nelson's Pocket Book of Pediatric Antimicrobial Therapy*. 14th ed. Philadelphia: Lippincott Williams & Wilkins; 2000.

Proceedings
4. Harrigan PR, Dong W, Weber AE, et al. Highly mutated RT and protease [Abstract I-115]. In: 38th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Diego, CA, September 24 to 27, 1998. Washington, DC: American Society for Microbiology; 1998.

Online journals
5. Friedman SA. Preeclampsia. Obstet Gynecol. [serial online]. January 1988;71:22–37.

World Wide Web
6. Gostin LO. Drug use and HIV/AIDS [JAMA HIV/AIDS web site]. June 1, 1996. Available at: http://www.ama-assn.org/special/hiv/ethics. Accessed June 26, 1997.

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HREC Reference No: S19/09/183
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After Ethical Review
Translation of the informed consent document(s) to the language(s) applicable to your study participants should now be submitted to the HREC.
Please note you can submit your progress report through the online ethics application process, available at: Links Application Form Direct Link and the
application should be submitted to the HREC before the year has expired. Please see *Forms and Instructions* on our HREC website (www.sun.ac.za/healthresearchethics) for guidance on how to submit a progress report.
The HREC will then consider the continuation of the project for a further year (if necessary). Annually a number of projects may be selected randomly for an
external audit.
Provincial and City of Cape Town Approval
Please note that for research at a primary or secondary healthcare facility, permission must still be obtained from the relevant authorities (Western Cape Department of
Health and/or City Health) to conduct the research as stated in the protocol. Please consult the Western Cape Government website for access to the online Health Research Approval Process, see: https://www.westerncape.gov.za/general-publication/health-research-approval-process. Research that will be conducted at
any tertiary academic institution requires approval from the relevant hospital manager. Ethics approval is required BEFORE approval can be obtained from
these health authorities.
We wish you the best as you conduct your research.
For standard HREC forms and instructions, please visit: Forms and Instructions on our HREC website https://applyethics.sun.ac.za/ProjectView/Index/10394
If you have any questions or need further assistance, please contact the HREC office at 021 938 9657.
Yours sincerely,
Mrs. Melody Shana
Coordinator
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HREC2
National Health Research Ethics Council (NHREC) Registration Number:
REC-130408-012 (HREC1)·REC-230208-010 (HREC2)
Federal Wide Assurance Number: 00001372
Office of Human Research Protections (OHRP) Institutional Review Board (IRB) Number:
IRB0005240 (HREC1)·IRB0005239 (HREC2)
The Health Research Ethics Committee (HREC) complies with the SA National Health Act No. 61 of 2003 as it pertains to health research. The HREC abides by the ethical
*norms and principles for research, established by the*World Medical Association (2013). Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects;*the South African* Department of Health (2006). Guidelines for Good Practice in the Conduct of Clinical Trials with Human Participants in South Africa (2nd edition);
as well as the Department of Health (2015). Ethics in Health Research: Principles, Processes andStructures (2nd edition).
The Health Research Ethics Committee reviews research involving human subjects conducted or supported by the Department of Health and Human Services, or other federal
departments or agencies that apply the Federal Policy for the Protection of Human Subjects to such research (United States Code of Federal Regulations Title 45 Part 46);
and/or clinical investigations regulated by the Food and Drug Administration (FDA) of the Department of Health and Human Services.
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Approval Letter
Progress Report
22/07/2021
Project ID: 10394
Ethics Reference No: S19/09/183
Project Title: Incidence, causes and outcome of management in cases of hepatocellular injury in paediatric patients on multidrug-resistant tuberculosis
therapy
Dear Dr J Duvenhage
We refer to your request for an extension/annual renewal of ethics approval dated 19/07/2021 09:33.
The Health Research Ethics Committee reviewed and approved the annual progress report through an expedited review process.
The approval of this project is extended for a further year.
Approval date: 22 July 2021
Expiry date: 21 July 2022
Kindly be reminded to submit progress reports two (2) months before expiry date.
Where to submit any documentation
Kindly note that the HREC uses an electronic ethics review management system, *Infonetica*, to manage ethics applications and ethics review process. To
submit any documentation to HREC, please click on the following link: https://applyethics.sun.ac.za.
Please remember to use your Project Id 10394 and ethics reference number S19/09/183 on any documents or correspondence with the HREC concerning
your research protocol.
Please note that for studies involving the use of questionnaires, the final copy should be uploaded on Infonetica.
Yours sincerely,
Ms Brightness Nxumalo
Coordinator: Health Research Ethics Committee 2 (HREC 2)
National Health Research Ethics Council (NHREC) Registration Number:
REC-130408-012 (HREC1)·REC-230208-010 (HREC2)
Federal Wide Assurance Number: 00001372
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